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CONTENTS

HUMAN LYSOSOMAL α-GALACTOSIDASE OPENS THE DOOR TO NEW PHARMACOLOGICAL CHAPERONES FOR FABRY DISEASE

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Abstract

Fabry Disease (FD) is a rare lysosomal storage impairment caused by mutations in the alpha-galactosidase-A gene (GLA), that leads to a decrease in α-galactosidase A (AGAL) enzyme activity. This abnormality promotes the accumulation of glycosphingolipids, particularly globotriaosylceramide (Gb3) in various vital organs such as heart, nervous system and kidneys etc. Increased Gb3 induces lysosomal dysfunction, resulting in impaired cellular signaling pathways and subsequently leads to the organ failure. Enzyme replacement therapy, is the usual treatment for FD is, but due to high cost recently chaperone therapy i,e Migalastat has been launched as a treatment option. Migalastat interacts with active site of α-GLA, stabilizes the abnormal forms and encourages the trafficking to lysosomes for substrate degradation. Taking the advantage of computational approach, present study aimed to identify chemically analogous compounds to Migalastat and to understand their molecular insights & binding potential against alpha-galactosidase A (PDB ID: 3GXT). Critical observations indicated the greater selectivity of (2R,3R,4S,5R)-2-(hydroxymethyl) piperidine-3,4,5-triol toward 3GXT, by affording D score -7.53 kcal/mol in comparison to reference drug mogalastat (-7.60 kcal/mol). *In silico* studies suggested (2R,3R,4S,5R)-2-(hydroxymethyl) piperidine-3,4,5-triol serves as possible pharmacological chaperones for Fabry disease.

Key words: Fabry Disease, Migalastat, in silico studies, chaperones.

1. Introduction

In 1898, Dortmund dermatologist Johannes Fabry and London dermatologist William Anderson reported identical patients with angiokeratoma corporis diffusum. The inherited condition was named Anderson–Fabry disease (AFD), nowadays generally referred as Fabry disease (FD) [Anderson ,1898; and Fabry J., 1898]. FD an X-linked lysosomal storage disorder with an incidence of approximately 1:40,000–1:117,000 among males and females wordwide. It is caused due to the absence or deficiency of the alpha-galactosidase A (α-Gal-A) that triggers an intracellular collection of toxic metabolites such as globotriaosylceramide (Gb3) and other glycosphingolipids present in the lysosomes of various cells including cardiac, endothelium, renal and nerve cells [Beirao I, et al., 2017]. Subsequently, ends with life-threatening complications and individual's

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premature death, due to irreversible damage to the affected organs [Burlina, A et al., 2023; Amodio F, et al., 2022]. Characteristic features includes peripheral neuropathic pain, cornea verticillata, abdominal pain, vomiting, diarrhea, angiokeratoma, left ventricular hypertrophy, cryptogenic stroke, anhidrosis, kidney failure, red spots on skin etc. shown in **Fig. 1** [Weidemann, F et al., 2022].

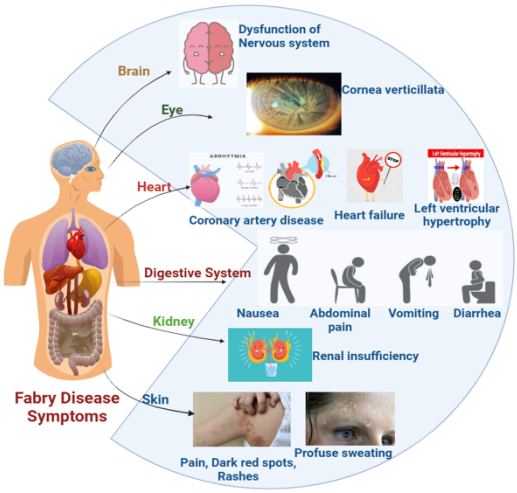


Fig. 1 Fabry disease: a multisystemic disease [6].

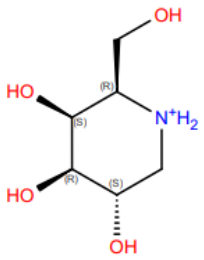
Fabry disease is categorized into two types: Classical and Later-onset, based on the degree of enzyme failure in affected individuals. Classic Fabry, the rarer variant, often appears in childhood, while the late-onset one, as indicated by its name generally begins to exhibit symptoms in individuals during their 30s or 40s [Arends M, et al., 2017].

Early identification and quick treatment of FD patients is critical to prevent irreversible tissue damage and organ failure. Gene/RNA and Enzyme replacement therapy (ERT), are treatments available for the patient with FD, unfortunately the cost of ERT is too high. The treatment requires lifelong treatment for the patients which involves the use of either Fabrazyme or Replagal. Due to high cost, In 2016, an oral chaperone therapy i,e. Megalastat also known as Galafold, was approved for the management of FD by the European Union and followed by approval in United States during 2018 [Miki Y, et al., 1994]. Migalastat reversibly binds to the active site and stabilizes mutant forms of α-Gal, which permits the trafficking into lysosomes and subsequently allows substrates (GL-3) breakdown [Keyzor I, et al ., 2023;]. This situation is continuously knocking the doors for affordable, safe molecules and new treatment options for patients with high medical needs. Thus, taking into consideration, the present study was aimed to identify structurally similar molecules to Migalastat and further, to investigate molecular binding interactions of Migalastat and similar molecues with alpha-galactosidase A (PDB ID: 3GXT) enzyme essential for lysosomes metabolic activity to identify its probable mechanism of action.

2.1. Material and methods

2.1.1. *In silico studies*

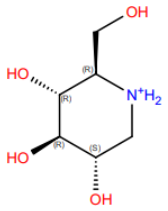
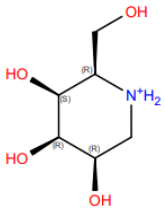
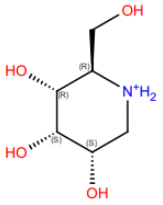
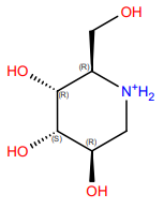
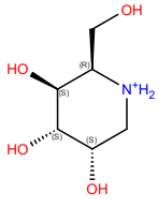
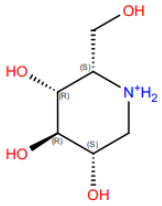
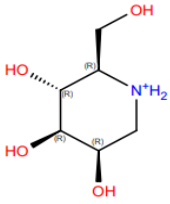
Data set of the molecules similar to migalastat, were taken from reaxys software using structure similarity tool (**Table 1**). Further, same dataset was used for the docking studies, conducted on HP Pentium 4 Processor 2.80 GHz system using Schrodinger Maestro 12.3 version.



$C_6H_{13}NO_4$

Migalastat (1-deoxygalactonojirimycin)

Table 1. Dataset of molecules.

Sr. No.	Structure	Chemical name	Reference
Compound 1		1-deoxynojirimycin	Kuska, J et al., 2021
Compound 2		1-deoxyallonojirimycin	Kuska, J et al., 2021
Compound 3		1-deoxytalonojirimycin	Kato A, et al., 2015
Compound 4		(2R,3R,4S,5R)-2-(hydroxymethyl)piperidine-3,4,5-triol	Azad CS et al., 2021
Compound 5		1-deoxygulonojirimycin	Li, M et al., 2018
Compound 6		1-deoxynojirimycin	Martins AM, et al., 2009
Compound 7		L-deoxymannojirimycin	Whybra C et al., 2006

The marketed drug Migalastat and its structural analogues (**compound 1-7**) 2D structures were converted into 3D geometry using Maestro module in Schrodinger software. All the ligands were geometrically & energetically minimized using MacroModel module. Further to achieve an RMSD of 1.8 A, all the stable conformations were allowed to optimized using Liquid Simulations-2005 force field at 7.4 pH. The optimised molecules were subjected for docking study.

The targeted enzyme alpha-galactosidase A, crystal structure (PDB ID: 3GXT, 2.70Å) was downloaded from Protein Data Bank (RCSB). Protein Preparation wizard was used to prepare and refine the protein structure. The protein structure integrity was assessed & refined for topologies, missing hydrogen atoms, bond ordering, and formal charges. The water molecules beyond 5 Å were removed from the protein and valency was maintained by addition of hydrogen molecules. Finally, OPLS2005 force field with inbuilt Root Mean Square Deviation (RMSD) of 0.30 was used for restrained minimization of target protein. The interaction site where ligand and protein can interact is depicted by the receptor grid, defined in terms of x, y, and z coordinates was created using receptor grid generation tool. Finally, the dimensions of the receptor grid, were defined at 14Å x 14Å x 14 Å. Finally the energetically minimized ligands were docked with receptor 3GXT using Glide extra-precision module. Obtained results were recorded in the form of D-scores, types of interactions and active amino acid residues involved.

3. Results and Discussion

Recently, molecular docking has gained considerable attention due to their potential to fasten up the drug discovery process. This method implies atom level predictions how a small molecule interacts & behaves within the binding site of target protein. Further, helps to optimize lead compound to discover novel physiologically active compound. One of the most significant treatment options for patients with Fabry disease is oral migalastat, the first and only oral medication available in market. Herein authors are reporting the first-ever binding relationship between migalastat and their analogues with alpha-galactosidase A (PDB ID: 3GXT) receptor using Glide module of the Schrodinger software. The docking outcomes compiled in the form of docking scores, interacting active amino acids, types of interactions involved are depicted in **Table 2**, accompanimented with **Figs. 2–3** indicating 2D and 3D ligand-receptor interactions. In ligand receptor interaction studies, binding affinity is projected by docking score that indicates the information about the degree of binding interactions between the ligand and receptor. The magnitude of negative score indicates ligand binding affinity, stability and compactness towards the binding site of receptor.

The docking scores for the selected ligands compound 1 to compound 7 were found to be in the range -6.65 and -7.53 respectively in comparison to Migalastat having docking score -7.60. Migalastat exhibited hydrogen bonding with ASP 170 amino acid residues by

interacting with the amine group at position (N-1) and ASP 92, ASP 93, ASP 231, LYS 168 amino acid residues by interacting with the amine group at OH group **Table 2** Amongst the selected compound the sequential order was found as: compound 4 > compound 2 > compound 1 > compound 7 was observed and found comparable binding affinity to the receptor protein by securing close docking score to reference drug Migalastat shown in **Table 2.**

Table 2. Docking score and various types of interactions involved between Migalastat, & selected compounds with alpha- galactosidase-A enzyme.

Compounds	D-score kcal/mol)	No. of residues	H-bonding	Pi-cation	Salt bridge
Migalastat	**-7.60**	**15**	ASP 231, ASP 170, LYS 168, ASP 93, ASP 92	TRP 47	ASP 170
Compound 1	**-7.32**	**13**	ASP 231, ASP 170, LYS 168, ASP 93, ASP 92	TRP 47	ASP 170
Compound 2	**-7.45**	**14**	ASP 231, GLH 203, ASP 170, LYS 168, ASP 93	TRP 47	ASP 170
Compound 3	**-6.65**	**13**	ASP 231, ASP 170, ASP 93, ASP 92	TRP 47	ASP 170
Compound 4	**-7.53**	**15**	ASP 231, GLH 203, ASP 170, ASP 93	TRP 47	ASP 170
Compound 5	**-6.67**	**14**	ASP 93, ASP 170, ASP 231, LYS 168	TRP 47	ASP 92, ASP 170
Compound 6	**-6.95**	**13**	ASP 231, GLH 203, ASP 170, LYS 168, ASP 92	TRP 47	ASP 170, ASP 93, ASP 92
Compound 7	**-7.364**	**12**	GLH 203, ASP 170, LYS 168, ASP 93, ASP 92	TRP 47	ASP 170

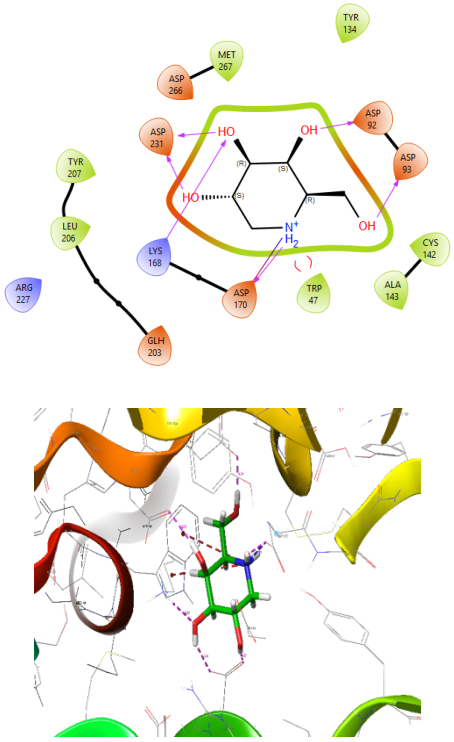


Fig. 2. 2D and 3D illustrations indicating binding interactions between **Migalastat** (---------- Hydrogen bonds, ---------- Pi cation, ---------- Salt bridge) and **3GXT receptor**.

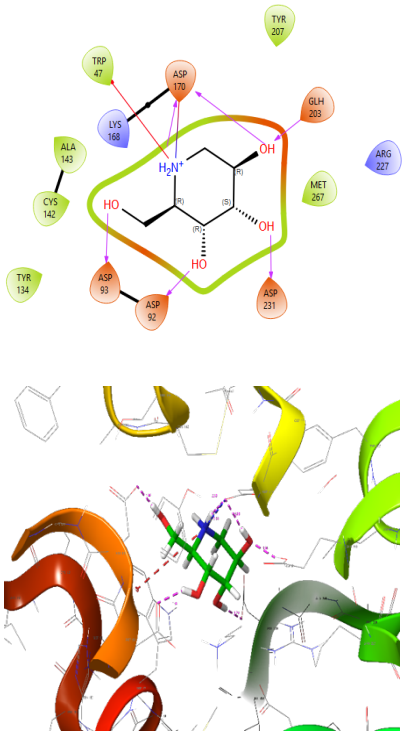


Fig. 3. 2D and 3D illustrations indicating binding interactions between **compound 4** (---------- Hydrogen bonds, ---------- Pi cation, ---------- Salt bridge) and **3GXT receptor**.

Docking analysis revealed **compound 4** [(2R,3R,4S,5R)-2-(hydroxymethyl) piperidine-3,4,5-triol] exhibited highest affinity with binding energy of **-7.530** Kcal/mol. This compound established hydrogen bond with ASP 93, ASP 170, ASP 231, GLH 203 and pi stacking with TRP 47 while engaging in Salt bridge with ASP 170. Overall observations from docking studies indicated that **compound 4** can potentially binds with the active site of α-GLA, and help in trafficking to lysosomes for substrate degradation.

Conclusion

Fabry disease is a genetic lysosomal pathological condition, characterized by decrease or lack of α-galactosidase A enzyme activity, which causes the accumulation of toxic metabolites like globotriaosylceramide and globotriaosylsphingosine. Our research indicates that the identification of structural analogues of Migalastat using computational approach provided a dataset offers an broader scope to mine new molecules for fabry disease. Further molecular docking studies indicated strongest binding affinity of (2R,3R,4S,5R)-2-(hydroxymethyl) piperidine-3,4,5-triol against alpha-galactosidase A receptor (3GXT), by affording D score -7.53 kcal/mol amongst all the structural analogues. This molecule may act as pharmacological chaperone for Fabry disease and can speed up the investigation towards fabry disease.

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UNDERSTANDING RESEARCH METRICS: MEASURING IMPACT OF SCIENTIFIC RESEARCH

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Abstract

The increasing volume of scientific publications necessitates robust methods to evaluate research quality, influence, and visibility. This paper examines the role of research metrics in assessing the impact of scientific work, focusing on traditional tools such as the H-index and journal impact factor, as well as modern approaches like Field-Weighted Citation Impact (FWCI), Altmetrics, and Article-Level Metrics (ALMs). By exploring the utility, strengths, and limitations of these metrics, the study emphasizes the importance of a multidimensional framework for research evaluation. Scientometrics are highlighted as essential methodologies for tracking trends, fostering collaboration, and identifying gaps in knowledge. However, challenges such as data quality, field variability, and the overreliance on quantitative indicators are critically addressed. This paper advocates for integrating quantitative metrics with qualitative evaluations to ensure a comprehensive understanding of research impact. The findings underscore the transformative potential of research metrics to inform funding decisions, academic benchmarking, and strategic planning while promoting innovation and addressing global challenges.

Keywords: Altmetrics, Bibliometrics, FWCI, H-index, Impact Factor, Scientometrics.

1. Introduction

The rapid surge in scientific publications has created an urgent demand for innovative tools to evaluate research quality, influence, and visibility effectively. Research metrics have emerged as indispensable instruments for gauging scientific productivity, and shaping decisions for researchers, institutions, and funding agencies alike (Bornmann, 2017). By spanning multiple dimensions—ranging from individual author contributions to journal prominence, article impact, and societal reach—these metrics provide a comprehensive lens to assess research performance (Moed, 2006). In many countries, there are frameworks within which the national evaluations are carried out to put more responsibility in the utilization of funds in research. Some countries like Australia, Belgium, France, Italy, New Zealand, United Kingdom are among those having the system with a view of determining if public funds used in funding scientific research have significant

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impacts (Abramo & D'Angelo, 2011; Derrick & Pavone, 2013). These frameworks assess the science achievements as well as other social impacts on the economy, environment, security and health in societies. A good example is the UK's Research Excellence Framework (REF) started in 2014 which uses a combination of Peer Reviews, cases and numerical performance of the academic institutions (King's College London & Digital Science, 2015; Thwaites, 2014).

In a recent release by Stanford, Ioannidis et al. (2019), ranked the top 2% of scientists globally across 22 scientific fields and 176 subfields. The ranks were based on composite indicator which was calculated using these research matrices – citations, h-index, h_m index, citation for single authored papers, citations for first authored papers, citation for single, first and last authored papers.

$$c = \frac{\ln(nc9617+1)}{\ln(nc9617max+1)} + \frac{\ln(h17+1)}{\ln(h17max+1)} + \frac{\ln(hm17+1)}{\ln(hm17max+1)} + \frac{\ln(ncs+1)}{\ln(ncsmax+1)} + \frac{\ln(ncsf+1)}{\ln(ncsfmax+1)} + \frac{\ln(ncsfl+1)}{\ln(ncsflmax+1)},$$

Where nc9617 is the total number of citations for the period of 1996- 2017, h17 is the h-index, hm17 is the hm index for year 2017, "ncs" is the number of citations to papers as a single author, "ncsf" is the number of citations to papers as single or first author, and "ncsfl" is the number of citations to papers as single, first, or last author.

This paper seeks to provide a concise overview of research metrics, encompassing journal-level, article-level, and researcher-level metrics. The objective is to enhance researchers' understanding of the methodologies used to evaluate the value and impact of scientific work within the context of today's dynamic academic and social landscape.

1.1 Scientometrics/Bibliometrics

In the landscape of scientific research, it is essential to adopt effective methods to assess the impact and dynamics of scholarly work. Scientometrics and bibliometrics are two powerful, quantitative approaches that offer valuable insights into research trends, the dissemination of knowledge, and the collaboration networks that drive scientific progress. Both approaches rely on rigorous, data-driven analysis to evaluate scientific publications, collaboration patterns, and citation trends. The core application of these methods lies in their ability to track and analyze research trends over time. By examining publication patterns and citation dynamics, these tools help identify emerging fields of study, shifts in scholarly focus, and areas of growing interest. For instance, bibliometric analysis has shown the increasing prominence of fields such as artificial intelligence and climate change, reflecting their heightened importance in contemporary research agendas (İRİ & Ünal, 2024). Scientometrics is the branch and subset of bibliometrics that deals with analysing of scientific publications for specifying publication tendencies, collaborations, or growth of elucidations in specific fields of study (Fitra, 2024).

Another significant contribution of scientometrics and bibliometrics is in collaboration mapping. These methods provide a comprehensive view of the interconnectedness between research

institutions, countries, and individual researchers. By mapping collaborative networks, both bibliometrics and scientometrics uncover the global exchange of knowledge and resources, which is critical for fostering international research partnerships. Such collaborations, often across geographical and institutional boundaries, are essential for addressing complex, interdisciplinary challenges (Rathika & Thanuskodi, 2021). Moreover, these approaches are instrumental in identifying under-researched areas. Through systematic analysis of existing literature, they reveal gaps in knowledge, guiding future research efforts to address overlooked or neglected domains (Matorevhu, 2024; Robledo, 2024).

The advantages of applying scientometrics in the evaluation of scientific research are manifold. One of the primary benefits is the provision of objective, reproducible metrics for assessing research impact. These metrics, such as citation counts, h-indices, and journal impact factors, offer a quantitative framework for evaluating the visibility and influence of scholarly work (Rathika & Thanuskodi, 2021). This objectivity is invaluable for funding bodies, policymakers, and academic institutions that rely on data-driven insights to make decisions regarding resource allocation, research funding, and strategic planning. Additionally, scientometrics and bibliometrics enable researchers and institutions to track their academic impact, thus enhancing their visibility within the scientific community and promoting the wider dissemination of innovative ideas (Krauss, 2024). These tools also facilitate long-term forecasting by identifying emerging trends and potential areas for future research, enabling researchers to align their efforts with evolving scientific priorities (Simion et al., 2023).

2.1 Research Metrics

Research matrices are structured frameworks that facilitate the organization and analysis of research projects across various disciplines. They serve as tools to categorize, manage, and visualize complex information, enhancing the understanding of research processes and outcomes.

2.1.1 Author-Level Metrics

Author-level metrics measure the research output and citation impact of individual researchers. Key metrics include:

1. h-index:

The H-index, developed by J.E. Hirsch in 2005, is one of the best-known scientometric measures calculated to determine the performance and citation impact of peer-reviewed published research. It has the advantage of ease of computation and this applies to individuals, researchers and institutions – (Kawimbe, 2024). Moreover, the h-index gives a comprehensive evaluation of a researcher in terms of the number of papers produced and citations, an evaluation that gives a correct picture of the contribution the research is making in the academic world (Mondal et al., 2023).

Calculation of H-index

- Order the publications in descending order based on the number of citations.
- **Determine h-index**: Identify the point where the number of citations is greater than or equal to the rank number. The h-index is the highest rank number where this

condition holds. (Mondal et al., 2023).

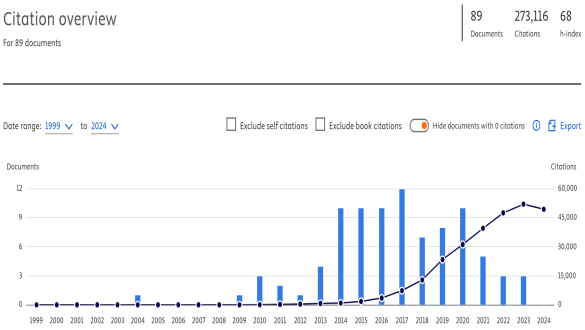

Image 1: H-index shown in the author's profile. (Source- Scopus)

Advantage of h-index

- **Simplicity:** The h-index is straightforward to compute and understand, making it accessible for researchers (Kawimbe, 2024).
- **Impact Assessment:** It provides a quantifiable measure of a researcher's influence within their field, often used in academic evaluations (Gupta, 2024).

Limitations of h-index

- The h-index ignores the actual quality and importance of publications measured by citation counts alone ignoring potentially vital contributions that may not attract many citations (Kawimbe, 2024; Mondal et al., 2023).
- The h-index fails to consider the quality of publications or the contributions of co-authors, leading to an incomplete picture of a researcher's impact (Formoso, 2022).
- It only provides valuable information when supported by other parameters evaluation of the academic in question as an over-concentration on such a kind h-index pointer may defeat objective valuable innovative approaches to problem-solving in the area, as yet unrecognized (Gupta, 2024).

Despite its utility, the h-index should be complemented with other metrics to provide a more comprehensive evaluation of a researcher's contributions. Alternative indices, like the ha-index, have been proposed to address some of these shortcomings, offering a more nuanced approach to measuring academic impact (Fassin, 2023).

2. I10-index:

The I-10 index is a bibliometric indicator that quantifies an author's scholarly productivity by enumerating the number of publications that have accrued at least ten citations. As a complementary metric to the widely utilized h-index, the I-10 index offers a refined lens through which an academic's impact can be assessed, particularly for individuals with substantial citation records.

Calculation of the I10 index

Its calculation involves a straightforward tally of an author's publications that meet the threshold of ten citations; for instance, an author with 15 papers, 8 of which have been cited at least 10 times, would possess an I-10 index of 8.

This metric is lauded for its simplicity and accessibility, providing an intuitive measure of academic influence while emphasizing the significance of works that have achieved a moderate degree of recognition. Nevertheless, the I-10 index is not without its limitations. It cannot differentiate effectively between authors with exceptionally high citation

counts, as it neither considers the aggregate number of citations nor their distribution across an author's body of work (Teixeira, 2021). Furthermore, its applicability is constrained by disciplinary variability in citation practices, which can distort its relevance in fields characterised by disparate citation norms. Although the I-10 index provides valuable insights into an author's academic contributions, its utility is maximised when interpreted with other evaluative metrics, such as the h-index, to develop a holistic understanding of scholarly productivity and influence.

3. G-index:

The G-index is one of the most recently developed bibliometric indicators and its goal is to measure the productivity and citation impact of researchers while especially taking into consideration the maximum number of citations for highly cited papers.

Calculation of the G-index:

It is determined by ranking a researcher's publications in descending order of citation counts and identifying the largest integer "g" such that the cumulative citations of the top "g" publications are at least g^2.

Publication year	Rank	Citations	Add up citations	Calculate square of Rank	Is the sum of Citations at least as large as square of the Rank?	g-index (the higest Rank where sum of Citations is larger than square of Rank)
2008	1	70	70	1 x 1 = 1	yes	
2009	2	12	70+12=82	2 x 2 = 4	yes	
2009	3	6	82+6=88	3 x 3 = 9	yes	
2010	4	5	88+5=93	4 x 4 =16	yes	
2011	5	5	93+5=98	5 x 5 = 25	yes	
2010	6	4	98+4=102	6 x 6 = 36	yes	
2011	7	4	102+4=106	7 x 7 = 49	yes	
2013	8	4	106+4=110	8 x 8 = 64	yes	
2013	9	2	110+2=112	9 x 9 = 81	yes	
2014	10	2	112+2=114	10 x 10 = 100	yes	g-index = 10

Image 2: Worked example of g-index calculation for an author with 10 publications

By supplementing the concentration of place accorded by the h-index of research productivity for each researcher, the G-index also substantially gratify its major drawbacks without losing its central benefits that favour high-profile papers (Schreiber, 2013; Zhang, 2010). Unlike the h-index where just a few papers with high impacts are neglected by the h-index, the G-index offers a more sensitive measure of scholarly influence because it offers the researcher those works that have earned exceptional citations (Zhang, 2010). In addition, it can be computed flexibly by scaling up or down prefactors to fit the requirements of the discipline or the context in which the metric is being used (Schreiber, 2013).

While the use of prefactors enhances the fineness of classification since it allows for customization, the element of arbitrariness can be disappointing when determining the final position of the institutions (Schreiber, 2013). Also, the calculation process might involve the creation of 'phantom' articles with no citations to make the evaluation criteria work mathematically, which makes its application difficult and is big on methodological issues (Zhang, 2010). However, if G-index lacks methodological flaws compared to other bibliometric indices and its scoring is partly based on the subjective adjustments, it should be used in combination with other bibliometric indices to avoid the under- or over-evaluation of the impact of research (Bonett, 2022).

4. The H_m Index:

Roots of Hm index: The h-index, introduced by Hirsch (2005), was designed to evaluate the impact of a scientist's publications based on

the number of citations received. However, one significant limitation of ranking scientists using the h-index is its lack of consideration for multiple co-authorships (Batista, Campiteli, Kinouchi, & Martinez, 2006; Bornmann & Daniel, 2007; Burrell, 2007; Hirsch, 2005, 2007; Imperial & Rodriguez-Navarro, 2007). To address this, Batista et al. (2006) proposed the h_i-index, which adjusts the h-index by dividing it by the average number of authors per paper within the h-core, the set of publications defining the h_i-index. While this adjustment has been widely adopted, particularly among Brazilian researchers, the author cautioned against potential pitfalls, noting that the averaging approach is sensitive to extreme values. Consequently, this normalization method may disadvantage researchers with a few papers co-authored by many individuals. Furthermore, it can disproportionately diminish the influence of single-author publications on the h-index.

The Hm-index

To mitigate such issues, Schreiber (2008) introduced the "H_m index," which employs a fractional counting method to account for authorship contributions. Unlike the H_i -index, which focuses on citations, the hm index determines a researcher's impact by dividing credit for each paper among its authors. Schreiber's comparative analysis of the "H_m index" (fractionalised counting of paper) with the H index and the H_i index (fractionalised counting of citations found the hm index superior due to its straightforward methodology and ability to address the limitations of the other measures.

Limitations of h_m index:

- H_m emphasis on first, single, or last authorship may inadvertently promote competition over collaboration, potentially discouraging interdisciplinary research.
- For researchers in collaborative environments, such as Indian academics engaged in team-based projects, this metric may undervalue their contributions.

Egghe et al. (2000), in their analysis of various bibliometric indices, emphasized that no single method provides an absolute standard for credit distribution among co-authors. The inherent subjectivity in determining the "correct" distribution of credit underscores the complexity of accurately assessing scientific impact.

2.1.2 Journal-Level Metrics

Journal-level metrics evaluate the reputation and influence of academic journals. Major indicators include:

1. **Impact Factor (IF)**:

The impact factor (IF) serves as a cornerstone metric in bibliometrics, widely utilized to evaluate the significance of scientific journals within their respective disciplines. By calculating the average citation rate of articles, the IF provides a numerical indicator of journal influence, derived annually using the formula IF = A/B, where A represents the number of citations received in a given year to articles published in the prior two years, and B denotes the total number of articles published in those years (Grzybowski, 2015).

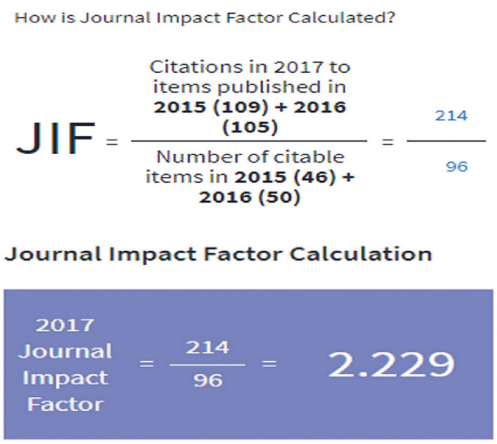

Image 3: Calculation of journal impact factor of Journal X for 2017 (released in 2018)

The IF is a commonly adopted measure for comparing journals, helping researchers select publications, as well as determining employees' promotions or funding (Moustafa 2015; Quintanilha & Cardoso 2018). They also apply it in identifying key journals for subscription or in decision-making on subscriptions (Grzybowski, 2015). However, IF assists in evaluating journals within the fields and identifies the most important ones (Hauptman, 2016; Grzybowski, 2015), although the citation distribution is distorted by outliers, which significantly increase ranks. It also ignores the quality of individual articles and may be gamed by self-citations and by privileging of review articles (Moustafa, 2015). Disciplinary differences in citation behaviors extend its cross-disciplinary use more and put low citation fields at a disadvantage (Grzybowski, 2015). IF misuse could mislead the researchers by shifting focus on the amount of research rather than quality, or by overshadowing innovative or cross-disciplinary research (Wanzala, 2018; Moustesta, 2015). Therefore, there is the need to combine quantitative measurements and qualitative assessments to achieve a balanced approach that will help in producing better estimates and improving the standard of studies.

2. *Five-Year Impact Factor*:

The five-year impact factor extends the traditional impact factor by incorporating citations over five years, offering a more comprehensive evaluation of a journal's influence. This metric addresses the limitations of the standard two-year impact factor, which often favours disciplines with rapidly evolving research landscapes, by providing a more accurate measure of long-term scholarly impact. Particularly beneficial for fields where research progresses at a slower pace, such as the humanities and some areas of social sciences, the five-year impact factor mitigates biases that undervalue journals in these disciplines. The calculation mirrors that of the traditional impact factor but expands the citation window to five years, dividing the total number of citations received in a given year by the number of citable items published during the previous five years (Della & Grafman, 2009; Scully, 2009). By capturing a longer citation horizon, this metric enhances the ability to assess the sustained relevance and influence of journals across diverse academic domains, making it a valuable tool for nuanced bibliometric analyses.

3. *Cumulative impact factor*

The cumulative impact factor is used in the analysis of research groups or individual scholars and characterizes their impact for a particular period. It aggregates the impact factors of journals where the research has been published, providing a comprehensive view of

the research's influence. Measured over time, this metric is especially helpful in assessing whether the journal's research and scholarly productions continue to generate readership and use over time – distinguished from current usage or impact which might be obtained from conventional impact factors. The subsequent parts provide information on the calculation of cumulative impact factors and their application, strengths and weaknesses. For research groups, metrics like Cumulative Journal Impact Factor (CJIF) are used, which aggregate the impact factors of all journals where the group's work is published. (Moll et al., 2023).

4. SCImago Journal Rank (SJR):

The SCImago Journal Rank (SJR) is a metric used to evaluate the prestige and influence of academic journals. It is based on the number of citations received by a journal's articles, with a higher weight given to citations from more prestigious journals. This metric is particularly useful for assessing the centrality of journals within their respective disciplines, offering a more nuanced view than traditional impact factors. The SJR is calculated using a three-year citation window, which helps to smooth out annual fluctuations in citation counts. Below are the key aspects of SJR, including its calculation, uses, advantages, and limitations.

Calculation of SJR

- SJR is calculated by considering citations received over a three-year period, with greater weight given to citations from highly prestigious journals (Montero, 2019).
- Self-citations are limited to 33% of the total citation count to prevent inflation of the metric (Montero, 2019).
- The metric is size-independent, meaning it does not favor larger journals over smaller ones (Kalita et al., 2018).

Advantages of SJR

- SJR accounts for the quality of citations, not just their quantity, offering a more refined measure of journal prestige (Kalita et al., 2018).
- It is considered more egalitarian than the impact factor, as it reduces the influence of highly cited articles on the overall metric (Aguilar-Velázquez et al., 2023).
- The metric is less biased by journal size, making it a fairer comparison across different fields (Kalita et al., 2018).

Limitations of SJR

- SJR may still be subject to biases related to time and subject domains, potentially affecting its reliability across different disciplines (Kalita et al., 2018).
- The reliance on the Scopus database means that journals not indexed there are excluded from the ranking (González-Mariño, 2022).
- The three-year citation window may not fully capture the long-term impact of certain research fields (Montero, 2019).

While SJR provides a valuable measure of journal prestige, it is important to consider its limitations and the context in which it is used. Other metrics, such as the h-index, offer

alternative perspectives on journal impact, focusing more on the quantity of citations and the number of published papers (Kalita et al., 2018).

Immediacy index

The Immediacy Index measures the usage where the articles in a journal are cited in the same year of the publication of the articles. It is a measure of the current research interest in a journal within its particular specialisation area. It requires the development of a ratio of the number of citations that an organization gets in a given year over the actual number of articles that the organization published in the same year. This index is very helpful in measuring the staggering of journals from one discipline to another and even other categories of journals such as library and information sciences as well as other scholarly disciplines.

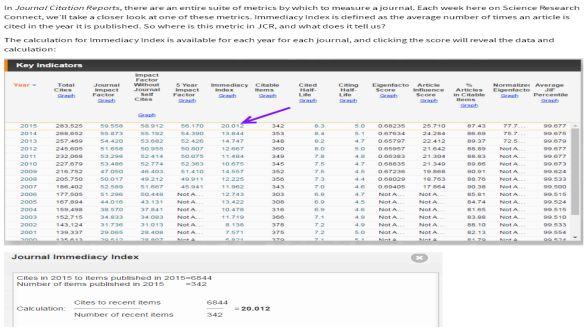

Image 4: Immediacy index from the Journal citation report – BYU Library.

2.1.3 Article-Level Metrics

Article-level metrics (ALMs) represent a progressive approach to assessing the impact and reach of individual scientific publications, extending beyond the limitations of traditional citation-based evaluations. These metrics integrate diverse indicators, including citation counts from academic databases, mentions in social media, and engagement metrics on platforms such as Mendeley. By capturing a broader spectrum of interactions, ALMs provide a nuanced understanding of a publication's influence, encompassing academic recognition as well as public and professional discourse. This comprehensive perspective positions ALMs as vital tools for evaluating the multidimensional impact of research in an increasingly interconnected and digital scholarly landscape.

1. **Field-Weighted Citation Impact (FWCI)**:

The Field-Weighted Citation Impact (FWCI) is a bibliometric measure that evaluates research impact by normalizing citation counts across fields and periods.

It is calculated by dividing the actual number of citations received by a publication by the expected number of citations for similar publications in the same field and year (Zanotto & Carvalho, 2021).

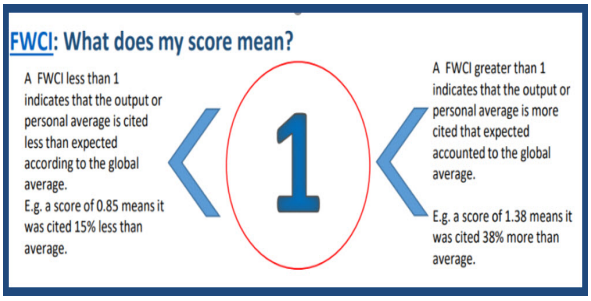

Image 5: Output interpretation of FWCI

This metric is widely used to assess the scientific visibility and influence of researchers, research groups, or institutions, facilitating fair comparisons across disciplines and timeframes. FWCI is also employed in

research evaluations and funding decisions to identify high-impact research outputs and benchmark performance against peers (Zanotto & Carvalho, 2021).

A significant advantage of FWCI is its ability to account for field-specific citation behaviors, providing a more nuanced view of research impact compared to raw citation counts, which are often biased by disciplinary norms and publication age (Gómez-Déniz & Dorta-González, 2024). However, FWCI is not without limitations. Misclassification of research topics can undermine its accuracy by skewing expected citation calculations (Zanotto & Carvalho, 2021). Additionally, it does not account for the quality or prestige of citing papers, which can influence the perceived impact of a publication (Chughtai et al., 2018). Despite its utility, these limitations highlight the need to use FWCI alongside other metrics to ensure a comprehensive and balanced evaluation of research impact.

2.1.4 Altmetrics

Altmetrics, or alternative metrics, represent a modern approach to evaluating the impact of scholarly work by focusing on online mentions and engagement beyond traditional citation-based methods. These metrics aggregate data from diverse online platforms, including social media outlets like Twitter and Facebook, blogs, and news articles (Robinson-García & Torres-Salinas, 2024; Thelwall, 2018). By capturing the volume and nature of interactions, the altmetrics provide a rapid assessment of research impact and immediate public interest, offering unique insights into societal engagement with scientific work (Thelwall, 2018; Fraumann, 2018). Their timeliness is a notable advantage, as they reflect the influence of research almost in real-time, unlike traditional citation metrics, which often require years to develop (Ciriminna et al., 2024). Furthermore, Altmetrics measure broader dimensions of impact, encompassing societal and media mentions, thereby providing a more comprehensive view of research influence (Fraumann, 2018). However, there use is not without limitations. Altmetric scores are susceptible to manipulation, raising concerns about gaming behaviors similar to those observed with citation metrics (Hassan et al., 2018). Additionally, the coverage of altmetrics can be uneven, as not all research outputs receive online attention, leading to potential gaps in representation (Thelwall, 2018). While Altmetrics serve as a valuable tool for capturing the societal and public engagement of research, their limitations highlight the need for careful and complementary use in academic evaluations.

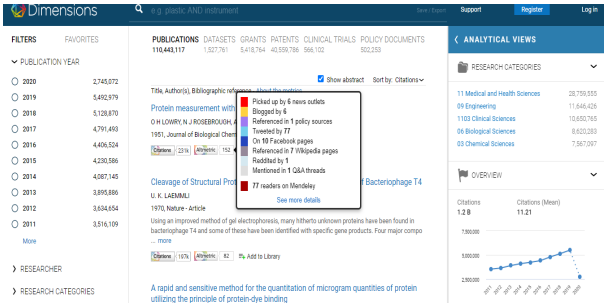

Image 6: Alternative metrics derived from open web resources (Dimensions)

3. *Conclusions:*

The study underscores the critical role of scientometrics in advancing our understanding of the impact, influence, and dynamics of scientific research. As a cornerstone of research evaluation, scientometrics provides a robust framework for tracking trends,

assessing scholarly output, and identifying underexplored areas. By utilizing tools such as citation counts, journal impact factors, and advanced metrics like the Field-Weighted Citation Impact (FWCI) and Altmetrics, scientometrics offers invaluable insights for researchers, institutions, and policymakers. However, the study also highlights the limitations of these methodologies, particularly their reliance on data accuracy, field-specific biases, and overemphasis on quantitative indicators.

While traditional metrics such as the H-index and journal impact factor remain foundational for assessing research productivity and influence, their shortcomings—such as the inability to capture qualitative aspects like societal impact and policy relevance—necessitate the adoption of more nuanced approaches. Modern tools like FWCI provide normalized assessments that account for disciplinary citation behaviors, while Altmetrics expand the scope of evaluation by measuring societal engagement and public discourse. These advancements reflect the evolution of scientometrics in addressing the multifaceted demands of contemporary research evaluation, where impact is no longer confined to academic citations but extends to broader societal and interdisciplinary relevance.

The study further emphasizes the importance of integrating scientometrics with bibliometric methods to inform strategic planning, funding decisions, and institutional benchmarking. However, challenges such as metric manipulation, coverage gaps, and inaccuracies in topic classification underscore the need for careful application of these tools. A balanced approach, combining quantitative metrics with qualitative assessments such as peer reviews and narrative evaluations, is essential to capture the holistic value of scientific contributions.

In conclusion, scientometrics is indispensable in guiding the development of research strategies and fostering international collaborations. By addressing its limitations and leveraging its strengths, scientometrics can continue to play a pivotal role in advancing academic excellence, bridging disciplinary divides, and aligning scientific efforts with global challenges. This multidimensional approach ensures that research evaluation remains equitable, comprehensive, and aligned with the broader goals of societal progress and innovation.

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ANTIBACTERIAL ACTIVITIES OF GREEN ZNO NANOPARTICLES AGAINST *ESCHERICHIA COLI*

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ABSTRACT

The present research investigated the production and analysis of Zinc oxide nanoparticles (ZnO NPs) to use them as anti-pathogenic agents against bacteria. Antibiotics, which were previously a lifesaver in treating multi-drug-resistant bacteria, are now useless; this could be attributed to either overuse or misuse of the drugs. Additionally, new approaches to treating microbial illnesses are needed to combat the growing issue of bacterial strains that are resistant to several drugs. ZnO nanoparticles were synthesized from aqueous extract of cinnamon bark and clove. The characterization of the nanoparticles was monitored using Ultraviolet-visible spectroscopy (UV-Vis), which examined the wavelength range and indicated a strong peak at 262.5 nm in the case of cinnamon while for clove extract, the largest absorption peak was found at about 321 nm wavelength. Synthesized green ZnO NPs found to be most effective against *E. coli* growth and showed remarkable antibacterial action against gram-negative bacteria even at low concentrations.

Keywords: Antimicrobial activity, Nanoparticles, bacteria , *E.coli*

INTRODUCTION

Escherichia coli is a diverse category of Gram-negative bacteria. They typically reside in both human and animal intestines. Among the most significant food-borne diseases in the food sector is caused by *E. coli* *Narayanan et al.*(2012). Most *E. coli* strains are benign and necessary for a human digestive system to function properly. However, some strains of *E. coli* are pathogenic, meaning they can make people sick. The *E. coli* infection can cause colon inflammation, which can result in diarrhoea, abdominal pain, and bloody stools. *E. coli* can thrive on acidic meals and only requires 10–100 cells to be infectious *Lin et al.* (1996). This food-borne pathogen has become more prevalent in outbreaks in the past few years. A wide range of foods have been linked to *E. Coli* outbreaks and infections, including raw milk, undercooked ground beef, roast beef, cantaloupe, alfalfa, lettuce, radish sprouts, pea salad, and cantaloupe (Dorman & Deans, 2000).

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Two types of antibacterial agents are now employed in the food industry: organic and inorganic reagents. Inorganic antibacterial reagents are more stable at high pressure and high temperatures than organic reagents *Reddy et al.* (2007). Recent years have seen a rise in interest in inorganic antibacterial reagents, such as metal oxides like zinc oxide (ZnO), due to their stability in the face of extreme processing conditions and general perception as safe materials for both humans and animals *Fratamico et al*. (2005). Green synthesis of nanoparticles provides several benefits, including reliable operation, reduced time and cost, environmental friendliness, and most importantly the ability to conduct the process without the use of any dangerous chemicals. ZnO NPs are very effective inorganic materials with antibacterial properties. *Kalemba et al.* (2003) described their use in pharmaceutical medications, sanitizers, cosmetics, and food packaging procedures Antibiotics, which were previously a lifesaver in treating multi-drug resistant bacteria, are now useless; this could be attributed to either overuse or misuse of the drugs. Additionally, new approaches to treating microbial illnesses are needed to combat the growing issue of bacterial strains that are resistant to several drugs. According to recent research, certain metal oxide nanoparticles, including ZnO NPs, may be hazardous to bacteria but do not affect human cells *Stoimenov et al*.(2002); *Raj et al.* (2021); Thill *et al.*(2006). ZnO's precise mode of action is still unknown, although it has been demonstrated that ZnO at the nanoscale exhibits greater antibacterial activity than ZnO at the micro-scale. Several studies have suggested mechanisms for ZnO NPs antibacterial activity. These include (i) the induction of reactive oxygen species, such as hydrogen peroxide (H_2O_2), which is a potent oxidizing agent that is harmful to bacterial cells. (ii) ZnO NP-induced damage to cell membranes and interactions with intracellular contents. Due to its huge surface volume ratio, ZnO NPs have strong antibacterial properties because they help bacteria dissolve and penetrate more easily *Reddy et al.*(2007). This study's goals were to examine the quick synthesis of green ZnO nanoparticles from plants, characterize them, and investigate the inhibitory concentration of ZnO NPs antibacterial capabilities against *E. coli*.

RESULTS AND DISCUSSION

A viable strategy to produce ZnO NPs with increased antibacterial action and less environmental effect is to employ green synthesis techniques. The physical and chemical characteristics of nanoparticles, such as their size, shape, surface charge, crystallinity, and surface functional groups, can all be learned via UV-vis. For example, ZnO NPs optical characteristics, such as band gap energy and absorption spectra, are often determined via UV-visible spectrophotometry. The UV-visible absorption's greatest wavelength falls between 200 and 500 nm. Using UV-Visible spectroscopy, the presence of nanoparticles was discovered at the conclusion of the ZnO NPs synthesis. When the plant extracts were added during synthesis, there was an instantaneous color shift that signalled the beginning of the ZnO NPs creation. The UV-Vis spectroscopy verified that the reaction's color changed from pale brown to brown, indicating the creation of ZnO NPs. For cinnamon extract, the spectra revealed a maximum absorption peak at

around 262.5 nm wavelength, while for clove extract, the largest absorption peak was found at about 321 nm wavelength. In the present study, we have reported the effectiveness of aqueous extract and green nanoparticles of cinnamon and clove spices against *E.coli*. Table 1 illustrates the outstanding antibacterial effect of ZnO NPs against gram-negative bacteria in our investigation, even at low doses results show maximum inhibition.

TABLE **1: Effect of different concentrations of GREEN ZnO NP on E.coli**

S. NO.	SAMPLE	ZONE OF INHIBITION (mm)	
		CINNAMON EXTRACT	CLOVE EXTRACT
1.	STANDARD DRUG	6.75 ± 0.5	13.7 ± 0.9
2.	CONTROL (water)	No zone of inhibition	No zone of inhibition
3.	PLANT EXTRACT	9.50 ± 3.1	22.5 ± 3.8
3.	0.1% ZnO NPs	5.75 ± 1.7	4.50 ± 0.5
4.	0.5% ZnO NPs	6.0 ± 0.81	5.2 ± 0.9
5.	1% ZnO NPs	7.5 ± 0.57	8.92 ± 2.1
6.	2% ZnO NPs	7.75 ± 0.95	10.25 ± 0.5
7.	3% ZnO NPs	8.5 ± 0.57	11.50 ± 1.0
8.	4% ZnO NPs	9.5 ± 0.57	12.25 ± 0.5
9.	5% ZnO NPs	10.75 ± 0.95	14.25 ± 3.8

To distinguish between the drug and the nanoparticles and determine their antibacterial activity against *E. coli*, the usual medication Azithromycin was also given, as indicated in Table 1. Different concentrations of green ZnO NPs and plant extracts of cinnamon and clove and Azithromycin drug were commercially available. They were inspected for their antimicrobial properties against gram-negative bacteria *E. coli and* through well diffusion method. Evaluation of the antimicrobial activity of plant extracts and nanoparticles is shown in table 1, representing the zone of inhibition. Test reveals that ZnO NPs, and extract shows potential antimicrobial activity against *E. coli* even at low doses, the values are statistically significantly different ($P < 0.05$). 5% ZnO NPs have the most potential of inhibiting *E. coli* growth, showing inhibition zone 10.75 ± 0.95 in case of cinnamon and 14.25 ± 3.8 in case of clove. Even at low doses, i.e. 0.1% ZnO NPs also shows a good inhibition. Because of variations in the bacterial structure, ZnO activity varies in cinnamon and clove. This is because different interactions between nanoparticles and cell surfaces affect membrane penetrability because the entry of nanoparticles inside bacterial cells causes oxidative stress, which in turn inhibits cell growth and ultimately results in cell death. The cytoplasmic membrane of all bacterial species was affected during the first 15 minutes of exposure to ZnO NPs *Mehendale et al*. (2013). Although the exact mode of action of ZnO NPs is yet unknown, it was anticipated that they would either electrostatically interact with the cell surface or cling to it and harm the cell membrane. ZnO NPs showed antibacterial activity against a variety of bacteria and fungi, including those that cause mastitis in cattle (*E.coli* and *A. flavus*) and skin infections in buffaloes *Parthasarathy et al*. (2017). ZnO NPs can penetrate microbial cell walls, causing cell death *Azam et al*.(2012). Positive charges from ZnO NPs are drawn to the cell surface by electrostatic interactions, and the difference in electrostatic gradient causes damage to the cell surface as observed by *Raghupathi et*

al.(2011). Furthermore, the activity of metal oxides may result in the production of oxygen species such as reactive oxygen species (ROS); in high concentrations, ROS can impair cellular proteins and lipids as well as cause damage to DNA and other macromolecules. The minimum concentration of inhibition was 0.1% where the mean zone of inhibition obtained was 5.75 ± 1.7 mm in case of cinnamon extract whereas in case of clove extract the mean zone of inhibition obtained was 4.50 ± 0.5, which further increased with the increase in concentration of the nanoparticles. Plant extracts derived from several medicinal plants exhibit antimicrobial properties against numerous food-borne, human, and plant infections and pests (*Ibrahim Khan* & *Khan*, 2017) ; *Mendes et al*.(2022). Multiple research projects have been undertaken to examine the antimicrobial activities of various herbs, spices, and their derivatives, such as essential oils, extracts, and decoction (Cowan,1999). *Brayner et al*. (2006) given that several plant extracts derived from medicinal plants have been utilized for both food preservation and therapeutic applications because of their antibacterial properties. Certain plants can serve as substitutes for disease control drugs already in use, as they contain an abundant number of bioactive compounds. The produced ZnO NPs exhibited antibacterial properties against *S. aureus* and *E. coli*, with inhibition zones of 19 mm and 14 mm, respectively when the quantity of ZnO NPs was 40µg/mL *Mohapatra et al*.(2020) . ZnO NPs had inhibitory effects against *E. coli* and *S. aureus* at t a dosage of 50 µg/mL, the inhibition zones for *E. coli* and *S. aureus* were measured to be 18 ± 0.5 mm and 16 ± 0.2 mm, respectively. The difference between our results and the previously reported results may be attributed to the utilization of different technologies for synthesizing ZnO NPs, resulting in variations in their sizes *Albukhaty et al*. (2020) Previous studies have suggested that the physical and chemical properties of nanoparticles, including their form, size, surface area, and other characteristics, can significantly influence their biological effects *Al-Holy et al*.(2006) and *Huq et al*. (2023) and (Sawai, 2003). The variation in these strains' genetic composition and enzyme ownership, as well as their ability to acquire antibiotic resistance mechanisms, may potentially account for the discrepancies in inhibition zone results. This is particularly true in pathological isolates collected from healthcare facilities as suggested by *Lin et al*.(1996); Liu et *al*.(2009).

CONCLUSIONS

Fighting infectious illnesses is a continuous phenomenon. Antibiotics, which were previously a lifesaver in treating multi-drug-resistant bacteria, are now useless; this could be attributed to either overuse or misuse of the drugs. Additionally, new approaches are needed to combat the growing issue of bacterial strains that are resistant to many drugs. Nanotechnology is one of the newest methods; it is an emerging technology that has sparked a new scientific revolution in every discipline. It works with nanoparticles ranging in size from 1 to 100 nm. All the main industrial sectors have been transformed by nanoparticles, including drug delivery, agriculture, food processing, pharmaceuticals, and many more. A viable strategy to produce Zn ONPs with increased antibacterial action and less environmental effect is to employ green synthesis techniques. Because of their

high surface-to-volume ratio and compact size, nanoparticles have a wide range of uses hence ZnO NPs antibacterial properties have drawn attention. Hence the physical and chemical properties of nanoparticles, including their form, size, surface area, and other characteristics, can significantly influence their biological effects. UV-Vis Spectrophotometry was used to evaluate the nanoparticles. The greatest absorption of cinnamon nanoparticles was at 262.5 nm and clove ZnO NPs was at 321 nm in the UV study. In conclusion, the development of novel antimicrobial agents to counter the growing problem of antimicrobial resistance is greatly encouraged by the antibacterial uses of ZnO NPs.

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FACTORS UNDERLYING INCREASING SUSCEPTIBILITY OF ESSENTIAL HYPERTENSION

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ABSTRACT

Hypertension is a known risk factor for increasing the incidence of cardiovascular diseases and stroke. It is also referred to as silent killer due to its asymptomatic nature for years. Hypertension persisting for longer time period damages blood vessel's elasticity and weakens the heart and affects other organs gradually. Sympathetic nervous system and Renin angiotensin aldosterone system forms the central theme with all the major risk factors for hypertension like weight gain, physical inactivity, mental stress and excess salt intake. Increasing prevalence of hypertension has definitely increased the awareness among general public about the consequences like cardiovascular diseases, stroke and heart attacks but still risk factors are not taken much seriously. This review highlights the importance of understanding the role of these risk factors which might also be the underlying cause of initiating the susceptibility towards hypertension in current times.

Keywords: Sympathetic nervous system, Essential hypertension, Susceptibility factors, Mental stress, Physical Inactivity.

INTRODUCTION

Hypertension or high blood pressure is a known risk factor for increasing the incidence of cardiovascular diseases and stroke. Blood pressure (BP) is defined as the force of the blood flowing against the arterial wall and when this force is constantly elevated (>120/80 mmHg) it is termed as high BP or hypertension. It is categorized as stage I, II and III depending on the degree of rise in the blood pressure, as per Joint National Committee (JNC VII). It is Primary/ Essential hypertension when underlying cause is unknown and termed as secondary hypertension when underlying cause is known such as primary aldosteronism (excess of aldosterone), Liddle's syndrome (abnormal kidney function) and others (Rimoldi, 2014). Among the two, Essential hypertension is more common (90-95%) in general population and tends to develop gradually with time, most often unidentified.

Hypertension is also one of the components which constitute metabolic syndrome (MetSyn), besides hyperglycemia, hypertriglyceridemia, higher abdominal weight and low HDL (High density lipoprotein). MetSyn is defined as presence of

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three out of five above mentioned conditions (Tanaka and Itoh, 2019). This cluster of condition increases the risk of CVD, stroke and type 2 diabetes. Remarkably, except for abdominal weight/waist circumference, none of these conditions show any symptoms/visible identifier and therefore, they remain unidentified until tested. Essential hypertension (EH) is also referred to as silent killer due to this character of being asymptomatic for years. Elevated blood pressure for longer time period not only damages blood vessel's elasticity but also weakens the heart which gradually progresses towards heart attack in conditions where heart has to work harder even temporarily like during running, strenuous work or under stress. Gradually other organs also tend to get affected in the long run leading to renal failure, brain hemorrhage, vision loss, vascular dementia, metabolic disor and others.

Blood pressure is determined mainly by two factors, first is the amount of blood pumped by the heart (cardiac output, CO) and second is the force with which blood flows (peripheral vascular resistance, PVR). All the factors altering these two or both may result into perturbed BP. Cardiac output depends on contractility of myocardium, preload and heart rate whereas PVR generally gets affected by the mean radius of the systemic vessels which gets altered due to contraction of smooth muscles of vascular system. This vasoconstriction also occurs due to sympathetic stimulation in case of stress or more commonly known, fight or flight condition. The effect of sympathetic stimulation on heart results in cardiac excitation via increased heart rate, heart contractility and coronary flow. Normally these stimulations are of short durations and their effects also phase out with time, returning heart to its normal state. If such conditions are for longer duration (chronic stress) complete physiology gets altered resulting into long term changes (DeLalio, 2020).

The pathophysiology of hypertension includes multiple organ system like Renin Angiotensin Aldosterone system (RAAS), Sympathetic nervous system (SNS), immune system, endocrine factors to name a few. It falls in the category of complex phenotype where both genetic and environmental factors have either unified or interactive effect. Several studies have indicated involvement of genetic factors but due to its complex architecture, even GWAS (Genome wide association studies) has not identified reproducible factors (Kelly, 2022). Only small proportion of EH confers to familial or monogenic inheritance (Raina, 2019) pattern leaving larger one as sporadic in nature. Enormous studies have reviewed the biological molecular pathways involved in the pathophysiology of the disease (Foex and Sear, 2004) therefore this review focusses on the other part which is factors leading to the buildup of susceptibility for hypertension.

SUSCEPTIBILITY FACTORS

Susceptibility factors do not directly cause the disease condition but raise the risk for its development either alone or in combination with other factors. Considering our nation which has transformed itself from a developing, economically weaker nation to almost reaching a developed, economically

stable state, a lot has got transformed in the terms of automation (instead of manual labor), fast food (in place of classical home-made) and modern culture (instead of traditional). In terms of biology, geographic area, surroundings play an important role in building up of body's physiology. Accordingly, our genome also organizes, reorganizes and functions as per stimuli received during the past years, in a slow but gradual process of evolution. In recent past, non-communicable diseases (NCDs) have substituted the communicable ones, in terms of population health hazard, primarily due to improved medical advancements and better understanding of infection biology but also due to transformation as mentioned above. As per WHO-India, NCDs account for 60% deaths in India where every forth individual has hypertension and more than 60% adolescents are physically inactive. This is in stark contrast to earlier times where work profile of Indians was either agriculture based or other labor-intensive works. This shift was far rapid than the pace of physiological changes and genome evolution, hence resulting into increase in the mismatch between current lifestyle and tendency to achieve physiological homeostasis. Following factors explain the above-mentioned changes leading to increase in higher susceptibility towards hypertension and other NCDs.

HIGH SALT INTAKE

High salt intake (>5gm sodium/day) evidently produces significant increase in the BP and its reduction similarly lowers the BP significantly (WHO). More sodium intake enhances retention of water in the body thereby increasing the fluid volume and high flow in arterial vessels, hence increase in BP. Long term effect results in microvascular endothelial inflammation, anatomic remodeling and functional abnormalities (Grillo, 2019, Marketou, 2019). Additionally, to conserve water in high salt situation, body requires energy, for which either more fuel is needed or body's own reserve (muscle mass) has to be utilized, therefore this leads to increased hunger, as muscle wasting would be costlier to avoid dehydration. This could be the reason that high BMI is often seen in hypertensive individuals and may further predispose to metabolic syndrome (Murray et al, 2023). Certain genetic polymorphisms involved in the sodium reabsorption in the nephron also predisposes individual towards high BP as seen in some of the monogenic forms. As per studies, blacks in comparison to whites have impaired ability to concentrate sodium in urine after high salt intake therefore salt sensitivity is more common cause for BP among them (Frohlic, 1990). Depending on the degree of salt sensitivity among different individuals, varied response to BP is observed on salt reduction therapy, which also forms the primary lifestyle change along with physical activity on primary diagnosis of hypertension before pharmacological intervention. Interestingly, studies also found sodium reduction is difficult to follow due to sodium's property to enhance taste and flavor despite its substantial potential to reduce the risk of CVD (Cobb et al 2012).

EXCESS BODY WEIGHT

Higher body mass index (BMI) and waist circumference are indicators for excessive body weight which is a known risk factor for hypertension. Investigations show weight

gain is associated with impaired flow-mediated dilation (FMD) which gets reversed on weight loss. FMD measures how much an artery widens in response to increased blood flow. FMD impairment was more related with visceral fat though increase in BP was not significant, indicating vascular response to be more sensitive or precedes the onset of hypertension (Romero-Corral, 2010). Increase in body weight and more appropriately visceral fat alters the material properties of the blood vessels before it affects the other mechanisms like SNS activation which ultimately increases BP. Though more individuals are aware about weight gain but still there is lesser understanding about visceral fat accumulation, which could be present among lean individuals also. Awareness about central/abdominal obesity, waist circumference, waist to hip ratio, in addition to BMI is necessary to increase the measures to control incidence of hypertension. Another related factor is dyslipidemia which again is predominant among individuals with hypertension (Dalal, 2012). The reason could again be visceral adiposity due to fast food (high calories and salt) consumption and physical inactivity (another risk factor). Abnormal weight gain/loss indicates poor energy metabolism which also depends on the time and regularity of food consumption in addition to the type of food and lifestyle.

PHYSICAL INACTIVITY

Sedentary lifestyle or physical inactivity is known to increase risk for hypertension, high abdominal weight and CVDs, still our understanding is incomplete regarding the complete mechanism of physical inactivity's role in Hypertension. BP reduction (almost for 24 hrs) as a result of acute/irregular regimen exercise is termed as post-exercise hypotension whereas frequent exercise results in exercise training response which produces sustained reduction in BP (Pescatello, 2004, 2005). Regular exercises also show beneficial effects on heart rate and insulin resistance via decreased sympathetic activity (Fagard, 2011). Other mechanisms such as oxidative stress, RAAS, inflammation, endothelial function have also shown positive correlation between regular exercise and BP reduction. All these investigations though demonstrate positive effect but they all vary in the types of exercise, regimens, environmental conditions, genetic factors and the resultant response to BP reduction (Islam et al., 2023). Considering India, as per WHO report, almost 60% adolescents are physically inactive and probably this accounts for the increasing incidences of hypertension even in youths. Interestingly, regular exercise regimen is also beneficial for reducing mental stress (another risk factor) by releasing neurotransmitters like serotonin and endorphins.

MENTAL STRESS

It is a form of stress dependent on how one perceives the events in their external and internal environment, resulting in psychological experience of distress and anxiety. This may accompany physiological responses in certain situations depending on the duration of stress condition. Most common example is that of fear which stimulates the sympathetic nervous system, leading to release of catecholamines which alter the physiological response by increasing the heart rate, cardiac output and BP via

vasoconstriction. This fear may be due to real life event (facing a tiger) or due to imagination (thinking of a ghost in dark room), both situations generate similar psychological experience and physiological response. The response as a result of acute stress generally rolls back to normalcy within a short span of time or when stressor is removed. But chronic stress and the resultant conditions like depression have been positively correlated with hypertension (Inoue, 2024), though complete mechanism is still not clear. As the psychological responses vary from person to person therefore most often investigations have focused on physiological responses of stressors in correlation with hypertension (Spruill, 2010). Nevertheless, most of these investigations vary in the type of stressor analyzed such as psychosocial stressor, chronic illnesses, occupational stress and others. Additionally, chronic stress and rumination of previous stress condition, both makes it difficult to reach normalcy of BP as a result of persisting stimulus. Several studies have reported coexistence of depression and hypertension, with higher percentage of depression reported among pre-hypertensives (Sundarrajan, 2022). This indicates that chronic mental stress predisposes towards hypertension. Treatment for both the conditions become difficult when they coexist, due to their synergistic effect on each other. Individuals under stress more often isolate themselves and underestimate the importance of psychological support or treatment thereby worsening their situation and individuals with hypertension show higher non-compliance with medication probably as a result of stress which often remains undiagnosed. There is a need for increasing awareness as well routine screening of psychological stress among individuals especially at a younger age to reduce the onset of susceptibility for such conditions.

CONCLUSION

The prevalence of Essential hypertension is increasing worldwide and more importantly in India irrespective of urban or rural areas. There is an underlying susceptibility for hypertension which is increasing mainly due to changes in the lifestyle. Technology driven comfortable life must be balanced with physically active life and work-life balance. Working for long duration to achieve work targets, skipping timely and nutritious meals and sitting for longer hours are some of the factors which disturbs the metabolism of the body gradually. These gradual and progressive changes affect the physiology of the body and initiates the susceptibility towards asymptomatic complex conditions like Essential hypertension, dyslipidemia and visceral fat accumulation. Efforts are essential to increase awareness, screen lipid profile, blood glucose, blood pressure and mental health among the youths of the nation to bring down this susceptibility.

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DISCRIMINATION OF ILLICIT LIQUOR BASED ON ADULTERANTS BY MANOEUVRING ANALYTICAL AND MULTIVARIATE CLASSIFICATION METHODS

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Abstract

Illicit liquors are being manufactured and sold in various parts of India.Considering its health and legal implications,this study has been designed to assist the forensic investigation of related crimes. In this study, ATR-FTIR and GC-FID have been used as potential analytical techniques to identify and discriminate illicit liquors based on their adulterants.The presence of adulterants like furfural, ethyl 2-bromomethyl acrylate, cyclohexane carboxamide, has been successfully detected through GC-FID. As expected, ethanol is found to be the common constituent detected in all the samples. Multivariate analysis using PCA showed 99.8% variance while HCA displayed the discriminating power of 77.18%. The methodology provided encouraging results in discriminating and classifying the samplesbased on the presence of adulterants.

Keywords: Forensic, Illicit liquor, Chemometrics, ATR-FTIR, GC-FID, Adulterants

1. Introduction

In India, the manufacturing of different forms of alcoholic beverages is increasing expansively. This is owing to the increase in demand, changing consumer tastes, and majorly alcohol dependence. Generally, an alcoholic beverage contains any amount of ethyl alcohol that is produced either via natural fermentation or is separately added during the manufacturing process. The natural fermentation of ethyl alcohol occurs when any bio-culture (yeasts, some specific bacteria, and microorganisms) acts on the sugar found in fruits, grains, and sugarcane in the absence of oxygen [Zamani, 2019].

The liquor industry on a broader scale comprises recorded (licit liquor) and unrecorded liquor (illicit liquor). A

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categorical classification of liquors is displayed in Figure-1 [Okaru, 2019]. Recorded liquors are prepared by the process of distillation from wine or other fermented fruit or plant juice or from a starchy substance (such as different grains) that has first been brewed and then fermented. On the contrary, unrecorded liquors are manufactured unlawfully without considering any standard protocol. Moreover, they do not undergo any quality control and hence are deemed harmful for human consumption. Substances that are generally contaminated and are reported to have been containing a potentially toxic chemical known as methylated spirit or methanol generally produce these liquors. Studies have also shown the presence of other harmful adulterants like furfural, urea, acetone, chloral hydrate, etc. [Pandey, 2018; Sankhla, 2018 & Kumar, 2018] [Abegg, 2019; Magro, 2019; Broek, 2019; Van Den, Pratsinis, 2019; & Güntner, 2019] [Kumar, 2020; Pandey, 2020 & Sankhla, 2020].

Alcohol is a psychoactive substance that acts as both a stimulant and a depressant. It is consumed for reasons like enjoyment, inducing sedation. In the long run of intake, the person gets addicted and settles for alcohol in any form. These practices have given rise to the production of illicit liquors that are inexpensive, accessible, induces effects similar to recorded liquor, and can be made at home [Topala, 2019 & Tataru, 2019]. The 2018 report issued by WHOstates that illicit liquor contributes to as much as 45% of all alcohol consumption in India [WHO 2019].

The higher rate of consumption of illegal liquors containing methanol has led to an increase in the death rate mainly associated with hooch tragedies and causes blindness. Illicit liquors are produced and sold in various parts of India. A large number of cases are reported every year related to illegal production and marketing of illicit liquor and deaths caused due to harmful adulterants [Chakrabarti, 2012; Rai, 2012 & Panda, 2012]. Chromatographic techniques such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), and Gas Chromatography Flame Ionization Detection (GC-FID) have been used to classify the liquors based on their aroma style, taste, and geographical origin [Bachmanov, 2003; Kiefer, 2003; Molina, 2003; Tordoff, 2003; Duffy, 2003; Bartoshuk, 2003; & Mennella, 2003], [Hida, 2001; Kudo, 2001; Nishida, 2001 & Ikeda, 2001], [Ma, 2017; Huo, 2017; Qin, 2017; Shen, 2017; Yang, 2017 & Hou, 2017]. In the past decades, IR including mid-IR (MIR) and near-IR(NIR) has developed to be more sensitive and rapid for the identification of alcoholic beverages [Berna, 2009; Trowell, 2009; Clifford, 2009; Cynkar, 2009 & Cozzolino, 2009].

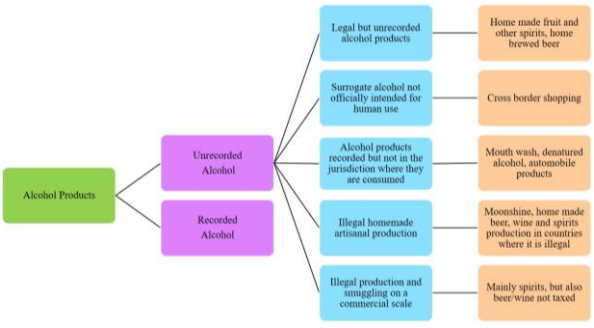

Figure 1: Categorical division of alcoholic beverages

In a related study, Y. Ma *et al.* classified 84 samples of Chinese liquors based on aroma styles and geographical origins by using Fluorescence spectroscopy coupled with chemometrics. In this study, the liquors were statistically discriminated by using principal component analysis (PCA), stepwise linear discriminant analysis (SLDA), and hierarchical cluster analysis (HCA) [11]. In another similar study, Silva*et al.* used MIR and NIR coupled with chemometrics for detecting adulterationin hydrated ethyl alcohol fuel. In addition, PLS-DA and LDA were used to identify adulterations in hydrated ethyl alcohol fuel samples with water or methanol [Silva, 2012; Lira Pontes, 2012; Pimentel, 2012 & Pontes, 2012].

The present study aims to characterize, identify, and classify 60 samples of illicit liquors fromthree close-knit states and one Union Territory (UT) of North India. Various chemical tests for the identification of alcohol and certain adulterants have been used for preliminary examination of the samples. Further, ATR-FTIR spectroscopy and GC-FID have been used for analytical examination. Additionally, chemometric methods including PCA and HCA have also been used as statistical tools to determine the variance in the data and classify them based on similarities and dissimilarities.

2. Materials and Methods

2.1. Sample collection

Sixty samples of illicit liquors were collected from three close-knit states and one Union Territory (UT) of North India including Punjab (Pb), Himachal Pradesh (HP), Haryana(HR), and Chandigarh (CHD) (UT), respectively. The samples were procured either directly from the shops or from people selling from their homes. On collection, each sample was well labeled and documented. All preventive measures were taken to avoid any chance of sample contamination. The sample details are presented in Table 1.

Table 1 List of illicit liquor samples

S. No.	States	Sample ID	Total Samples
1	Jalandhar (Pb)	J1-J5	05
2	Hoshiarpur (Pb)	H1-H4	04
3	Patiala (Pb)	P1-P2	02
4	Ludhiana (Pb)	L1-L5	05
5	Ropar (Pb)	R1-R6	06
6	Chamba (HP)	CA1-CA4	04
7	Rori (HR)	RI1-RI10	10
8	Ambala (HR)	A1-A13	13
9	Chandigarh (CHD)	C1-C11	11

2.2. Chemical examination

Chemical tests were conducted on all the samples of illicit liquor. The methods used in this study are based on the Indian Standard of Alcoholic Drinks that demonstrates the procedures for examining unrecorded alcohols [BIS IS, 2009]. Five different chemical tests were applied viz. iodoform and dichromate test for detecting the presence of ethanol and furfural test, potassium ferrocyanide test for the presence of copper and iron respectively [Manual 2015]. These tests were specifically chosen because these

are the most common impurities found in illicit liquors.

2.3 Instrumentation methods

2.3.1. Chromatographic Analysis

The samples were examined by using GC-2014 Shimadzu gas chromatograph coupled with FID. The gas chromatograph was equipped with a capillary column RESTEX Rtx-624 (30m, 0.32mm i.d.). The GC oven was set primarily at 40°C for 10 minutes and then increased to the final temperature at 245°C for further 10 minutes at the rate of 20°C/min. The injector temperature was held at 200°C while the detector temperature was set at 235°C. A sample injector value of 1µl has been used in this study.

2.3.2. Spectroscopic Analysis

All the samples were analyzed using the 'Spectrum Two' PerkinElmer ATR-FTIR spectrophotometer. A resolution of $4cm^{-1}$ with sixteen scans for both background and sample having absorbance mode at 4000-$400cm^{-1}$ was set. For sampling, an ATR unit housing a diamond crystal (ZnSe) as the focusing element has been used. A micropipette was used to disperse each sample evenly around the surface of the diamond crystal. After analyzing each sample, the diamond crystal was cleaned with acetone and dried with a tissue. For data acquisition and processing, the inbuilt software 'Spectrum' was used. To check the uniformity and repeatability of spectra, each sample was analyzed five times and the average spectra was considered for spectral examination.

2.4. Data pre-processing

The spectroscopic dataset was pre-processed using the OriginPro 8.5 software before proceeding with the statistical analysis. Pre-processing helps in removing any baseline shift or noise signal in the dataset. The smoothing, baseline shift correction, and removal of the noise signal from the data were done using the Savitzky-Golay filter, peak analyzer, and Standard Normal Variate (SNV) respectively [Yao, S., 2018; Li, T., 2018; Li, J. Q., 2018; Liu, H.G., 2018 & Wang, Y.Z. (2018).]. Normalization of the dataset is essential to elude any kind of noise that is resultant of the varied quantity of the liquor samples. For statistical analyses, the IBM SPSS Statistics-25 software was used to perform Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA).

The main objective of HCA is to group all the samples of liquor in different clusters based on the similarities or dissimilarities in their chemical composition. For example, samples placed in one cluster denote that they share a resemblance and are different from those placed in the other [Brown, 2010; Brereton, 2003]. All the objects are classified in a 2-dimensional tree-like diagram also known as a Dendrogram. To link the objects (or samples) from the other object in the dendrogram, a most common approach of linkage known as agglomerative clustering is used. In a dendrogram aligned horizontally, the vertical axis denotes the objects/clusters and the horizontal axis denotes the distance between the clusters. The larger the distance between the clusters the more is the

dissimilarity they share and vice-versa [Forina, 2002; Armanino, 2002 & Raggio, 2002; Verma, 2019; Sharma, 2019; Kumar, 2019; Sharma, 2019; Joshi, 2019; Umapathy, 2019; Ohja, 2019 & Chopra, 2019].

PCA is an unsupervised pattern recognition statistical technique that reduces the dimensions of the variables in a dataset. It works by transforming or rotating the original data into new orthogonal variables in a manner that shows the maximum variance in the data. The new variables thus derived are known as principal components (PC) and represent a normalized linear combination (first PC) [Sinelli, 2007; Cosio, 2007; Gigliotti, 2007 & Casiraghi, 2007; [Silva, 2018; Pimentel, 2018; Amigo, 2018; García-Ruiz, 2018 & Ortega-Ojeda, 2018; Gál, 2020; Oravec, 2020; Kiššová, 2020; Gemeiner, 2020 & Čeppan, 2020]. The remaining variance in the data is calculated by finding another linear combination of variables (second PC and so on) that shows a maximum variance from the other components. The size of each component can be determined by their eigenvalues that can be represented using a score plot. The first PC has an eigenvalue greater than the second PC and so on [Brown, 2010; Brereton, 2003; Broda, 2019 & Popescu, 2019; Bro, 2014 & Smilde, 2014]

3. Result and discussion

3.1. Preliminary examination

Chemical tests were applied on all 60 samples. The presence of furfural in a sample was indicated by the development of red color. In the case of iodoform test, the appearance of a yellow crystalline precipitate specified the presence of ethanol. In detecting the presence of copper ions, a light brown precipitate was developed by applying the potassium ferrocyanide test. The green color as the product of the dichromate test indicated the presence of ethanol in the sample. Iron was not detected in any of the tested samples. The samples that displayed positive chemical tests have been summarised in Table 1 and the experimental images are shown in Figure 2.As chemical tests do not provide a definite identification of the adulterants, they were only used for the preliminary examination. Further, more reliable and confirmatory techniques including GC-FID and ATR-FTIR were applied to the samples.

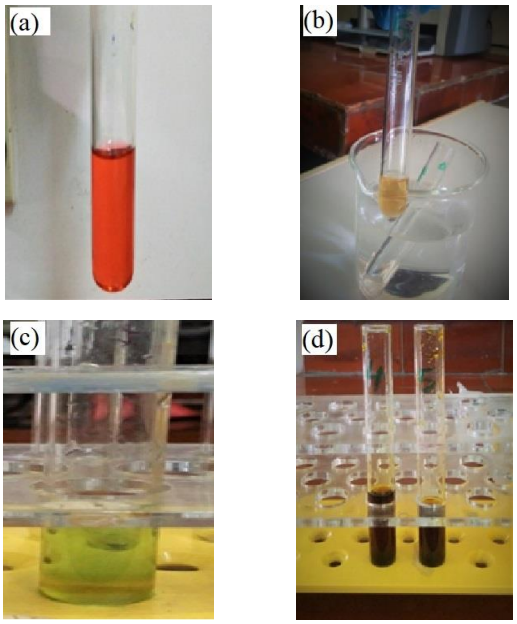

Figure 2: Experimental results of (a) furfural test (b) iodoform test for ethanol (c) potassium ferrocyanide test for copper (d) dichromate test for ethanol.

Table 2 Samples of illicit liquor displaying positive chemical tests

States	Chemical Tests				
	Iodoform Test	Dichromate Test	Furfural Test	Copper Test	Iron Test
Jalandhar (Pb)	J1-J5	J1-J5	J1-J4	J3-J5	Nil
Hoshiarpur (Pb)	H1-H4	H1-H4	Nil	Nil	Nil
Patiala (Pb)	P1-P2	P1-P2	P1, P2	P1	Nil
Ludhiana (Pb)	L1-L5	L1-L5	L1-L5	L2	Nil
Ropar (Pb)	R1-R6	R1-R6	Nil	Nil	Nil
Chamba (HP)	CA1-CA4	CA1-CA4	CA4	Nil	Nil
Ambala (HR)	A1-A13	A1-A13	6-A8, A10	Nil	Nil
Rori (HR)	RI1-RI10	RI1-RI10	RI3-RI5, RI7-RI9	RI4, RI5, RI7, RI9, R1(	Nil
Chandigarh (UT)	C1-C11	C1-C11	Nil	Nil	Nil

3.2. GC-FID

GC- FID was used to analyze all the samples of illicit liquor to determine the commonly added adulterants. The reported data were used as a reference for determining the presence of a specific adulterant based on the retention time (RT) [Bhupinder Singh punia, 2017; Praveen Kumar Yadav, 2017; Gurvinder Singh BumBrah, 2017 & Rakesh mohan, 2017; Restek. 2002]. As a representative of all the samples, the chromatogram of sample J2 and sample CA4 have been presented in Figures 3 and 4 respectively.

The results of GC-FID showed the presence of a variety of adulterants in each sample belonging to the class group of aldehyde, esters, alcohols, nitrogen and other tionalities (Table 3). However, ethanol was commonly present in all the samples of illicit liquor at 2.613 and 2.628 RT. Other distinguishing adulterants detected by GC-FID have been enlisted in Table 3. It can be observed that there are either one or more adulterant detected in each state that is only specific to that particular state. Therefore, these observations were used as a basis to discriminate the samples from each other.

Table 3 List of adulterants along with their respective retention time detected using GC-FID

S. No.	States	Adulterants	RT
1	Jalandhar (Pb)	Cyclohexanecarboxamide, N-decyl-N-methyl Ethyl 2-bromomethyl acrylate	14.123 17.669
2	Hoshiarpur (Pb)	Acetamide, 2-chloro- N-(1-methylethyl)	17.005
3	Patiala (Pb)	1-Propanamine, N,2 - dimethyl Benzoic acid, 3-fluoro-4-nitro-, methyl ester	5.731 12.26
4	Ludhiana (Pb)	Octanal Octadecanoic acid, 8-oxo-, methyl ester	9.466 13.46
5	Ropar (Pb)	Carbonic acid, pentadecyl 2,2,2-trichloroethyl ester	16.757
6	Chamba (HP)	Furfural, Cholest-8-ene-3,6 diol, 14methyl, Cholroaceticacid, 4-hexadecyl ester, N –hydroxy methyl fluoroacetamide	4.84 14.134 15.095 16.550
7	Ambala (HR)	3-Bromo-3-buten-1-ol, 5-Nitrothiophene- 2-aldehyde	14.54 14.409
8	Rori (HR)	Ethanal, 2-(3-ethyl-2,2-dimethylcyclobutyl)-, semicarbazone	11.319
9	Chandigarh (UT)	3-(1 H-Imidazol-4-yl)-propan-1-ol	17.05

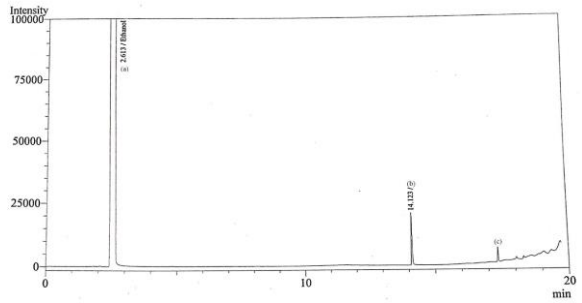

Figure 3: Total ion chromatogram of sample J2 (Jalandhar) indicating the presence of (a) ethanol (b) cyclohexane carboxamide, N-decyl-N-methyl (c) ethyl 2-bromomethyl acrylate

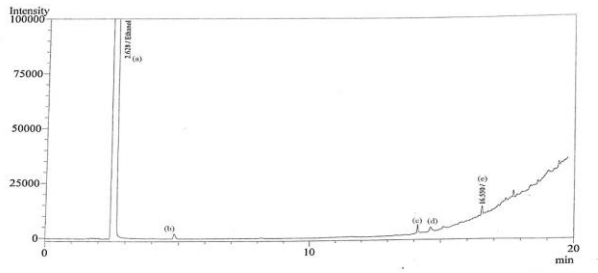

Figure 4: Total ion chromatogram of sample CA4 (Chamba) indicating the presence of (a) ethanol (b) Furfural (c) cholest-8-ene-3,6 diol, 14methyl (d) Cholroaceticacid,4-hexadecyl ester (e) N –hydroxy methyl fluoroacetamide

3.3. ATR-FTIR

ATR-FTIR was used to characterize the samples of illicit liquor. The spectral bands obtained in ATR-FTIR were analyzed to deduce significant findings and discriminate samples from one another. The IR spectra of sample P1, J3, L4, and CA4 has been displayed in Figure 5 as representative of all the samples. The spectra of these samples show moderate to strong absorbance peaks at 3304 cm^{-1}, 2982 cm^{-1}, and 2906 cm^{-1} corresponding to O-H stretching owing to the presence of secondary alcohol. The spectral band at 2120 cm^{-1} is attributed to the presence of alkyne. The band at 1640 cm^{-1} and 870 cm^{-1} corresponds with alkenes. Likewise, the band at 1450 cm^{-1} is attributed to the characteristic C-H stretching in alkanes. The band at 1084 cm^{-1}corresponds with C-O stretching in primary alcohol. The spectral peak at 1044 cm^{-1} is attributed to the presence of CO-O-CO stretching in anhydrides. The band peak at 513 cm^{-1} is associated with the presence of C-I stretching in halo compounds.

On the intercomparison of spectra of all the samples, the differences and similarities between the samples were determined. Although the visual spectra did not bear very characteristic differences, the dataset obtained from FTIR helped in conducting statistical analysis of the data using chemometric methods.

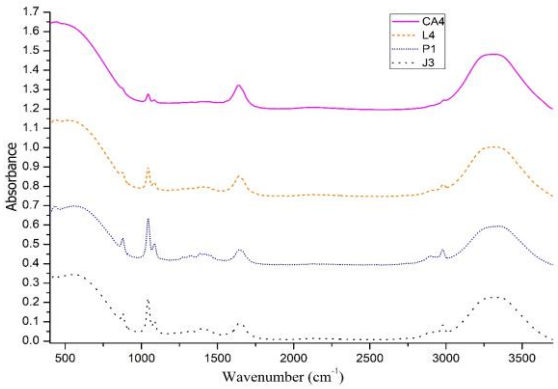

Figure 5:IR spectra of sample P1, J3, L4 and CA4

3.4. Statistical analysis

3.4.1. Hierarchical Cluster Analysis (HCA)

An agglomerative clustering algorithm was used to group samples into clusters. The variance between two clusters was measured using Ward's linkage method and the distance was measured using the Squared Euclidean distance criterion [Brown, 2010]. To measure the number of clusters formed, the stage number displaying the maximum variance was manually subtracted from the total stages. Clusters can also be calculated by carefully studying the dendrogram presented in Figure 6. In Table 4, it can be observed that all the samples are divided into eight clusters.

To evaluate the discriminating capability of the clustering algorithm, discriminating power in percentage was calculated using the equation [Williamson, 2016; Raeva, 2016 & Almirall, 2016].

$$\text{No. of possible sample pairs} = \frac{\text{Total no.samples} \times (\text{Total no.of samples} - 1)}{2}$$

$$= \frac{60 \times 59}{2} = 1770 \text{ pairs}$$

$$\text{Discriminating Power} = \frac{\text{No.of discriminated sample pairs}}{\text{Total no. possible sample pairs}} \times 100 = \frac{1366}{1770} \times 100 = 77.18\%$$

From the above equation, it was calculated that HCA showed a discriminating power of 77.18%.

Table 4 Grouping of illicit liquor samples in different clusters formed by HCA

Cluster No.	Sample ID	No. of samples in each cluster	No. of sample pairs not discriminated
1	A4, A5, A6, A7, A8, A9, A10, A12, C2, C4, C6, C9, R2, R6, H3, L3, L4	17	136
2	CA1, C1, C3, C5, C8, C10, C11, A1, A2, A3, A11, RI9, RI10, L1, L2, J1, J5, R1, R3, R4, R5, H2	22	231
3	CA2, CA3, CA4, RI7	4	6
4	A13, P2, RI3, RI6	4	6
5	RI1, J2, H1, RI4, H4	5	10
6	RI2, RI5, RI8, L5, J3, J4	6	15
7	P1	1	0
8	C7	1	0

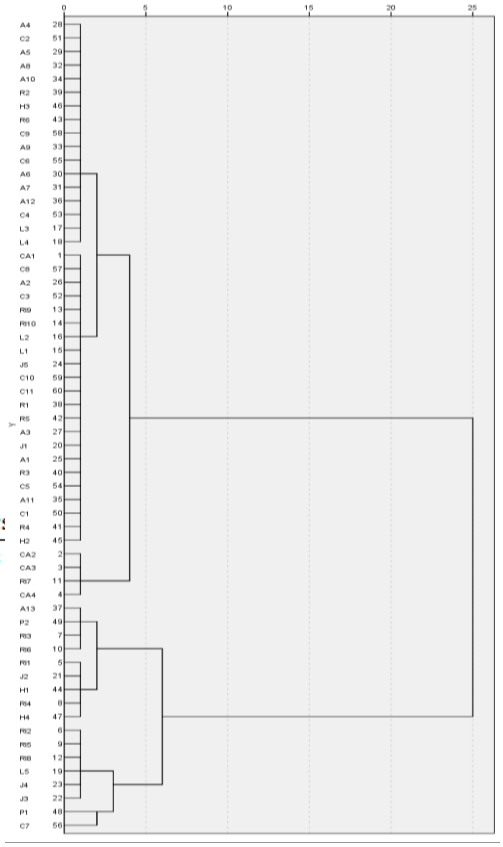

Figure 6: Dendrogram showing hierarchical clustering in different samples of illicit liquor

3.4.2. Principal Component Analysis (PCA)

To check the applicability of PCA on the data under analysis, it is first crucial to assess the adequacy of the sample and to establish that the variables have no inter-relationship. Therefore, Kaiser-Meyer-Olkin (KMO) and Bartlett's test of sphericity is first applied to the data. To proceed with PCA, the KMO value should lie within 0.5 – 1 and the Bartlett value should be <0.05. In this study, the values for the aforementioned tests were 0.952 and 0.00 respectively. Taking in accord the Kaiser's rule, only the PC with an eigenvalue greater than unity was considered to measure the variance in data [Karamizadeh, 2013; Abdullah, 2013; Manaf, 2013; Zamani, 2013 & Hooman, 2013; Hair, 2010; Anderson, 2010; Babin, 2010 & Black, 2010; Dziuban, 1974 & Shirkey, 1974].

In this study, PCA explained a variance of 99.8%. The score plot in Figure 7 shows the variance explained by PC 1 and PC 2 in the dataset representing the illicit liquor samples. It can be observed that samples showing dissimilarities are placed at a higher distance from each other. Nevertheless, many samples were placed closer to each other showing similarities between them. This can be associated with the presence of similar substances used in the manufacturing of illicit liquors in different regions.

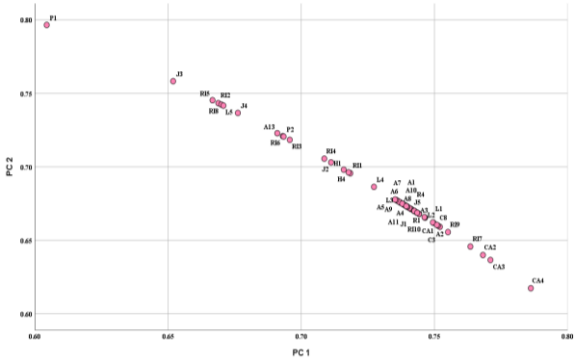

Figure 7: Score plot of PC1 and PC2 representing the variance in the dataset

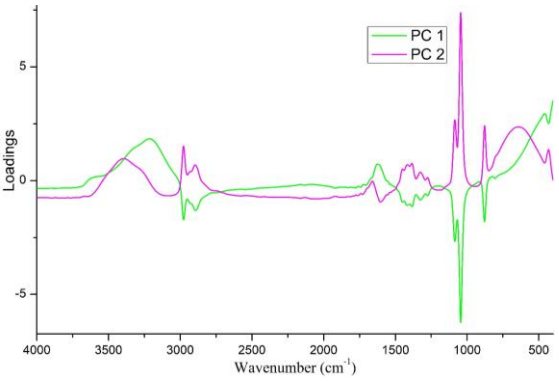

Figure 8: Loading plot of PC1 and PC2 obtained by PCA.

The regression factor values of PC1 and PC2 were plotted against the loadings in a linear graph to produce a loading plot (Figure 8). The plot displays those spectral bands that facilitated the discrimination of the samples and were associated with the respective PC. Both the PCs indicate the spectral band at 3304 cm^{-1} and 2982 cm^{-1}that corresponds to the O-H stretching due to secondary alcohol. The band at 1273 cm^{-1} and 1084 cm^{-1}can be attributed to the C-H stretching and C-O stretching of alkane compounds and primary alcohols respectively. The peak at 1044 cm^{-1} is linked to the stretching of CO-O-CO in

esters. Similarly, the spectral band at 513 cm^{-1} corresponds to C-I stretching in halo compounds. As a result, the absorption bands reveal a good concentration of primary and secondary alcohol, alkane, esters, and halo compounds in the samples of illicit liquors that correlates with various adulterants detected in the samples.

4. Conclusion

Earnest maiden attempt has been made to discriminate the samples of illicit liquors based on the presence of adulterants. Chemical color tests have been conducted as a part of the preliminary examination that indicated the presence of adulterants like copper, furfural, and ethanol. GC-FID successfully detected the adulterants in each sample thereby allowing their discrimination from each other. GC-FID furnished very distinguishable results depicting the presence of ethanol in all the samples alongwith adulterants belonging to the class group of aldehyde, esters, alcohols, nitrogen and other functionalities.ATR-FTIR spectral analysis depicted the presence of halo compounds, aromatic esters, and anhydrides which are deemed harmful for human consumption. Chemometric methods including HCA, classified the samples in eight clusters/groups displaying a discriminating power of 77.18 %, whilePCA, showed a variance of 99.8 % in the dataset. The combination of chemometric and analytical methods portrayed encouraging results in characterizing and discriminating the illicit liquor samples based on the adulterants. This study can have a potential application in real-time cases related to the forensic examination of illicit liquors. For future studies, a larger set of samples from across the country can be incorporated to create a database.

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Credit authorship contribution statement

Shweta Sharma- Writing - review & editing, Supervision. Bharti Jain- Conceptualization, Methodology, Writing - original draft. Anjali Tomar- Validation, Writing - review & editing. Nussrat Shiekh- Formal analysis.

Declaration of Competing Interest

None

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LECTINS: CURRENT STATUS AND FUTURE PERSPECTIVE

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Abstract

Lectins are group of proteins that bind non-covalently and reversibly with carbohydrates and glycoproteins. These have been classified on the basis of structural homology and evolutionary similar sequences into different classes like B-type lectin, C- type lectin , L- type lectin etc. Lectins have anti-cancer, anti-viral and anti-microbial potential; the molecular pathway of these activity has been investigated through different studies. These have been already used to study glycan structure by lectin blotting. As lectin microarray and biosensor it aids in disease diagnosis by analysing glycoproteins on the cell membrane.

Keywords: Lectin, Carbohydrates, Classification, Glycoproteins, Biosensor.

Introduction

Lectins are diverse group of non-immune origin proteins that can bind and recognize specific carbohydrates and glycoproteins reversibly and are widespread in all kingdoms of life. These proteins have crucial role in survival and functioning like plant defense system, cell-cell recognition, immune system activation and cell communication (*Lis et al.*, 1998; Drickamer 1999). Lectins are known for more than a century and were first found in plants and now have been reported in different animals and microorganisms. Lectin term was first introduced by William C. Boyd & Elizabeth Shapleigh in 1954 from a latin word "*lego*" which means chosen as they find its specific precipitating activity. Although lectins have role in biological processes, these can be toxic as in first ever studies of lectin byPeter Hermann Stillmark in 1888, a protein named ricin isolated from castor seed (*Ricinus communis*) which was toxic in nature.Later, in 1907, Landsteiner & Raubitschek discovered non-toxic lectin from leguminous plant seeds (*Phaseolus vulgaris, Pisum sativum* and *Lens culinary*). During the Second World- war the interest in blood typing for blood transfusion resulted in the discovery of some lectins specific to various blood types (*de Juan et al*., 2017). Different chromatographic techniques can be used, including affinity chromatography, ion-exchange chromatography, hydrophobic interaction chromatography, and gel filtration. Based on carbohydrate-binding capacity lectins can be grouped as mannose-binding, glucose binding, galactose binding, N-acetyl galactosamine binding, N-acetyl glucosamine binding, fucose binding, and sialic-acid binding. The hemagglutination activity can be inhibited in the presence of sugars.

Lectins are different from enzymes due to their carbohydrate-binding property never changing and they are unlike antibodies not induce an immune response (*El-Araby et al*.,

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2020). In plants, lectins are mainly expressed in stress conditions and are stored in seeds, vegetative storage compartment and roots. The structural and biological studies of the plant lectins also contributed in understanding lectin interactions and it is believed that plant lectins may have interaction with noncarbohydrate hydrophobic ligands (*Komath et al*., 2006). Some studies indicate that legume lectins are involved in root-nodule interactions but endogenous role of the plant lectins is yet to be fully explored and study (*Peumans et al.,* 1995. *Navarro-Gochicoa et al.,* 2003). While, in the animals and micro-organisms intensive studies has been done to understands its role as a therapeutic agent and has been reasonably understood. The carbohydrate binding properties of the lectins helps in understanding cell-cell communication and their vital role in immune systems eg. mannose binding lectin (MBL) has a crucial role in innate immune system by binding with the sugars on the surface of the pathogens. Different molecular pathways that are involved in the anticancer activity of the lectins have been thoroughly investigated where it reduces the growth of tumor cells or ultimately leads to the apoptosis of the cancer cells. Lectins from different families like legume lectin, jacalin- related lectin, *Galanthus nivalis* agglutinin (GNA) related lectin can recognize the glycoproteins on the envelop of the viruses that inhibit the viral interaction with host cells and act as an antiviral agent (*François* & *Balzarini* 2012; *Sarkar et al*., 2022; *de Camargo et al* 2020). Lectins also possess insecticidal properties. There are several studies that suggest that lectins bind with the glycan structure in the midgut of the insects which leads to the retard growth and loss of the fecundity of the insects (*Vandenborre et al.,* 2011). Its potential as an anti-tumor and anti-viral drug makes it useful in the therapeutic industry.

Lectins have been thoroughly studied for the therapeutic properties as these can bind with carbohydrates present on the cell surface; therefore, can be used as a recognition tool for the disease diagnosis and target drug delivery. Lectins can be used as probes for the characterization and isolation of simple or complex sugars and glycosylated macromolecules. The structural elucidation of the lectin by high resolution technology like nuclear magnetic resonance (NMR) and X-ray crystallography allows the better understanding of carbohydrate-lectin interactions as the structure of the lectins ultimately influence their vast functional abilities. There is abundant of research on lectin is going on to fully understand its potential and the mechanisms that are involved in its biological activities.

Classification of lectins

Classification of lectins based on sequence and structure homology (*Kumar et al*., 2012)

Beta prism lectins (B type):β-prism I-fold lectins are also known as jacalin-related lectins. β-prism II-fold lectins are also called as monocot mannose-binding lectins and beta-trefoil fold lectins. The characterization of β-prism I-fold initially as a lectin fold was done through the X-ray analysis of jacalin (*Sankaranarayanan et al*., 1996). Artocarpin, another lectin, with the b-prism fold from the seeds (*Pratap et al*., 2002). Various β-prism

fold lectins' crystal structures are available (Bourne et al., 1999, Bourne et al., 2004, Rao et al., 2004, Rabijns et al., 2005, Huang et al., 2006). β-prism II-fold was first discovered in snowdrop lectin (*Hester et al*., 1995). Snowdrop lectin is tetramer perhaps the second lectin analyzed β-prism II analyzed by X-ray crystallography, is dimeric garlic lectin (Chandra et al., 1999). The structure of some β-prism II-fold lectins has been reported (Chantalat et al., 1996, Wood et al., 1999). These lectins bind to mannose. The threefold symmetry of β-prism II-fold lectins is reflected in the sequence unlike β-prism I-fold. Each subunit has three carbohydrate binding sites (Sharma et al., 2007).

Calcium-dependent lectins (C type): C-type lectins are lectins that require calcium ion for binding to carbohydrates. C-type lectins bind to various carbohydrate and non-carbohydrate ligands. Each CRD is roughly 110-130 amino acids long and contains conserved cysteines and hydrophobic cores (Drickamer et al., 1993). C-type lectins are either found as transmembrane proteins or secreted as soluble proteins. Members of the collectins, selectins, asialoglycoproteins etc family come under the category of soluble C-type lectins (Lu et al., 2002). Examples are lung surfactant protein A (SP-A) and lung surfactant protein D(SP-D) (Wintergerst et al., 1989), which are secreted at the luminal surface of pulmonary epithelial cells and the mannose-binding protein also known as MBL, a collection present in plasma (Kawasaki et al., 1983). Transmembrane C-type lectins have been divided into two groups, depending on the orientation of their amino (N) terminus. Type-1 and Type-2 C-type lectins are divided depending on the N-terminus pointing outwards or inwards into the cytoplasm of the cell respectively. Some transmembrane C-type lectins with examples are the selectins (Ley and Kansas., 2004), the mannose receptor family (MMR) family (East and Isacke., 2002), and the dendritic cell-specific ICAM-3 grabbing non-integrin (DC-sign) (Geijtenbeek et al., 2003).

Ficolins: Ficolins are known as Fibrinogen/collagen domain containing lectins (F type). F-type lectins are recent addition to the lectin family and its characteristic feature is unique CRD amino acid sequence motif and structural fold and specificity for L-fucose (Odom et al., 2006, Bianchet et al., 2002, Bianchet et al., 2010). The structure of AAA/L-Fuc (*Anguillaanguilla* agglutinin; AAA,) complex contains a novel CRD sequence motif and a novel fold (F-type fold) for an animal lectin, which is found with a beta-jelly roll sandwich consisting of three and five stranded b-sheets (Bianchet et al., 2002). A sperm-binding protein, from the Japanese oyster (*Crassostrea gigas*) has been identified as F-type lectin. F-type lectins participate in immune recognition (Bianchet et al, 2010, Honda et al, 2010).

Garlic and snowdrop lectins (G type): *Galanthis nivalis* (snowdrop) lectin is a tetramer of identical subunits belonging to the non-seeded family of mannose-specific lectins from Amaryllidaceae which is known as structurally unique major lectin plant family (Van Damme et al., 1987). In previous studies, it has been reported that GNA plays a protective role against plant predators (Hilder et al., 1995). Due to mannose binding

properties, GNA has been used for glycoprotein isolation and purification (Shibuya &Bundesen, 1988).

Hyaluronin binding proteins or hyal adherins (H type): The H-type lectin term was introduced by Sanchez et al when they elucidated first crystal structure of an H-type lectin i.e *Helix pomatia* agglutinin (HPA). HPA has a quaternary structure which is a hexamer (Sanchez et al., 2006). *Helixpomatia* agglutinin (HPA) (Sanchez et al., 2006, Lescar et al., 2007), *Helix aspersa* agglutinin (Pietrzyk et al., 2015), *Sinularia lochmodes* lectin (SLL-2) (Kita et al., 2015), *Dictyostelium discoideum* discoidin DSC1 and DSC 2 (Mathiu et al., 2010) have their structure solved. H-type lectins play different biological roles in invertebrates (Jimbo et al., 2005). In snails, the role of H-type lectins is to protect fertilized eggs from bacteria because of their secretion by the albumin gland as a component of perivitelline fluid. HPA is a useful lectin for the visualization of glycoconjugates present in dental biofilm (Tawakoli et al., 2017).

Immunoglobulin superfamily lectins (I type): TheI-type lectin term was introduced by Powell and Varki (Powell et al., 1995). They are carbohydrate-binding proteins that belong to the immunoglobulin superfamily (IgSF). Several I-type lectins can specifically recognize sialic acids (Angata et al., 2002). I-type lectins recognizing sialic acids are known as Siglecs (Sialic acid-binding immunoglobulin superfamily lectins) which is a structurally distinct subclass of I-type lectins (Crocker et al., 1998). IgSF members contain at least one immunoglobulin (Ig)-like fold and contain features of fibronectin 3 repeats. The Ig fold discovered in antibodies and is made up of antiparallel b-strands organized into b-sandwich containing 100-120 amino acids, is often stabilized by an intersheet disulfide bond (Varki &Crocker, 2009).

Jacob and related lectins (J type): Jacob is a glycoprotein found on two-dimensional protein gels cyst walls purified from *Entamoeba invadens*. Jacob is an acidic protein with molecular weight of 100KDa containing sugar which can bind to Concanavalin A and ricin (Frisardi et al., 2000). The most abundant protein in the cyst wall of *Entamoebainvadens* is a lectin called EiJacob1. EiJacob 1 is a glycoprotein containing five tandemly arrayed chitin-binding domains (CBDs). Each EiJacob 1 possesses six conserved cysteine residues and various conserved aromatic amino acids. Galactose lectins present on plasma membrane can bind to carbohydrates on Jacob, and Jacob lectin in turn cross-links chitin fibers (Mann et al., 1991, Van dellen et al., 2006).

Legume seed lectins (L type): The most common fold in lectins is the legume lectin fold, which was initially found in concanavalin A (ConA) (Hardman et al., 1973, Edelman et al., 1972). The structure contains two hydrophobic cores, one between the two large sheets and the other between the curved b-sheet loops (Banerjee et al., 1996). From *Dolichosbiflorus*, in two legume lectins, half the subunits have an additional C-terminal helix (Vijayan & Chandra, 1999). Legume lectin folds and their variants are present in animal lectins, for example, galectins, pentraxins, and some other proteins

from microbial and animal sources (Srinivasan et al., 1996).

Alpha mannosidase-related lectins (M type): M-type lectins are associated with mannosidase in the endoplasmic reticulum, but they lack mannosidase activity due to the absence of some conserved residues and a disulfide bond (Hosokawa et al., 2001, Hirao et al., 2006)

Nucleotide phosphohydrolases (N-type): The lectin nucleotide phosphohydrolase (LNP) is a membrane protein present on the peripheral surface of epidermal root hairs (Kalsi et al., 2000). The characteristic feature of LNP for the establishment of the rhizobial symbiosis was indicated by the pre-treatment of roots with antiserum against recombinant LNP that inhibited root hair deformation and nodulation in response to rhizobia (Etzler &Esko, 2009). The lectin isolated from the roots of *Dolichosbisflorus* binds to Nod factors produced by rhizobial strains that nodulate this plant and contains amino acid sequence with no significant homology to any lectin reported to date. This lectin also contains enzyme activity that catalyzes the hydrolysis of phosphoanhydrous bonds of nucleoside di tri-phosphates, enzyme activity is enhanced with the presence of sugars. LNP contains a substrate specificity characteristic of the apyrase category of phosphohydrolases, and its sequence possesses four motifs, which is a feature of this enzyme. LNP is found on the surface of root hairs and the treatment of antiserum to these roots inhibits their ability to undergo root hair deformation and to form nodules on exposure to rhizobia (Etzler et al., 1999).

Ricin lectin (R type): R-type lectins were named after the ricin was discovered. Ricin was the first lectin to be discovered. Ricin has been isolated from *Ricinuscommunis*. Ricin can be easily isolated from castor beans and can kill humans at very small doses (Cummings et al., 2022)

Tachypleus tridentatus (T type): The lectin has been isolated from the hemolymph of Japanese horseshoe crab, which can agglutinate mammalian erythrocytes (Shimizu et al., 1977). Five types of lectins have been purified from Japanese horseshoe crab, tachylectin-1 can bind with gram-negative bacteria. Tachylectin -1 can also bind to polysaccharides like agarose and dextran. Tachylectin -2 can bind to N-acetyl glucose and N-acetyl galactose and it can recognize staphylococcal lipoteichoic acids and LPS from gram-negative bacteria. Tachylectin 3 and 4 can bind to S-type LPS from various gram-negative bacteria through a certain sugar moiety on O-specific polysaccharide (O-antigen). Tachylectin-5 in the hemolymph is found with the strongest bacterial agglutination activity among the five types of tachylectins. One of the major defense systems in Japanese horseshoe crab, *tachypleus tridentatus* is handled by hemolymph, which contains granular hemocytes composed of 99% of total hemocytes (Toh et al., 1991).

Wheat germ agglutinin (W type): Wheat germ agglutinin is a protein categorized in the hevein class with specificity for N-acetylglucosamine. It is isolated from wheat germ, a low-quality by-product of the wheat industry (Balčiūnaitė-Murzeine et al., 2021). Due to specificity for NAG, wheat germ

agglutinin contains antifungal properties and cytotoxic properties. In plant immunity, WGA plays an important role against pathogenic fungi (Tonkal, 2009, Leidke et al., 2017). A recombinant in combination with the effector Fc region of murine IgG2A was used for mycosis treatment. Due to specificity for chitin, WGA-Fc antibodies can bind to chitin standard fungal cultures and retard the growth of *Histoplasmacapsulatum*, *Candidaalbicans*, *Cryptococcusneoformans*, and *Saccharomycescerevisiae* (Leidke et al., 2017)

P-type lectin: P-type lectins are distinguished from other lectins by their ability to recognize phosphorylated mannose residues (Dahms & Hancock, 2002). Two members of the P-type lectin family members are the cation-dependent mannose-6-P receptor (CD-MPR) and the insulin-like growth factor 2/ mannose-6-P receptor (IgF 2/MPR) (Nair et al., 2005). P-type lectins play a role in the generation of functional lysosomes within the cell of eukaryotes by guiding newly synthesized lysosomal enzymes bearing the mannose-6-P signal to lysosomes (Hancock, 2002).

Potential applications of lectin

Anti-viral activity

The antiviral properties of the lectins explored a lot specially in the treatment against HIV (Human immunodeficiency virus) and coronaviruses (François, K.O., et.al., 2012). The HIV envelope surface is covered with glycoprotein gp120 and gp40 which is recognized by lectins from different families: legume lectins, cyanovirins, jacalin-related lectins, GNA-related lectins, Hevein-related lectins. The binding of lectin with N glycan on gp40 and gp120 inhibit or block the infection of HIV-1. There are some reports that lectins are also potent inhibitors of coronaviruses (Keyaerts, et.al. 2007). Cyanovirin strongly interact with high mannose N glycans present on the surface of the viral gp120 glycoprotein irreversibly binding and inactivation of HIV.

Anti-tumor activity

Lectins are most investigated for the diagnosis and treatment of cancer from past years. Ricin-B lectins and WGA (wheat germ agglutinin) belongs to legume lectin family extensively studied for their anticancer activity, few lectins including ConA (concanavalin A), PHA (Phytohemagglutinin), and WGA (wheat germ agglutinin) are in a pre-clinical phase (Liu, et.al., 2010). Lectins coupling to drugs have been examined which results to the increase in ligand targeted toxins and immunotoxins that allow targeted delivery. There are few plant lectins which are successfully used as adjuvants during radio- or chemotherapy for reducing the side-effect of the therapies. Plant lectins has been investigated for the trigger apoptotic or autophagy processes in vitro and in vivo.

Anti-microbial activity

Microorganism's outer membrane are covered with complicated varieties of glycoconjugates (glycoproteins and glycolipids) which are protruding to the extracellular side of the cell represent probable receptors for lectins. The lectin isolated from *Helianthus annuus* seeds changes the permeability of the membrane of

different Candida species and inhibits their growth (Regente et al, 2014). Many lectins can bind to Gram-positive or Gram-negative bacteria cell wall components such as teichoic and teichuronic acids, peptidoglycans, lipopolysaccharides, muramic or N-acetylmuramic acids and muramyl dipeptides. There are different mechanisms by which lectins show its antimicrobial activity like by blocking the entry, infection, adhesion, or migration of the bacteria and inhibition of microbial growth. Fungi are unicellular or multicellular microorganisms having a rigid cell wall composed of chitin, α- and β- linked glucose (β1–4-linked homopolymer of N-acetylglucosamine residues) and chitosan (copolymer of N-acetyl-D-glucose amine and D-glucosamine polymer) that provide stability for the fungal cell wall.

Application and uses of lectins

Lectin as gut stimulants

Dietary lectins are resistant to proteolysis to varying degrees in gut. They have different effects when passing through different regions of gut, because they are intact. They stimulate production of gut hormones. Dietary lectins can be, potentially, used to reverse the grave condition of small bowel atrophy in parenterally fed (fed with other methods instead of involvement of intestines) patients (Jordinson et al, 1999) (Pusztai et al 2008). Kidney bean (*Phaseolusvulgaris*) agglutinin (PHA) can be used to control gastric acid (Kordas et al 2001). Kidney bean (*Phaseolusvulgaris*) agglutinin (PHA) also leads to production of cholecystokinin leading to increase pancreatic enzyme secretion in duodenum (Kordas et al 2000) (Pusztai, A., 1999). Kidney bean (*Phaseolusvulgaris*) agglutinin (PHA) induces growth and maturation of gastrointestinal tract in suckling animals, this can be used to ensure smoother transition to adult feed in animals (Linderoth et al 2005).

Obesity

Obese rats have lower lipid accumulation when fed high lectin kidney bean diet (Pusztai,1998). This opened the possibility of use of kidney bean lectin as a dietary adjunct or therapeutic agent to reduce obesity.

Anti-viral properties

Mushroom lectins have antiviral activities against pathogenic viruses (El-Maradny et al 2021).

Drug delivery systems

Lectins can be used as potential drug delivery systems due to their ability to specifically recognize and bind to glycan structures on cell surfaces. This unique property allows lectins to facilitate targeted drug delivery, enhancing bioavailability and therapeutic efficacy while minimizing systemic side effects. Lectin-based drug delivery systems have been explored for oral, nasal, ocular, and targeted cancer therapies, leveraging their mucoadhesive and cell-specific binding capabilities. Additionally, lectins can be conjugated with nanoparticles or other drug carriers to improve drug stability and controlled release. Odorranalectin have potential in drug targeting and delivery (Li et al 2008).

Lectin affinity chromatography (LAC)

Lectins are used as affinity matrix for the purification of glycoconjugates. In LAC, a

protein is bound to an immobilized lectin, the unbound protein is washed away, and the bound protein is eluted. Newer techniques like Serial lectin affinity chromatography are also being developed (Satish & Surolia, 2001).

Lectin blotting

This technique is used for visualizing glycoproteins on western blot. In lectin blotting, lectins are used to analyse the glycan structures of glycoproteins on membranes after electrophoresis or chromatography. The lectins are biotinylated and then visualized by binding streptavidin-alkaline phosphatase. This technique detects glycans without releasing them (Sato, 2014).

Enzyme linked lectin assay

ELLA stands for Enzyme linked lectin assay. ELLA has same principle as ELISA with difference of using an enzyme linked lectin (Surya et al, 2020).

Glycan analysis by lectin microarray

In this technique lectins of known specificity are immobilized on the glass slide. A variety of samples can be used including biomolecules and cells. If a particular ligand binds to lectin, then it can be detected. This technique has helped in detection of aggressive cancer and has other applications (Surya et al, 2020).

Lectins as biosensors

Detection and onset of diseases can be done by monitoring glycoproteins and glycans. This technique measures signals based on carbohydrate lectin interaction. Electrochemical-based biosensors are coated with lectins with help of nanoparticles and polymers. EIS (Electric impedance spectroscopy) is used to measure the signals (Surya et al, 2020).

Outlook

Lectins have distinctive carbohydrate binding properties which draw interest of researchers to study them structurally and functionally. This binding ability of lectins allow them to be part of many biological process because carbohydrates play fundamental role as essential molecules for cellular structure and processes. The carbohydrate binding specificity and potential as therapeutic agent is determined by its amino acid sequence and structure. Therefore lectins are classified in different groups of protein on the bases of its structure and sequences. There are few specific potential of classified lectins which has been studied for example banana lectin (beta prism lectin) has been studied intensively for its anti-viral activity against HIV-1, influenza virus etc. The crystal structure of banana lectin helped in its molecular engineering which reportedly inhibits the ebola virus. C- type lectins which need calcium ions for their activity play vital role in the immune system by interacting with pathogen and in reconstruction of nervous system. These are important class of lectins which also play important role in gut health. There is an another very important lectin concanavalin A (legume lectin), which is commercially available currently used as affinity chromatography for purification of glycosylated macromolecules. Lectins can bind to carbohydrates glycoproteins and

glycolipids present on cell membrane, therefore can be used as tumour marker, recognition tool and in targeted drug delivery. Lectins are present in the plants which we regularly eat on daily bases, therefore are very interesting molecule to be studied further structurally and functionally.

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THE IMPACT OF CLIMATE CHANGE ON AGRICULTURAL-CROPS IN AFGHANISTAN

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Abstract

Climate change poses a significant threat to global agriculture, with profound implications for crop production, food security, and rural livelihoods. Crop yields were also very low due to the negative effects of climate change. The yields of crops like wheat, rice, and corn have recently continued to decline because of the recent drought related to agriculture, livestock, connected dynamics of desertification, land degradation, water, economic sectors, urban, and energy were the most likely negative effects of climate change in Afghanistan. In this research 50 participants were surveyed by simple randomly sampling method in Afghanistan. This study employed a mixed-methods approach, combining both quantitative and qualitative methods to assess the impact of climate change on agricultural crops. The quantitative analysis involved the use of historical climate data and crop yield records, while the qualitative component included interviews with farmers and agricultural experts to gather insights on adaptive practices and perceptions of climate impact. The findings reveal that 75.90% of respondents are familiar with climate change, reflecting a high level of awareness. Key causes identified include industrial pollution (37%), fossil fuel emissions (28%), and agricultural activities (21%), with deforestation recognized by only 15%, indicating a potential gap in understanding its role. Observed climate changes include frequent droughts (32%) and increased temperatures (26%), with corresponding impacts on crop production such as lower yields (32%) and increased pest/disease problems (26%).

Keywords: Agricultural-Crops, Climate, Change, Impact

Introduction

Climate change poses a significant threat to global agriculture, with profound implications for crop production, food security, and rural livelihoods. Rising temperatures, changing precipitation patterns, increased frequency of extreme weather events, and elevated atmospheric CO_2 levels have direct and indirect effects on agricultural crops. These changes alter growing seasons, disrupt pollination, reduce crop yields, and exacerbate pest and disease pressures. (Alley, W. M., Reilly, and Lehn. R, 2009)

With the rapid increase in the world's population, there is a corresponding increase in food demand owing to concerns about the stability of the global environment. Water availability, air pollution, and soil fertility have a large impact on agriculture productivity. (Noyal, 2017)

Although predictions of large climate impacts to developing country agriculture have been

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long standing, there have been few economic studies that actually measured climate impacts in these countries. A handful of studies were conducted using existing Agricultural Census data from India and Brazil. (Robert Mendelsohn and Ariel Dinar, 2001)

Sustainable agricultural and economic systems are needed against climatic disasters to meet a growing population, rising demand, and extreme weather events. A sustainable agricultural system can be achieved through investment, infrastructure development, and irrigation system building. (World Bank, 2021)

Agriculture is the largest economic sector in the country and is expected to remain in the medium term. Though increasing world population, changing climate conditions and economics activities are growing with each passing day making it more important than water. (Bellitürk, 2016) Increasing or decreasing changes in climatic values affect living things negatively and cause a decrease in productivity, especially in agriculture productions. Agriculture is highly dependent on the weather pattern, because agriculture is likely to be affected positively and negatively by climate change, and change in weather patterns (climate) is expected to have an adverse effect on food security, agricultural productions, and rural livelihoods. (Omerkhil & Pandey, 2020)

Afghanistan, with its largely agrarian economy, is significantly vulnerable to the effects of climate change. The agricultural sector employs around 60-70% of the population and contributes approximately 25% of the GDP. However, the increasing frequency of extreme weather events, changes in temperature, and precipitation patterns have been disrupting agricultural productivity, threatening food security, and impacting the livelihoods of farmers. (Omerkhil, 2020)

Afghanistan has faced extreme climatic crises such as drought, rising temperature, and scarce precipitation, and these crises will likely worsen in the future. Reduction in crop yield can affect food security in Afghanistan, where the majority of population and economy are completely dependent on agriculture. (Raoufi, 2023)

The objective of this study is to analyze the impact of climate change on Agricultural – Crops in Afghanistan. A sustainable agricultural system can be supported by adaptation strategies, including cultivation practice, field management, cultivating seed selection, ecological conservation, and water resources management.

Material and Methods

Afghanistan is a landlocked country in southern Asia that borders with China, Iran, Pakistan, Tajikistan, Turkmenistan, and Uzbekistan. The geography of Afghanistan is arid and mountainous; the Hindu Kush mountains run northeast to southwest and divide the northern provinces from the rest of the country.

This study employs a mixed-methods approach, combining both quantitative and qualitative methods to assess the impact of climate change on agricultural crops. The quantitative analysis involves the use of historical climate data and crop yield records, while the qualitative component includes interviews with farmers and agricultural experts to gather insights on adaptive practices

and perceptions of climate impact. The method which was used in this research was a descriptive method. The selection of respondents was used simple randomly sampling technique based on the considering the respondents. A total of 120 respondents were interviewed. Total populations are unknown to draw a statically based sample therefore we have come up with 120 responds which provided enough information for statically conclusion.

Results and Discussion

Figure 1. Respondents familiarity with the concept of climate change.

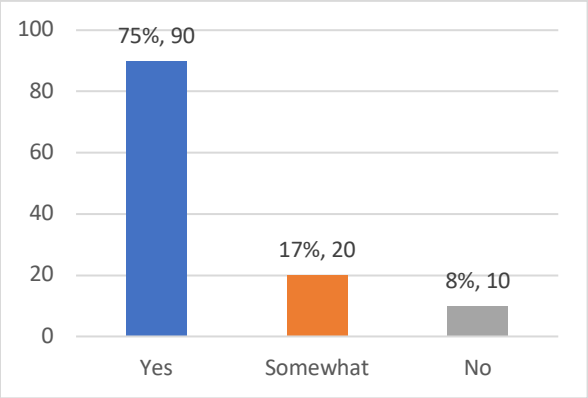

Figure 1 shows that the majority of respondents (75.90%) report being familiar with climate change, which shows a strong awareness of the issue. A smaller portion (17.20%) falls into the 'Somewhat Familiar' category, which could indicate that some people may be aware of climate change in a general sense but lack deeper knowledge. A very small percentage (8.10%) are 'Not Familiar,' suggesting that only a few respondents are completely unaware of the concept of climate change.

Figure 2. Main causes of climate change according to the participants.

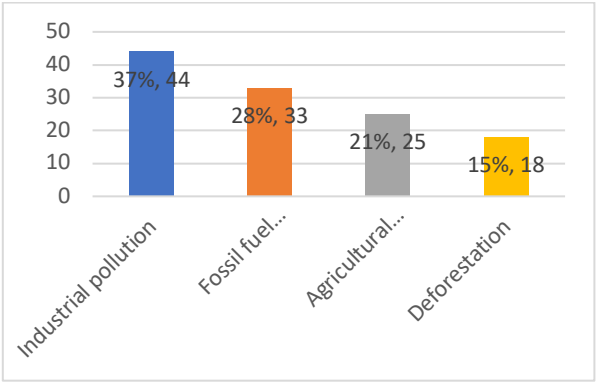

Figure 2 shows the main causes of climate change as perceived by the respondents. Industrial pollution leads with 37%, followed by fossil fuel emissions at 28%. Agricultural activities are the third most recognized cause at 21%, while deforestation is noted by 15% of respondents. The data indicates that a majority of people attribute climate change to industrial activities and fossil fuel use, highlighting a strong awareness of these sectors' contributions to greenhouse gas emissions. The relatively lower percentage for deforestation suggests a potential gap in understanding the full impact of forest loss on climate change.

Figure 3. Types of climate changes observed by participants

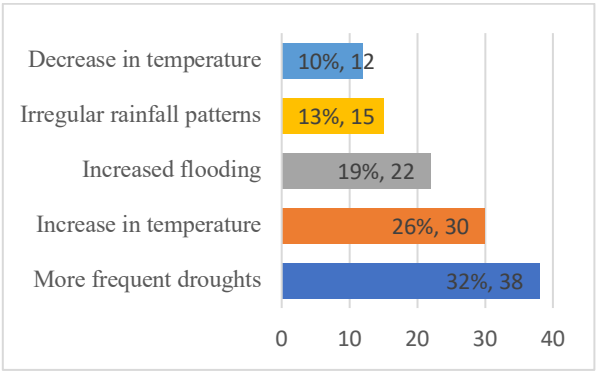

Figure 3 highlights the types of climate changes observed by respondents. The most

commonly reported change is more frequent droughts (32%), followed by increased temperature (26%). Increased flooding was noted by 19% of respondents, while irregular rainfall patterns were observed by 13%. A smaller portion of respondents (10%) reported decreased temperature. These findings indicate that most respondents perceive changes consistent with the effects of global warming, particularly more frequent droughts and higher temperatures. This suggests a strong awareness of the shifting climate and its impacts, although fewer respondents noted cooling trends, reflecting the predominant global warming narrative.

Figure 4. The impact of climate change on crop - production over recent year.

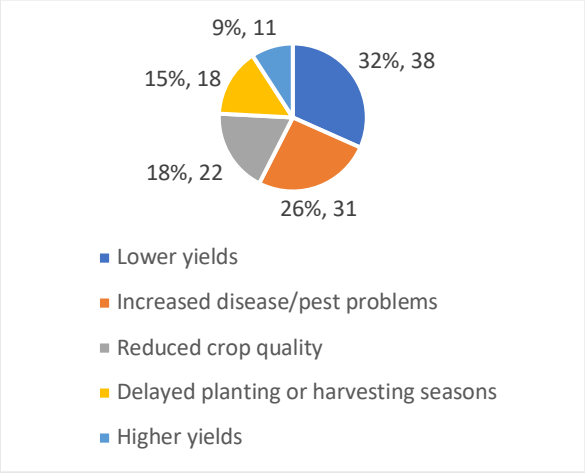

Figure 4 shows the perceived impact of climate changes on crop production in recent years. The most commonly reported effect is lower yield (32%), indicating significant reductions in crop productivity. Increased disease/pest problems follow at 26%, suggesting that climate variability is contributing to more frequent pest and disease outbreaks. Reduced crop quality was reported by 18%, reflecting concerns about the declining quality of produce. Delayed planting or harvesting seasons accounted for 15%, highlighting disruptions in traditional agricultural schedules. Interestingly, only 9% of respondents observed higher yields, indicating that while some may benefit from changing conditions, the overall impact on crop production is largely negative. These results underscore the importance of developing adaptive measures in agriculture to cope with the challenges posed by climate change.

Figure 5. Strategies adopted by farmers to cope with climate change.

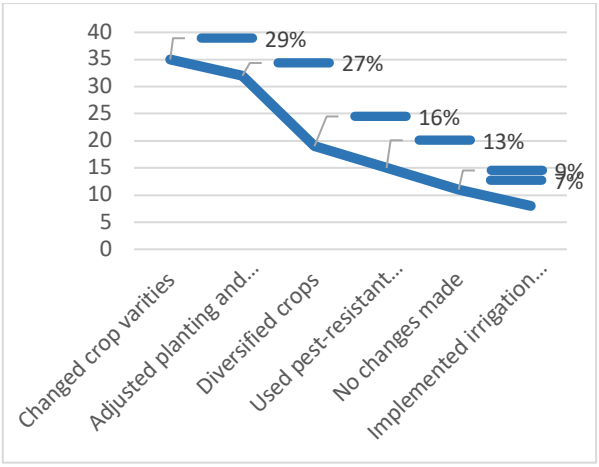

Figure 5 reported the strategies adapted by farmers to cope with climate change. Changing crop varieties is the most common strategy, adopted by 29% of farmers. This suggests a shift towards crop varieties that are more resilient to changing climate conditions, such as drought-resistant or heat-tolerant species, to maintain or improve yields. Adjusting planting and harvesting times is the second most adopted strategy at 27%. This indicates that farmers are modifying their agricultural calendars to better align with shifting seasonal patterns, such as earlier or delayed rains. Diversifying crops was reported by 16% of farmers, reflecting an effort to spread risk by

growing a variety of crops. This strategy can help ensure that if one crop fails due to climate stress, others may still thrive. Using pest-resistant crop varieties accounts for 13% of responses, suggesting that some farmers are adopting crop types that can withstand increased pest and disease pressure, which is often exacerbated by changing climate conditions. No changes made were reported by 9% of respondents, indicating a small proportion of farmers have not yet adapted any specific strategies. This could be due to a lack of resources, information, or perceived need for adaptation. Implementing irrigation or water conservation methods was the least common strategy at 7%, highlighting that while water management is critical, it may be less accessible or costlier for many farmers compared to other adaptation methods.

Table 1. Garret's Ranking Technique for ranking the cases of climate change

No	Causes of climate change	Garrett's Score	Rank
1	Cutting down forests	77	1
2	Using transportation	63	2
3	Manufacturing goods	54	3
4	Generating Power	46	4
5	Powering building	36	5
6	Producing food items	23	6

The above –mentioned table indicated the causes of climate change. The responses were asked to rank the causes of climate change in order of importance so the rates of the causes were analyzed by using Garrett's ranking technique. The first important cause was found cutting down forests with a mean score of 77. Cutting down forests to create farms or pastures, or for other reasons, causes emissions. Since forests absorb dioxide, destroying them also limits nature's ability to keep emissions out of the atmosphere. The second most important cause was using transportation a mean score of 63. Most cars, trucks, and planes run on fossil fuels. That makes transportation a major contributor of greenhouses gases, especially carbon dioxide emission. Road vehicles account for the largest part, due to the combustion of petroleum-based products, like gasoline, internal combustion engines. The third important cause was manufacturing goods with a mean score of 54. Manufacturing and industry produce emissions, mostly from burning fossil fuels to produce energy for making things like cement, iron, steel, electronics, plastics, and other goods. The fourth important cause was generating power with a mean score of 46. Generating electricity and heat by burning fossil fuels causes a large chunk of global emissions. Most electricity is still generated by burning coal, oil, gas, which produces carbon dioxide and nitrous oxide – powerful greenhouse gases that blanket the Earth and trap the sun heat. The fifth important cause was powering buildings with a mean score 36. Residential and commercial buildings consume over half of all electricity. As they

continue to draw on coal, oil, and natural gas for heating and cooling, they emit significant quantities of greenhouse gas emissions. The sixth important cause was producing food items with a mean score of 23.

Conclusion

The findings highlight a strong overall awareness of climate change among respondents, with most individuals recognizing its causes, impacts, and the need for adaptive strategies. Industrial pollution, fossil fuel emissions, and agricultural activities were widely acknowledged as significant contributors, although deforestation and other critical causes may not be as well understood. Respondents perceived climate change primarily through observable phenomena such as frequent droughts, rising temperatures, and irregular rainfall patterns, which align with global warming trends. These changes are also seen to negatively impact agricultural productivity, with lower yields, increased pest problems, and disrupted farming schedules being the most commonly reported consequences. Adaptation strategies employed by farmers reveal a proactive approach to addressing challenges. Changing crop varieties, adjusting planting times, and diversifying crops are popular methods, though limitations in resources and knowledge prevent some from implementing effective measures like irrigation or water conservation. Finally, the ranking of causes using Garrett's technique underscores deforestation, transportation, and manufacturing as the top-contributors to climate change, reinforcing the need for targeted action in these areas. The results emphasize the necessity for enhanced education on climate-related issues, investment in sustainable agricultural practices, and robust policy measures to mitigate the adverse impacts of climate change and build resilience across communities.

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STATISTICAL INFERENCES AND RENYI`S ENTROPY OF ALPHA POWER TRANSFORMED GENERALIZED EXPONENTIAL DISTRIBUTION BASED ON ORDERED STATISTICS WITH APPLICATION

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ABSTRACT

Alpha power transformed generalized exponential (APTGE) distribution has three parameters (two shapes and one scale) contains some sub-models like exponentiated exponential and truncated exponential distribution. This distribution is more useful for consistently increasing, decreasing, constant, upside down and bathtub hazard rates. For this distribution, we find the mathematical equations for single moments, product moments, marginal and joint moment-generating functions of order statistics. Further, We also compute the expected value, product moments, covariances, and variances based on the concept of order statistics for sample sizes up to 10 for various values of the parameters. The unknown parameters are obtained by the method of maximum likelihood estimation (MLEs) based on Type-II censored sample. Moreover, we obtain the best linear unbiased estimators for scale and location parameters based on Type II right censoring. We compute the numerical values of alpha power transformed generalized exponential distribution using Monte Carlo simulations to show the adequacy of our findings.

Keywords: APTGE distribution, order statistics, best linear unbiased estimators, Maximum Likelihood estimators, entropy.

1. Introduction

Over the recent years, a massive growth has been observed in the use of order statistics because of their characteristic, also order statistics are essential in a large variety of theoretical and real world problems like analysis of censored samples, entropy estimation, income inequality, quality control, reliability analysis, strength of materials, and selecting the best. *Arnold et al*. (2003), David and *Nagaraja* (2003) have given the characterization of probability distribution and goodness of fit tests. The applicability of moments of order statistics can be observed in various areas such as reliability, quality control, auction theory etc. For example, a cord composed of many threads, the failure of one thread will cause the cord to break, but more likely the cord will function as long as

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selected threads remain unbroken. These predictions are frequently based on moments of order statistics. Many authors have addressed the problems based on the moments of order statistics among different probability distributions, one can see *Balakrishnan* and *Cohan* (1991), *Balakrishnan* and *Sultan* (1998), *Sultan* and *Balakrishnan* (2000, 2000a), *Genc* (2012), *Jabeen et al*. (2013), *Mir Mostafaee*, (2014), *Balakrishnan et al*. (2015), *Kumar* (2015), *Kumar et al*. (2017), and *Kumar* and *Goyal* (2019,2019a) for the related works over the years in this domain. Many scholars have studied statistical properties and developed best linear unbiased estimators (BLUEs) for the scale and location parameters of the distributions based on Type-II right censored data like *Kumar et al.* (2022, 2021, 2020). They also created point prediction and goodness of fit tests.

The Shannon entropy of the random variable Y is the mathematical measure of information which measures the average reduction of uncertainty of random variable X. Renyi`s entropy is an extension of Shannon entropy, which has an important role in cryptography (1997), resolution in time frequency (2000), high-resolution scalar quantization (2011) and signal segmentation in time-frequency plane (2013) etc.

The two-parameter generalized exponential distribution initially proposed by *Gupta* and *Kundu* (1999), is a suitable model for analyzing positive lifetime data, demonstrating superior performance compared to the gamma, Weibull, and log-normal distributions. Many variations of the generalized exponential distribution have been utilized by researchers under multiple scenarios, as can be found in *Merovci* (2013) proposed the transmuted exponential distribution, *Barreto-Souza et al.* (2010) studied the beta generalized exponential distribution, *Tahir et al.* (2015) introduced the Odd generalized exponential family of distributions, *Ristic* and *Kundu* (2015) studied the Marshall-Oklin generalized exponential distribution and *Mahdavi* and *Kundu* (2017) introduced the α-power exponential distribution.

Th $APTGE$ distribution (Dey et al. (2017)) pdf's is given as:

$$f(x) = \frac{\beta\lambda\, log\alpha}{\alpha - 1} e^{-\lambda x}\left(1 - e^{-\lambda x}\right)^{\beta-1} \alpha^{\left(1-e^{-\lambda x}\right)^{\beta}}, \alpha \neq 1, \beta, \lambda > 0, x > 0, \qquad (1)$$

and the cumulative distribution function (cdf) is

$$F(x) = \frac{\alpha^{\left(1-e^{-\lambda x}\right)^{\beta}} - 1}{\alpha - 1}, \qquad \alpha \neq 1, \beta, \lambda > 0, x > 0 \qquad (2)$$

1) As $\alpha \to 1$, the $APTGE$ distribution reduces to generalized exponential distribution (1999).

2) As $\alpha \to 1$ and $\beta = 1$, the $APTGE$ distribution reduces to an exponential distribution.

3) For $\beta = 1$, the $APTGE$ distribution reduces to α-power exponential distribution.

4) As $x \to 0$, $f(x) \sim \left(1 - e^{-\lambda x}\right)^{\beta-1}$, and as $x \to \infty$, $f(x) \sim e^{-\lambda x}$.

Now, we added two more parameters (location and scale) to the $APTGE$ distribution, the pdf of the $APTGE$ distribution's location parameter is

$$f(x) = \frac{\beta\lambda\, log\alpha}{\alpha-1} e^{-\lambda(x-\mu)}\left(1 - e^{-\lambda(x-\mu)}\right)^{\beta-1} \alpha^{\left(1-e^{-\lambda(x-\mu)}\right)^{\beta}}, \alpha \neq 1, \beta, \lambda,\ x > \mu, \quad (3)$$

and the pdf of the $APTGE$ distribution's scale parameter is

$$f(x) = \frac{\beta\lambda\, log\alpha}{\alpha-1} e^{-\lambda\left(\frac{x}{\sigma}\right)}\left(1 - e^{-\lambda\left(\frac{x}{\sigma}\right)}\right)^{\beta-1} \alpha^{\left(1-e^{-\lambda\left(\frac{x}{\sigma}\right)}\right)^{\beta}}, \alpha \neq 1, \beta, \lambda, \sigma > 0, x > 0, \quad (4)$$

while the pdf of the $APTGE$ distribution's location-scale parameter is

$$f(x) = \frac{\beta\lambda\, log\alpha}{\sigma(\alpha-1)} e^{-\lambda\left(\frac{x-\mu}{\sigma}\right)}\left(1 - e^{-\lambda\left(\frac{x-\mu}{\sigma}\right)}\right)^{\beta-1} \alpha^{\left(1-e^{-\lambda\left(\frac{x-\mu}{\sigma}\right)}\right)^{\beta}}, \alpha \neq 1, \beta, \lambda, \sigma > 0, x > \mu. \quad (5)$$

There are multiple applications for the scale-parameter and location-scale parameter distributions, including statistical inference, see *Arnold et al.* (2003), *Meyer* (1987) studied the two-moment decision model, and *Wasserman* (2002) studied reliability and life testing.

This paper majorly divided into two parts, the first part we obtained the explicit expressions for the single and product moments of order statistics from the $APTGE$ distribution. The best linear unbiased estimate of the model's location (mean) and scale (standard deviation) parameters based on order statistics is obtained, along with the variances and covariances of the best linear unbiased estimate covered in second part. Also obtained the maximum likelihood estimators (MLEs) of unknown (α, β, λ) parameters based on Type-II censored sample. The probability density graph of $APTGE$ distribution for different values of parameter is shown in Figure 1.

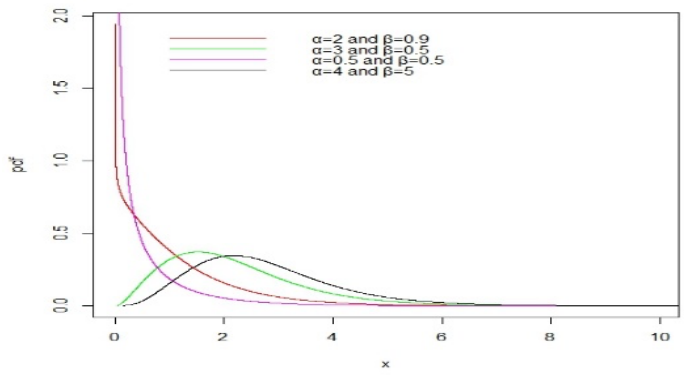

Figure 1. The density graph of $APTGE$ distribution for different parameters and $\lambda = 1$.

In this paper, we work out the moments of order statistics for $APTGE$ distribution . We include numerous technical lemmas in Section 2 that are used to develop explicit formulations for order statistics. In Section 3, we find the mathematical formulas for single and product moments, and we also construct the precise formulations for moment-generating functions for each of these order statistics. In Section 4, We utilise these moments to calculate the BLUEs for mean (μ) and standard deviation (σ). In Section 5, we obtained the MLEs of the parameters based on Type-II censored samples. We obtained the Renyi entropy of order statistics in section 6. In Section 7, a numerical study utilizing R software shows that these expressions yield accurate numerical evaluations. In

Section 8, we also included a graph and a real-life data set showing the results. In Section 9, we conclude the paper with remarks.

2. Technical Lemma

Here, we show the four technical lemmas.

Lemma 1: Let $f(x)$ and $F(x)$ be pdf and cdf of $APTGE$ distribution respectively. For $a > 0, b > 0$ and $p > 0$, let

$$K(a,b,p) = \int_0^\infty x^p\,[F(x)]^a[1-F(x)]^b f(x)dx$$

then,

$$K(a,b,p) = \frac{\beta\,\Gamma(p+1)}{\lambda^p}\sum_{i_4=0}^{\infty}\sum_{i_3=0}^{\infty}\sum_{i_1=0}^{b}\sum_{i_2=0}^{a+i_1}\frac{(-1)^{a+i_2+i_4}}{(\alpha-1)^{a+i_1+1}}\binom{b}{i_1}\binom{a+i_1}{i_2}(i_2+1)^{i_3} \times\binom{\beta(i_3+1)-1}{i_4}\frac{(log\alpha)^{(i_3+1)}}{i_3!}\frac{1}{(i_4+1)^{(p+1)}},$$

where $\Gamma(a)$ denotes the complete gamma function defined by $\Gamma(a) = \int_0^\infty t^{a-1}\,e^{-t}\,dt$.

Proof: Using the binomial expansion in $[1-F(x)]^b$ and equation (1) and (2),

$$K(a,b,p) = \sum_{i_1=0}^{b}(-1)^{i_1}\binom{b}{i_1}\int_0^\infty x^p\,[F(x)]^{a+i_1}f(x)dx$$

$$= \sum_{i_1=0}^{b}\binom{b}{i_1}\frac{\beta\lambda\,log\alpha\,(-1)^{i_1}}{\alpha-1}\int_0^\infty x^p\left[\frac{\alpha^{(1-e^{-\lambda x})^\beta}-1}{\alpha-1}\right]^{a+i_1} e^{-\lambda x}\left(1-e^{-\lambda x}\right)^{\beta-1}\alpha^{(1-e^{-\lambda x})^\beta}dx$$

$$= \beta\lambda\sum_{i_3=0}^{\infty}\sum_{i_1=0}^{b}\sum_{i_2=0}^{a+i_1}\frac{(-1)^{a+2i_1+i_2}}{(\alpha-1)^{a+i_1+1}}\binom{b}{i_1}\binom{a+i_1}{i_2}\frac{(log\alpha)^{i_{3+1}}}{i_3!}(i_2+1)^{i_3}\int_0^\infty x^p e^{-\lambda x} \times\left(1-e^{-\lambda x}\right)^{\beta(i_3+1)-1}dx$$

Now, if $|z| < 1$ and $b > 0$ is a real non integer number then we have the series representation

$$(1-z)^{b-1} = \sum_{j=0}^{\infty}\frac{(-1)^j\,\Gamma(b)}{\Gamma(b-j)\,j!}\,z^j, \qquad (6)$$

using the equation (6), we get the result.

Lemma 2: Let $f(x)$ and $F(x)$ be pdf and cdf of the $APTGE$ distribution respectively. For $a > 0, b > 0, c > 0, p > 0,\ q > 0$, and $0 < x < y < \infty$, let

$$L(a,b,c,p,q) = \int_0^\infty \int_x^\infty x^p y^q [F(x)]^a [F(y)-F(x)]^b [1-F(y)]^c\, f(x) f(y) dy dx,$$

then,

$$\begin{aligned}
&L(a,b,c,p,q)\\
&= \frac{\beta^2\,\Gamma(q+1)}{\lambda^{p+q}} \sum_{i_9=0}^{\infty}\sum_{i_6=0}^{\infty}\sum_{i_4=0}^{\infty}\sum_{i_7=0}^{\infty}\sum_{i_8=0}^{q}\sum_{i_1=0}^{b}\sum_{i_2=0}^{c}\sum_{i_5=0}^{b+i_2-i_1}\sum_{i_3=0}^{a+i_1}\binom{b}{i_1}\binom{c}{i_2}\binom{b+i_2-i_1}{i_5}\binom{a+i_1}{i_3}\\
&\times \binom{\beta(i_6+1)-1}{i_7}\binom{\beta(i_4+1)-1}{i_9}\frac{(-1)^{a+b+i_1+i_3+i_5+i_7+i_9}}{(\alpha-1)^{a+b+i_2+2}}(i_7+1)^{i_8}\,(i_3+1)^{i_4}\,(i_5+1)^{i_6}\\
&\times \frac{(log\alpha)^{i_4+i_6+2}}{i_6!\;i_4!\;i_8!}\frac{\Gamma(p+i_8+1)}{(i_7+i_9+2)^{p+i_8+1}(i_7+1)^{q+1}}
\end{aligned}$$

Proof: Using the binomial expansion,

$$\begin{aligned}
&L(a,b,c,p,q)\\
&= \sum_{i_1=0}^{b}\sum_{i_2=0}^{c}(-1)^{i_1+i_2}\binom{b}{i_1}\binom{c}{i_2}\int_0^\infty\int_x^\infty x^p y^q [F(x)]^{a+i_1}[F(y)]^{b+i_2-i_1} f(x)f(y)dydx\\
&= \sum_{i_1=0}^{b}\sum_{i_2=0}^{c}\frac{(-1)^{i_1+i_2}}{(\alpha-1)^{a+b+i_2+2}}\binom{b}{i_1}\binom{c}{i_2}(\beta\lambda)^2(log\alpha)^2\int_0^\infty\int_x^\infty x^p y^q\left[\alpha^{(1-e^{-\lambda x})^\beta}-1\right]^{a+i_1}e^{-\lambda x}\\
&\qquad\times\left[\alpha^{(1-e^{-\lambda y})^\beta}-1\right]^{b+i_2-i_1}\left(1-e^{-\lambda x}\right)^{\beta-1}\alpha^{(1-e^{-\lambda x})^\beta}e^{-\lambda y}\left(1-e^{-\lambda y}\right)^{\beta-1}\\
&\qquad\times\alpha^{(1-e^{-\lambda y})^\beta}dydx\\
&= \sum_{i_1=0}^{b}\sum_{i_2=0}^{c}\frac{(-1)^{i_1+i_2}}{(\alpha-1)^{a+b+i_2+2}}\binom{b}{i_1}\binom{c}{i_2}(\beta\lambda)^2(log\alpha)^2\int_0^\infty x^p\left[\alpha^{(1-e^{-\lambda x})^\beta}-1\right]^{a+i_1}e^{-\lambda x}\\
&\qquad\times\left(1-e^{-\lambda x}\right)^{\beta-1}\alpha^{(1-e^{-\lambda x})^\beta}\left(\int_x^\infty y^q\left[\alpha^{(1-e^{-\lambda y})^\beta}-1\right]^{b+i_2-i_1}e^{-\lambda y}\right.\\
&\qquad\left.\times\left(1-e^{-\lambda y}\right)^{\beta-1}\alpha^{(1-e^{-\lambda y})^\beta}dy\right)dx
\end{aligned}$$

$$= \sum_{i_6=0}^{\infty}\sum_{i_7=0}^{\infty}\sum_{i_8=0}^{q}\sum_{i_1=0}^{b}\sum_{i_2=0}^{c}\sum_{i_5=0}^{b+i_2-i_1}\frac{(-1)^{b+i_5+i_7+2i_2}}{(\alpha-1)^{a+b+i_2+2}}\binom{b}{i_1}\binom{c}{i_2}\binom{b+i_2-i_1}{i_5}\binom{\beta(i_6+1)-1}{i_7}(\beta\lambda)^2$$
$$\times\frac{(log\alpha)^{i_6+2}}{i_6!}(i_5+1)^{i_6}\frac{\lambda^{i_8}(i_7+1)^{i_8}}{i_8!\,(\lambda(i_7+1))^{q+1}}\,\Gamma(q+1)\int_0^{\infty}x^{p+i_8}\left[\alpha^{\left(1-e^{-\lambda x}\right)^{\beta}}-1\right]^{a+i_1}e^{-\lambda x}\times\left(1-e^{-\lambda x}\right)^{\beta-1}\alpha^{\left(1-e^{-\lambda x}\right)^{\beta}}e^{-\lambda(i_7+1)x}\,dx$$

$$= \sum_{i_9=0}^{\infty}\sum_{i_4=0}^{\infty}\sum_{i_6=0}^{\infty}\sum_{i_7=0}^{\infty}\sum_{i_1=0}^{b}\sum_{i_8=0}^{q}\sum_{i_3=0}^{a+i_1}\sum_{i_2=0}^{c}\sum_{i_5=0}^{b+i_2-i_1}\frac{(-1)^{a+b+i_1+i_3+i_5+i_7+i_9}}{(\alpha-1)^{a+b+i_2+2}}\binom{b}{i_1}\binom{c}{i_2}\binom{b+i_2-i_1}{i_5}(\beta\lambda)^2$$
$$\times\binom{\beta(i_6+1)-1}{i_7}\binom{\beta(i_4+1)-1}{i_9}\frac{(log\alpha)^{i_4+i_6+2}}{i_8!\;i_6!\;i_4!}(i_3+1)^{i_4}(i_5+1)^{i_6}\frac{\lambda^{i_8}(i_7+1)^{i_8}}{(\lambda(i_7+1))^{q+1}}\times\Gamma(q+1)\int_0^{\infty}x^{p+i_8}\,e^{-\lambda(i_7+i_9+2)x}\,dx$$

To calculate the above integral, we use the complete gamma function and get the mathematical expression.

Lemma 3: Let $f(x)$ and $F(x)$ be pdf and cdf of the $APTGE$ distribution respectively. *For* $a>0, b>0$ and $t>0$, *let*

$$K(a,b,t)=\int_0^{\infty}e^{tx}\,[F(x)]^a[1-F(x)]^b f(x)dx,$$

then,

$$K(a,b,t)=\sum_{i_3=0}^{\infty}\sum_{i_1=0}^{b}\sum_{i_2=0}^{a+i_1}\frac{\beta(-1)^{a+i_2}(log\alpha)^{i_3+1}}{i_3!\,(\alpha-1)^{a+i_1+1}}\binom{b}{i_1}\binom{a+i_1}{i_2}(i_2+1)^{i_3}B\left(1-\frac{t}{\lambda},\beta(i_3+1)\right)$$

where $B(a,b)=\frac{\Gamma(a)\Gamma(b)}{\Gamma(a+b)}$.

Proof: Analogous to the proof of Lemma 1.

Lemma 4: Let $f(x)$ and $F(x)$ be pdf and cdf of the $APTGE$ distribution respectively. For $a>0, b>0, c>0$, $t_1>0$ and $t_2>0$. Let

$$L(a,b,c,t_1,t_2)=\int_0^{\infty}\int_x^{\infty}e^{t_1x}e^{t_2y}[F(x)]^a[F(y)-F(x)]^b[1-F(y)]^c\,f(x)f(y)dydx$$

then,

$$L(a,b,c,t_1,t_2)$$
$$= \beta^2 \sum_{i_7=0}^{\infty}\sum_{i_6=0}^{\infty}\sum_{i_4=0}^{\infty}\sum_{i_1=0}^{b}\sum_{i_2=0}^{c}\sum_{i_5=0}^{b+i_2-i_1}\sum_{i_3=0}^{a+i_1}\binom{b}{i_1}\binom{c}{i_2}\binom{b+i_2-i_1}{i_5}\binom{\beta(i_6+1)-1}{i_7}\binom{a+i_1}{i_3}$$
$$\times \frac{(-1)^{a+b+i_1+i_3+i_5+i_7}}{(\alpha-1)^{a+b+i_2+2}}\frac{(log\alpha)^{i_6+i_{4+2}}}{i_6!\times i_4!}(i_3+1)^{i_4}\,(i_5+1)^{i_6}\frac{1}{\left(i_7+1-\frac{t_2}{\lambda}\right)}$$
$$\times \mathrm{B}\left(\left(i_7+2-\frac{t_1}{\lambda}-\frac{t_2}{\lambda}\right),\beta(i_4+1)\right)$$

Proof: Analogous to the proof of Lemma 2.

3. Moments of Order Statistics

Let $X_1, X_2, X_3, \ldots, X_n$ be n random sample from distribution with pdf $f(x)$ and cdf $F(x)$ and $X_{1:n} \leq X_{2:n} \leq X_{3:n} \leq \ldots \leq X_{n:n}$ be the corresponding order statistics then the pdf of the r^{th} order statistic is

$$f_{X_{r:n}}(x) = C_{r:n}[F(x)]^{r-1}[1-F(x)]^{n-r}f(x), \tag{7}$$

and the joint probability density function (pdf) of the r^{th} and s^{th} order statistics is

$$f_{X_{r:n},X_{s:n}}(x,y) = C_{r,s:n}[F(x)]^{r-1}[F(y)-F(x)]^{s-r-1}[1-F(y)]^{n-s}\,f(x)f(y), \tag{8}$$

for $x < y$. Where $C_{r:n} = \frac{n!}{(r-1)!(n-r)!}$ and $C_{r,s:n} = \frac{n!}{(r-1)!(s-r-1)!(n-s)!}$.

3.1. Single moments of rth order statistic

In order to compute the variance and create the inferential methods for the distribution, the single moments of order statistics are required. We will initially obtain direct expressions for each r^{th} order statistics moments., $E\left(X_{r:n}^{(p)}\right) = \mu_{r:n}^{(p)}$ and marginal moment generating function from the $APTGE$ distribution.

Theorem 1. Let $f(x)$ and $F(x)$ be pdf and cdf of the $APTGE$ distribution respectively. For $1 \leq r \leq n$

$$\mu_{r:n}^{(p)} = \frac{C_{r:n}\beta\,\Gamma(p+1)}{\lambda^p}\sum_{i_4=0}^{\infty}\sum_{i_3=0}^{\infty}\sum_{i_1=0}^{n-r}\sum_{i_2=0}^{r-1+i_1}\frac{(-1)^{(r-1+i_2+i_4)}}{(\alpha-1)^{(r+i_1)}}\binom{n-r}{i_1}\binom{r-1+i_1}{i_2}$$
$$\times\binom{\beta(i_3+1)-1}{i_4}(i_2+1)^{i_3}\frac{(log\alpha)^{(i_3+1)}}{i_3!}\frac{1}{(i_4+1)^{(p+1)}}$$

(9)

where Γ(.) is the complete gamma function.

Proof: Using the equation (7), we have

$$\mu_{r:n}^{(p)} = C_{r:n} \int_0^\infty x^p \,[F(x)]^{r-1}[1-F(x)]^{n-r} f(x)dx \tag{10}$$

The result is obtained by using the lemma 1.

Special Cases:

1) If $p = 1$ and $r = 1$, in (9) we get

$$\mu_{1:n}^{(1)} = \frac{n!}{(n-1)!}\frac{\beta\,\Gamma(2)}{\lambda}\sum_{i_4=0}^{\infty}\sum_{i_3=0}^{\infty}\sum_{i_1=0}^{n-1}\sum_{i_2=0}^{i_1}\frac{(-1)^{(i_2+i_4)}}{(\alpha-1)^{i_1+1}}\binom{n-1}{i_1}\binom{i_1}{i_2}\binom{\beta(i_3+1)-1}{i_4} \times (i_2+1)^{i_3}\frac{(log\alpha)^{(i_3+1)}}{i_3!}\frac{1}{(i_4+1)^2}$$

$$= \frac{n\beta}{\lambda}\sum_{i_4=0}^{\infty}\sum_{i_3=0}^{\infty}\sum_{i_1=0}^{n-1}\sum_{i_2=0}^{i_1}\frac{(-1)^{(i_2+i_4)}}{(\alpha-1)^{i_1+1}}\binom{n-1}{i_1}\binom{i_1}{i_2}\binom{\beta(i_3+1)-1}{i_4}(i_2+1)^{i_3} \times \frac{(log\alpha)^{(i_3+1)}}{i_3!}\frac{1}{(i_4+1)^2}$$

2) If $p = 1$ and $r = n$, in (9) we get

$$\mu_{n:n}^{(1)} = \frac{n!}{(n-1)!}\frac{\beta\,\Gamma(2)}{\lambda}\sum_{i_4=0}^{\infty}\sum_{i_3=0}^{\infty}\sum_{i_2=0}^{n-1}\frac{(-1)^{(n-1+i_2+i_4)}}{(\alpha-1)^n}\binom{n-1}{i_2}\binom{\beta(i_3+1)-1}{i_4} \times (i_2+1)^{i_3}\frac{(log\alpha)^{(i_3+1)}}{i_3!}\frac{1}{(i_4+1)^2}$$

$$= \frac{n\beta}{\lambda}\sum_{i_4=0}^{\infty}\sum_{i_3=0}^{\infty}\sum_{i_2=0}^{n-1}\frac{(-1)^{(n-1+i_2+i_4)}}{(\alpha-1)^n}\binom{n-1}{i_2}\binom{\beta(i_3+1)-1}{i_4}(i_2+1)^{i_3}\frac{(log\alpha)^{(i_3+1)}}{i_3!} \times \frac{1}{(i_4+1)^2}$$

3.2. Moment generating function of r^{th} order statistic

Theorem 2. Let $f(x)$ and $F(x)$ be pdf and cdf of the $APTGE$ distribution respectively. For $1 \leq$

$r \leq n$ and $t > 0$

$$E[e^{tX_{r:n}}] = C_{r:n}\,\beta \sum_{i_3=0}^{\infty}\sum_{i_1=0}^{n-r}\sum_{i_2=0}^{r-1+i_1} \frac{(-1)^{r-1+i_2}}{(\alpha-1)^{r+i_1}}\binom{n-r}{i_1}\binom{r-1+i_1}{i_2}\frac{(log\alpha)^{i_3+1}}{i_3!} \times (i_2+1)^{i_3}\, B\left(1-\frac{t}{\lambda}, \beta(i_3+1)\right).$$

Proof: Using the equation (7), we have

$$E[e^{tX_{r:n}}] = C_{r:n}\int_0^{\infty} e^{tx}\,[F(x)]^{r-1}[1-F(x)]^{n-r} f(x)dx$$

The result is obtained by using the lemma 3.

3.3. Product moments of rth and sth order statistics

In order to compute the variance and create the inferential methods for the distribution, the single and product moments of order statistics are required. We will initially obtain the direct expressions for the product moment of r^{th} and s^{th} order statistics, $E\left(X_{r,s:n}^{(p,q)}\right) = \mu_{r,s:n}^{(p,q)}$ and joint moment generating function from the $APTGE$ distribution.

Theorem 3. Let $f(x)$ and $F(x)$ be pdf and cdf of the $APTGE$ distribution respectively. For $1 \leq r < s \leq n$

$$\mu_{r,s:n}^{(p,q)} = C_{r,s:n}\frac{\beta^2\,\Gamma(q+1)}{\lambda^{p+q}} \sum_{i_9=0}^{\infty}\sum_{i_6=0}^{\infty}\sum_{i_4=0}^{\infty}\sum_{i_7=0}^{\infty}\sum_{i_8=0}^{q}\sum_{i_1=0}^{s-r-1}\sum_{i_2=0}^{n-s}\sum_{i_5=0}^{s-r-1+i_2-i_1}\sum_{i_3=0}^{r-1+i_1} \frac{(-1)^{s-2+i_1+i_3+i_5+i_7+i_9}}{(\alpha-1)^{s+i_2}} \times \binom{s-r-1}{i_1}\binom{n-s}{i_2}\binom{s-r-1+i_2-i_1}{i_5}\binom{\beta(i_6+1)-1}{i_7}\binom{r-1+i_1}{i_3}\binom{\beta(i_4+1)-1}{i_9} \times (i_3+1)^{i_4}\,(i_5+1)^{i_6}\,(i_7+1)^{i_8}\frac{(log\alpha)^{i_4+i_6+2}}{i_6!\;i_4!\;i_8!}\frac{\Gamma(p+i_8+1)}{(i_7+i_9+2)^{p+i_8+1}}\frac{1}{(i_7+1)^{q+1}}$$

Proof: Using the equation (8), we have

$$\mu_{r,s:n}^{(p,q)} = C_{r,s:n}\int_0^{\infty}\int_x^{\infty} x^p y^q [F(x)]^{r-1}[F(y)-F(x)]^{s-r-1}[1-F(y)]^{n-s}\, f(x)f(y)dydx \tag{11}$$

The result is obtained by using the lemma 2.

3.4. Joint moment generating function of rth and sth order statistics

Theorem 4. Let $f(x)$ and $F(x)$ be pdf and cdf of the $APTGE$ distribution respectively. For $1 \leq$

$r < s \leq n \; and \; t_1 > 0 \; and \; t_2 > 0$

$$E[e^{t_1 X_{r:n} + t_2 X_{s:n}}]$$

$$= C_{r,s:n}\, \beta^2 \sum_{i_7=0}^{\infty} \sum_{i_6=0}^{\infty} \sum_{i_4=0}^{\infty} \sum_{i_1=0}^{s-r-1} \sum_{i_2=0}^{n-s} \sum_{i_5=0}^{s-r-1+i_2-i_1} \sum_{i_3=0}^{r-1+i_1} \frac{(-1)^{s-2+i_1+i_3+i_5+i_7}}{(\alpha-1)^{s+i_2}} \binom{s-r-1}{i_1} \binom{n-s}{i_2}$$

$$\times \binom{s-r-1+i_2-i_1}{i_5} \binom{\beta(i_6+1)-1}{i_7} \binom{r-1+i_1}{i_3} \frac{(log\alpha)^{i_6+i_{4+2}}}{i_6! \times i_4!} (i_3+1)^{i_4} \, (i_5+1)^{i_6}$$

$$\times \frac{1}{\left(i_7 + 1 - \frac{t_2}{\lambda}\right)} \mathrm{B}\left(\left(i_7 + 2 - \frac{t_1}{\lambda} - \frac{t_2}{\lambda}\right), \beta(i_4+1)\right)$$

Proof: Using the equation (8), we have

$$E[e^{t_1 X_{r:n} + t_2 X_{s:n}}]$$

$$= C_{r,s:n} \int_0^{\infty} \int_x^{\infty} e^{t_1 x} e^{t_2 y} [F(x)]^{r-1} [F(y) - F(x)]^{s-r-1} \; [1 - F(y)]^{n-s} f(x) f(y) dy dx$$

The result is obtained by using the lemma 4.

3.5. Survival function and hazard rate function of r^{th} order statistic

The survival function of the r^{th} order statistic is given by

$$S_{X_{r:n}}(x) = 1 - F_{X_{r:n}}(x), x > 0 \text{ and } 1 \leq r \leq n$$

$$= 1 - \sum_{i_1=r}^{n} \sum_{i_2=0}^{n-i_1} \sum_{i_3=0}^{i_1+i_2} (-1)^{i_1-i_3} \binom{n}{r} \binom{n-i_1}{i_2} \binom{i_1+i_2}{i_3} \frac{\alpha^{i_3 \left(1-e^{-\lambda x}\right)^{\beta}}}{(\alpha-1)^{i_1+i_2}}$$

and the corresponding hazard function of r^{th} order statistic is given by

$$h_{X_{r:n}}(x) = \frac{f_{X_{r:n}}(x)}{1 - F_{X_{r:n}}(x)} = \frac{f_{X_{r:n}}(x)}{S_{X_{r:n}}(x)}$$

The survival function and hazard function of r^{th} order statistics are shown in Figure 2.

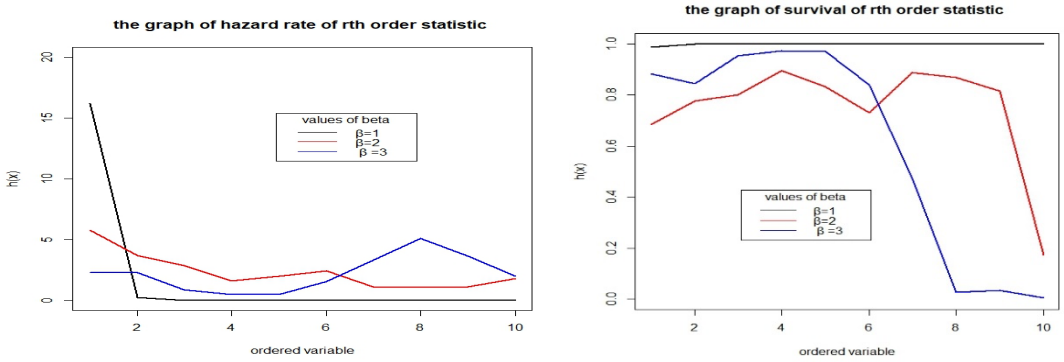

Figure 2. The Hazard rate function and Survival rate of r^{th} order statistic of $APTGE$ distribution for $\alpha = 1.5$. and $\lambda = 2$.

4. Estimation of the location (μ) and scale (σ) parameters

The relationships discovered in earlier sections enable us to assess the best linear unbiased estimates (BLUEs) of the location (mean) and scale (standard deviation) parameters based on the Type-II right censored samples. Let $X_{1:n} \leq X_{2:n} \leq X_{3:n} \leq \cdots \leq X_{n-c:n}$, $c = 0,1,2, \dots, n-1$ be the Type-II right censored sample from the location-scale parameter $APTGE$ distribution. Let us denote $Z_{r:n} = \frac{(X_{r:n}-\mu)}{\sigma}$, $E(Z_{r:n}) = \mu_{r:n}^{(1)}$, $1 \leq r \leq (n-c)$, and $Cov(Z_{r:n}, Z_{s:n}) = \sigma_{r,s:n} = \mu_{r,s:n}^{(1,1)} - \mu_{r:n}^{(1)}\mu_{s:n}^{(1)}$, $1 \leq r < s \leq (n-c)$.We use the following notations:

$$\boldsymbol{X} = (X_{1:n}, X_{2:n}, X_{3:n}, \dots, X_{n-c:n})^T$$

$$\mu = (\mu_{1:n}, \mu_{2:n}, \mu_{3:n}, \dots, \mu_{n-c:n})^T$$

$$\mathbf{1} = \underbrace{(1, 1, 1, \dots, 1)^T}_{n-c}$$

and

$$\sum = \left(\left(\sigma_{r,s:n}\right)\right), 1 \leq r < s \leq n-c.$$

The BLUEs of μ and σ are (see Balakrishnan and Cohen (1991), Sultan et al. (2000,2000a))

$$\mu^* = \left\{\frac{\mu^T\Sigma^{-1}\mu 1^T\Sigma^{-1} - \mu^T\Sigma^{-1}1\mu^T\Sigma^{-1}}{(\mu^T\Sigma^{-1}\mu)(1^T\Sigma^{-1}1) - (\mu^T\Sigma^{-1}1)^2}\right\}X = \sum_{r=1}^{n-c} a_r X_{r:n} \tag{12}$$

$$\sigma^* = \left\{\frac{1^T\Sigma^{-1}1\mu^T\Sigma^{-1} - 1^T\Sigma^{-1}\mu 1^T\Sigma^{-1}}{(\mu^T\Sigma^{-1}\mu)(1^T\Sigma^{-1}1) - (\mu^T\Sigma^{-1}1)^2}\right\}X = \sum_{r=1}^{n-c} b_r X_{r:n} \tag{13}$$

Now, These BLUEs variances and covariance are provided by

$$Var(\mu^*) = \sigma^2\left\{\frac{\mu^T\Sigma^{-1}\mu}{(\mu^T\Sigma^{-1}\mu)(1^T\Sigma^{-1}1) - (\mu^T\Sigma^{-1}1)^2}\right\} = \sigma^2 V_1 \tag{14}$$

$$Var(\sigma^*) = \sigma^2\left\{\frac{1^T\Sigma^{-1}1}{(\mu^T\Sigma^{-1}\mu)(1^T\Sigma^{-1}1) - (\mu^T\Sigma^{-1}1)^2}\right\} = \sigma^2 V_2 \tag{15}$$

And

$$Cov(\mu^*, \sigma^*) = \sigma^2 \left\{ \frac{-\mu^T \Sigma^{-1} 1}{(\mu^T \Sigma^{-1} \mu)(1^T \Sigma^{-1} 1) - (\mu^T \Sigma^{-1} 1)^2} \right\} = \sigma^2 V_3 \quad (16)$$

The coefficients of the BLUEs for Type II right censored samples with sample sizes $n = 7,10$, $\alpha = 0.5,1.5$, $\beta = 1$, $\lambda = 2$ and different censoring cases $c = 0(1)\left(\left[\frac{n}{2}\right] - 1\right)$ are shown in Table 4 and 5 and also satisfy these conditions

$$\sum_{i=1}^{n-c} a_i = 1$$

And

$$\sum_{i=1}^{n-c} b_i = 0$$

Example: We demonstrate the utility of the BLUEs coefficients in Table 4 and 5 by simulating a random of order statistics from the $APTGE$ distribution of size $n = 10, c = 0$ when $\mu = 0, \sigma = 1, \alpha = 1.5, \lambda = 2$ and $\beta = 1$ as: 0.166235, 0.1750422, 0.2381468, 0.293755, 0.528574, 0.581131, 0.65979, 0.8072376, 0.8195305, 1.822205. We calculate the BLUEs of μ and σ using Tables 4 and 5.

$$\mu^* = \sum_{r=1}^{n-c} a_r X_{r:n}$$

$$\begin{aligned} &= (1.095945 \times 0.166235) + (-0.006515395 \times 0.1750422) \\ &+ (-0.00781575 \times 0.2381468) + (-0.009023513 \times 0.293755) \\ &+ (0.01013073 \times 0.528574) + (-0.01112391 \times 0.581131) \\ &+ (-0.01200061 \times 0.65979) + (-0.0127112 \times 0.8072376) \\ &+ (-0.01322347 \times 0.8195305) + (-0.01340003 \times 1.822205) \end{aligned}$$

$$= 0.112792$$

and

$$\sigma^* = \sum_{r=1}^{n-c} b_r X_{r:n}$$

$$= (-1.724404 \times 0.166235) + (0.154536 \times 0.1750422) + (0.1677855 \times 0.2381468) + (0.1795619 \times 0.293755) + (0.1899056 \times 0.528574) + (0.1985239 \times 0.581131) + (0.2053057 \times 0.65979) + (0.2097824 \times 0.8072376) + (0.2112655 \times 0.8195305) + (0.2077375 \times 1.822205)$$

$$= 0.9053281$$

5. **Maximum likelihood estimation of unknown parameters (α, β, λ) based on Type-II censored sample**

In this Section, The maximum likelihood estimation (MLEs) of the unknown parameters (α, β, λ) of the distribution based on the Type-II censored sample. Let $X_{1:n} \leq X_{2:n} \leq X_{3:n} \leq \cdots \leq X_{r:n}$ be a Type-II random sample from (1), then the likelihood function can be written as

$$L = L(\underline{t}, \alpha, \beta, \lambda) = \frac{n!}{(n-r)!} \prod_{i=1}^{r} f(x_{i:n})[1 - F(x_{r:n})]^{n-r}$$

Where $x_{i:n}$ be the i^{th} order statistic and $0 < x_{1:n} < x_{2:n} < \cdots < x_{r:n} < \infty$

$$L = \frac{n!}{(n-r)!} \prod_{i=1}^{r} \left(\frac{\beta\lambda \log \alpha}{\alpha - 1} \, e^{-\lambda x_{in}} \left(1 - e^{-\lambda x_{i:n}}\right)^{\beta-1} \alpha^{\left(1-e^{-\lambda x_{i:n}}\right)^{\beta}} \right) \left[1 - \frac{\alpha^{\left(1-e^{-\lambda x_{r:n}}\right)^{\beta}} - 1}{\alpha - 1} \right]^{n-r}$$

The Log-likelihood function can be written as

$$\log(L) = \log(n!) - \log\left((n-r)!\right) + r\log(\beta) + \mathrm{r}\log(\lambda) + \mathrm{r}\log(\log(\alpha)) - \lambda \sum_{i=1}^{r} x_{i:n} + (\beta - 1) \sum_{i=1}^{r} \log\left(1 - e^{-\lambda x_{in}}\right) + \log(\alpha) \sum_{i=1}^{r} \left(1 - e^{-\lambda x_{in}}\right)^{\beta} - n\log(\alpha - 1) + (n-r)\log(\alpha - \alpha^{\left(\left(1-e^{-\lambda x_{r:n}}\right)^{\beta}\right)})$$

The MLE of α, β and λ are denoted by $\hat{\alpha}$, $\hat{\beta}$ and $\hat{\lambda}$ respectively can be obtained by solving these three non-linear equations

$$0 = \frac{\partial \log(L)}{\partial \alpha} = \frac{r}{\alpha \log \alpha} + \sum_{i=1}^{r} \frac{\left(1 - e^{-\lambda x_{i:n}}\right)^{\beta}}{\alpha} - \frac{n}{\alpha - 1} + (n-r) \times \left[\frac{1 - \alpha^{\left(1-e^{-\lambda x_{r:n}}\right)^{\beta}-1} \left(1 - e^{-\lambda x_{r:n}}\right)^{\beta}}{\alpha - \alpha^{\left(1-e^{-\lambda x_{r:n}}\right)^{\beta}}} \right] \quad (17)$$

$$0 = \frac{\partial \log(L)}{\partial \beta} = \frac{r}{\beta} + \sum_{i=1}^{r} \log\left(1 - e^{-\lambda x_{i:n}}\right) + \log(\alpha) \sum_{i=1}^{r} \left(1 - e^{-\lambda x_{i:n}}\right)^{\beta} \log\left(1 - e^{-\lambda x_{i:n}}\right) - (n-r)\left[\frac{\alpha^{\left(1-e^{-\lambda x_{r:n}}\right)^{\beta}} \left(1 - e^{-\lambda x_{r:n}}\right)^{\beta} \log\left(1 - e^{-\lambda x_{r:n}}\right) \log \alpha}{\alpha - \alpha^{\left(1-e^{-\lambda x_{r:n}}\right)^{\beta}}}\right] \quad (18)$$

$$0 = \frac{\partial \log(L)}{\partial \lambda} = \frac{r}{\lambda} - \sum_{i=1}^{r} x_{i:n} + (\beta - 1) \sum_{i=1}^{r} \frac{x_{i:n} e^{-\lambda x_{i:n}}}{\left(1 - e^{-\lambda x_{i:n}}\right)} + \beta \log(\alpha) \sum_{i=1}^{r} x_{i:n} e^{-\lambda x_{i:n}} \left(1 - e^{-\lambda x_{i:n}}\right)^{\beta - 1} - (n-r) \times \left[\frac{\beta\, \alpha^{\left(1-e^{-\lambda x_{r:n}}\right)^{\beta}} \left(1 - e^{-\lambda x_{r:n}}\right)^{\beta - 1} e^{-\lambda x_{r:n}} \log(\alpha)}{\alpha - \alpha^{\left(1-e^{-\lambda x_{r:n}}\right)^{\beta}}}\right] \quad (19)$$

6. Renyi entropy of Order Statistics

Entropy is used to measure the variation of the uncertainty of the random variable X. If X has the probability distribution function $f(.)$, then Renyi entropy defined as

$$H_{\tau}(x) = \frac{1}{1-\tau} \log\left(\int_{-\infty}^{\infty} \left(f(x)\right)^{\tau} dx\right), \tau > 0\,, \tau \neq 1$$

Now, The Renyi entropy of the r^{th} order statistic of the $APTGE$ is defined as

$$H_{\tau}(X_{r:n}) = \frac{1}{1-\tau} \log\left(\int_{0}^{\infty} \left(f_{X_{r:n}}(x)\right)^{\tau} dx\right), \tau > 0\,, \tau \neq 1$$

Where $f_{X_{r:n}}(x)$ is the pdf of r^{th} order statistic and $1 \leq r \leq n$.

$$H_{\tau}(X_{r:n}) = \frac{1}{1-\tau} \log\left(\int_{0}^{\infty} \left(C_{r:n}[F(x)]^{r-1}[1 - F(x)]^{n-r} f(x)\right)^{\tau} dx\right)$$

$$= \frac{1}{1-\tau} \log\left(\int_{-\infty}^{\infty} (C_{r:n})^{\tau} [F(x)]^{(r-1)\tau} [1 - F(x)]^{(n-r)\tau} \left(f(x)\right)^{\tau} dx\right)$$

$$= \frac{1}{1-\tau} \log\left(\int_{-\infty}^{\infty} (C_{r:n})^{\tau} \left[\frac{\alpha^{\left(1-e^{-\lambda x}\right)^{\beta}} - 1}{\alpha - 1}\right]^{(r-1)\tau} \left[1 - \frac{\alpha^{\left(1-e^{-\lambda x}\right)^{\beta}} - 1}{\alpha - 1}\right]^{(n-r)\tau} \times \left(\frac{\beta\lambda\, log\alpha}{\alpha - 1} e^{-\lambda x} \left(1 - e^{-\lambda x}\right)^{\beta - 1} \alpha^{\left(1-e^{-\lambda x}\right)^{\beta}}\right)^{\tau} dx\right)$$

$$= \frac{1}{1-\tau}\log\left((C_{r:n})^{\tau} \sum_{i_1=0}^{\tau(n-r)} \sum_{i_2=0}^{i_1+\tau(r-1)} \sum_{i_3=0}^{\tau(\beta-1)} (-1)^{\tau(r-1)-i_2+i_3} \binom{\tau(n-r)}{i_1} \binom{i_1+\tau(r-1)}{i_2} \right.$$
$$\left. \times \binom{\tau(\beta-1)}{i_3} \left[\frac{\beta\lambda\log(\alpha)}{\alpha-1}\right]^{\tau} \int_{-\infty}^{\infty} \frac{e^{-\lambda(i_3+\tau)x}}{(\alpha-1)^{i_1+\tau(r-1)}} \alpha^{(i_2+\tau)\left(1-e^{-\lambda x}\right)^{\beta}} dx \right)$$

$$= \frac{1}{1-\tau}\log\left((C_{r:n})^{\tau} \sum_{i_1=0}^{\tau(n-r)} \sum_{i_2=0}^{i_1+\tau(r-1)} \sum_{i_3=0}^{\tau(\beta-1)} \sum_{i_4=0}^{\infty} \sum_{i_5=0}^{\beta i_4} (-1)^{\tau(r-1)-i_2+i_3+i_5} \binom{\tau(n-r)}{i_1} \binom{i_1+\tau(r-1)}{i_2} \right.$$
$$\left. \times \binom{\tau(\beta-1)}{i_3} \binom{\beta i_4}{i_5} \left[\frac{\beta\lambda\log(\alpha)}{\alpha-1}\right]^{\tau} \frac{\left(\log(\alpha)\ (i_2+\tau)\right)^{i_4}}{i_4!\,(\alpha-1)^{i_1+\tau(r-1)}} \int_{-\infty}^{\infty} e^{-\lambda(i_3+i_5+\tau)x}\, dx \right)$$

$$= \frac{1}{1-\tau}\log\left((C_{r:n})^{\tau} \sum_{i_1=0}^{\tau(n-r)} \sum_{i_2=0}^{i_1+\tau(r-1)} \sum_{i_3=0}^{\tau(\beta-1)} \sum_{i_4=0}^{\infty} \sum_{i_5=0}^{\beta i_4} (-1)^{\tau(r-1)-i_2+i_3+i_5} \binom{\tau(n-r)}{i_1} \binom{i_1+\tau(r-1)}{i_2} \right.$$
$$\left. \times \binom{\tau(\beta-1)}{i_3} \binom{\beta i_4}{i_5} \left[\frac{\beta\lambda\log(\alpha)}{\alpha-1}\right]^{\tau} \frac{\left(\log(\alpha)\ (i_2+\tau)\right)^{i_4}}{i_4!\,(\alpha-1)^{i_1+\tau(r-1)}} \frac{1}{\lambda(i_3+i_5+\tau)} \right) \quad (20)$$

Put $r = 1$ in equation (20), we get

$$H_{\tau}(X_{1:n}) = \frac{1}{1-\tau}\log\left((n)^{\tau} \sum_{i_1=0}^{\tau(n-1)} \sum_{i_2=0}^{i_1} \sum_{i_3=0}^{\tau(\beta-1)} \sum_{i_4=0}^{\infty} \sum_{i_5=0}^{\beta i_4} (-1)^{-i_2+i_3+i_5} \binom{\tau(n-1)}{i_1} \binom{i_1}{i_2} \right.$$
$$\left. \times \binom{\tau(\beta-1)}{i_3} \binom{\beta i_4}{i_5} \left[\frac{\beta\lambda\log(\alpha)}{\alpha-1}\right]^{\tau} \frac{\left(\log(\alpha)\ (i_2+\tau)\right)^{i_4}}{i_4!\,(\alpha-1)^{i_1}} \frac{1}{\lambda(i_3+i_5+\tau)} \right)$$

and also put $r = n$ in equation (20), we get

$$H_{\tau}(X_{n:n}) = \frac{1}{1-\tau}\log\left((n)^{\tau} \sum_{i_2=0}^{\tau(n-1)} \sum_{i_3=0}^{\tau(\beta-1)} \sum_{i_4=0}^{\infty} \sum_{i_5=0}^{\beta i_4} (-1)^{\tau(n-1)-i_2+i_3+i_5} \binom{\tau(n-1)}{i_2} \right.$$
$$\left. \times \binom{\tau(\beta-1)}{i_3} \binom{\beta i_4}{i_5} \left[\frac{\beta\lambda\log(\alpha)}{\alpha-1}\right]^{\tau} \frac{\left(\log(\alpha)\ (i_2+\tau)\right)^{i_4}}{i_4!\,(\alpha-1)^{\tau(n-1)}} \frac{1}{\lambda(i_3+i_5+\tau)} \right)$$

Table 7 shows that the Renyi entropy of the n^{th}(largest) order statistics is always more than the Renyi entropy of the 1^{st} order statistic in $APTGE$ distribution.

7. Numerical results

7.1. Tabulation of Means, Variances, Covariances, Coefficient of BLUEs of the location and Scale Parameters and Variances and Covariances of the BLUEs

We can evaluate the 1^{st} order moments (expected values), 2^{nd} order moments, product moments, variances, and covariances of order statistics from samples of sizes up to 10 for different values of the parameter using the relationships derived in the preceding sections. For sample sizes $n = 1,\ldots,10$. the relation in (9) can be utilized to obtain the 1^{st} order moments, 2^{nd} order moments, and variances of all order statistics. In Tables 1 and 2, the 1^{st} order moments, 2^{nd} order moments and variance of the r^{th} order statistic from the $APTGE$ distribution have been shown for different values for n and different values for $\alpha, \beta,$ and λ. It is clear that the mean and variances are decreasing with respect to n and increasing with respect to α. The product moments and covariances of the r^{th} and s^{th} order statistics form the $APTGE$ distribution for $n = 1, \ldots,10$ and $\alpha = 0.5,1.5$ and $\beta = 1$, $\lambda = 2$. Tables 3 shows that product moments are decreasing with respect to n but increasing with respect to α. Tables 4 and 5 exhibit the coefficients of the BLUEs for Type-II right censored samples of sample sizes $n = 7,10$, $\alpha = 0.5,1.5$, $\beta = 1$, $\lambda = 2$, and different censoring cases $c = 0(1)\left(\left[\frac{n}{2}\right] - 1\right)$. We see that the variances of the BLUEs are directly proportional to the values of α and censoring level and We also see that the covariances of the BLUEs are inversely proportional to the censoring level, while the covariances of the BLUEs are directly proportional to the parameter α in Table 6. In Table 7, we see that the Renyi entropy of order statistics is increasing and decreasing, when α, β increase and λ decreasing respectively. In Table 8, we obtained the average value MLEs of the Type-II censored sample by simulation. R software was used to do the computations in this section.

Table 1. Expected values, second moments and variance of the r^{th} order statistic from $APTGE$ distribution for $n = 1, \ldots,10$, $\alpha = 0.5, \beta = 1, \lambda = 2$ (Sim.= simulated)

r	n	$E(X)$	$Sim.E(X)$	$E(X^2)$	$Sim.E(X^2)$	$V(X)$	$Sim.V(X)$
1	1	0.417231	0.405396	0.380015	0.383766	0.205933	0.219420
	2	0.197405	0.193592	0.083721	0.083993	0.044752	0.046515
	3	0.128291	0.128545	0.034832	0.036085	0.018373	0.019585
	4	0.094836	0.097748	0.018834	0.018494	0.009840	0.008939
	5	0.075171	0.073062	0.011744	0.011161	0.006094	0.005823
	6	0.062242	0.061995	0.008007	0.008292	0.004134	0.004449
	7	0.053101	0.052733	0.005804	0.005872	0.002985	0.003091
	8	0.046296	0.045025	0.004397	0.004639	0.002254	0.002611
	9	0.041036	0.041255	0.003446	0.003456	0.001763	0.001754
	10	0.036848	0.036262	0.002773	0.002895	0.001415	0.001581
2	2	0.637054	0.626880	0.676309	0.674185	0.270470	0.281207
	3	0.335639	0.336716	0.181500	0.173475	0.068847	0.060097
	4	0.228652	0.227857	0.082825	0.082470	0.030543	0.030551
	5	0.173501	0.173996	0.047192	0.046971	0.017090	0.016697

	6	0.139814	0.140506	0.030427	0.030029	0.010879	0.010287
	7	0.117092	0.116892	0.021231	0.021479	0.007520	0.007816
	8	0.100727	0.100339	0.015649	0.015984	0.005503	0.005916
	9	0.088377	0.089859	0.012010	0.011805	0.004199	0.003730
	10	0.078727	0.079007	0.009507	0.009901	0.003309	0.003659
3	3	0.787762	0.793063	0.923713	0.935122	0.303144	0.306174
	4	0.442625	0.441026	0.280175	0.283218	0.084258	0.088714
	5	0.311378	0.318319	0.136274	0.134648	0.039317	0.033321
	6	0.240875	0.238399	0.080722	0.079959	0.022701	0.023125
	7	0.196620	0.196052	0.053419	0.053771	0.014760	0.015334
	8	0.166187	0.166857	0.037975	0.037663	0.010357	0.009821
	9	0.143949	0.144968	0.028386	0.027558	0.007664	0.006543
	10	0.126981	0.127426	0.022024	0.021829	0.005899	0.005591
4	4	0.902808	0.900378	1.138226	1.098241	0.323164	0.287561
	5	0.530129	0.538860	0.376109	0.385314	0.095073	0.094944
	6	0.381881	0.384165	0.191825	0.193329	0.045992	0.045747
	7	0.299882	0.300744	0.117126	0.114720	0.027197	0.024273
	8	0.247343	0.246583	0.079161	0.080514	0.017982	0.019711
	9	0.210661	0.210033	0.057152	0.057975	0.012774	0.013861
	10	0.183544	0.184641	0.043231	0.044148	0.009543	0.010056
5	5	0.995978	1.001015	1.328755	1.354382	0.336781	0.352351
	6	0.604245	0.606592	0.468252	0.467794	0.103139	0.099840
	7	0.443381	0.444304	0.247849	0.246628	0.051263	0.049221
	8	0.352421	0.353286	0.155092	0.152733	0.030892	0.027922
	9	0.293196	0.292326	0.106671	0.106176	0.020707	0.020721
	10	0.251337	0.248686	0.078034	0.075922	0.014863	0.014077
6	6	1.074326	1.089427	1.500856	1.499587	0.346680	0.312736
	7	0.668591	0.671146	0.556413	0.552122	0.101940	0.101685
	8	0.497957	0.497871	0.303503	0.292116	0.055542	0.044241
	9	0.399799	0.397973	0.193829	0.192418	0.033989	0.034035
	10	0.335054	0.336446	0.135308	0.139932	0.023047	0.026736
7	7	1.419480	1.137825	1.658263	1.657965	0.354218	0.363319
	8	0.725469	0.731575	0.640716	0.653332	0.114410	0.118131
	9	0.547035	0.548583	0.358341	0.363553	0.059093	0.062610
	10	0.442962	0.446982	0.232842	0.227747	0.036626	0.027955
8	8	1.201445	1.204409	1.803627	1.820821	0.360157	0.370220
	9	0.776450	0.779592	0.721395	0.733638	0.118520	0.125875
	10	0.591638	0.590536	0.412126	0.405749	0.062090	0.057016
9	9	1.254569	1.231408	1.938906	1.948127	0.364963	0.431761
	10	0.822653	0.825311	0.798712	0.794814	0.121954	0.113576
10	10	1.302560	1.301200	2.065594	2.063950	0.368931	0.370829

Table 2. Expected values, second moments and variance of the r^{th} order statistic from $APTGE$ distribution for $n = 1, \ldots, 10$, $\alpha = 1.5, \beta = 1, \lambda = 2$ (Sim. = simulated)

r	n	$E(X)$	$Sim.E(X)$	$E(X^2)$	$Sim.E(X^2)$	$V(X)$	$Sim.V(X)$
1	1	0.551706	0.556352	0.579124	0.579515	0.274745	0.269987
	2	0.285447	0.286325	0.155608	0.156598	0.074128	0.076155
	3	0.193713	0.193240	0.072087	0.072335	0.034562	0.034993
	4	0.146892	0.145026	0.041658	0.041694	0.020081	0.020066
	5	0.118399	0.116711	0.027174	0.027457	0.013155	0.013836
	6	0.099206	0.098309	0.019139	0.018321	0.009298	0.008657
	7	0.085388	0.085127	0.014217	0.014269	0.006925	0.007023
	8	0.074959	0.075575	0.010980	0.010653	0.005361	0.004942
	9	0.066807	0.064954	0.008738	0.008635	0.004275	0.004416
	10	0.060257	0.060187	0.007120	0.007229	0.003489	0.003607
2	2	0.817964	0.804949	1.002639	1.014649	0.333574	0.366706
	3	0.468916	0.460845	0.322649	0.318441	0.102767	0.106062
	4	0.334177	0.341337	0.163373	0.158187	0.051698	0.041676
	5	0.260864	0.260075	0.099598	0.102154	0.031548	0.034515
	6	0.214364	0.214597	0.067343	0.068698	0.021392	0.022647
	7	0.182115	0.182239	0.048677	0.047243	0.015511	0.014032
	8	0.158388	0.157244	0.036872	0.036913	0.011785	0.012187
	9	0.140178	0.140801	0.028919	0.029374	0.010269	0.009596
	10	0.125751	0.123758	0.023300	0.022989	0.007486	0.007673
3	3	0.992488	0.989352	1.342635	1.336874	0.357603	0.358057
	4	0.603654	0.606260	0.481925	0.492161	0.117530	0.124609
	5	0.444148	0.449008	0.259035	0.260819	0.061768	0.054474
	6	0.353865	0.350573	0.164109	0.163512	0.038888	0.040611
	7	0.294985	0.294710	0.114010	0.115391	0.026993	0.028537
	8	0.253295	0.253000	0.084092	0.084273	0.019934	0.020263
	9	0.222123	0.222550	0.064708	0.065260	0.015369	0.015731
	10	0.197888	0.195002	0.051394	0.051055	0.012234	0.013030
4	4	1.122099	1.119964	1.629538	1.621112	0.370432	0.366793
	5	0.709991	0.715283	0.630518	0.638382	0.126432	0.126752
	6	0.534430	0.539049	0.353596	0.359218	0.068345	0.068645
	7	0.432372	0.434450	0.230907	0.227267	0.043962	0.038521
	8	0.364470	0.362264	0.163873	0.167670	0.031034	0.036435
	9	0.315639	0.316408	0.122861	0.122121	0.023236	0.022007
	10	0.278671	0.278337	0.095573	0.095465	0.018116	0.017993
5	5	1.225126	1.218751	1.879293	1.885254	0.378359	0.399900
	6	0.797771	0.805582	0.768797	0.774568	0.132358	0.125606

	7	0.610974	0.611691	0.446251	0.442906	0.072962	0.068740
	8	0.500273	0.499748	0.297942	0.293439	0.047668	0.043691
	9	0.425509	0.426488	0.215138	0.211098	0.034066	0.029207
	10	0.371091	0.373338	0.163492	0.163724	0.025783	0.024342
6	6	1.310597	1.309870	2.101392	2.143314	0.383728	0.427555
	7	0.842490	0.870540	0.897816	0.899165	0.136577	0.141326
	8	0.677394	0.679867	0.535237	0.539071	0.076373	0.076851
	9	0.560084	0.561837	0.364184	0.365159	0.050490	0.049498
	10	0.479926	0.476682	0.266785	0.263915	0.036455	0.036689
7	7	1.383615	1.388861	2.301988	2.368741	0.387598	0.439806
	8	0.937522	0.934025	1.018676	1.018620	0.139728	0.146218
	9	0.736049	0.739953	0.620763	0.619405	0.078994	0.071874
	10	0.613523	0.613567	0.429117	0.422539	0.052707	0.046075
8	8	1.447343	1.444640	2.485319	2.479057	0.390517	0.392072
	9	0.995086	1.006128	1.132365	1.139160	0.142169	0.126866
	10	0.788561	0.788808	0.702897	0.712470	0.081069	0.090251
9	9	1.503875	1.506456	2.654438	2.677355	0.392798	0.407953
	10	1.046717	1.043355	1.239732	1.245696	0.144116	0.157106
10	10	1.554670	1.559875	2.811627	2.848963	0.394628	0.415753

Table 3. Product moments and Covariances of order statistics for $n = 2, \dots, 10$ and $\alpha = 0.5, 1.5$, $\beta = 1$ and $\lambda = 2$

$\boldsymbol{\alpha}$	$\boldsymbol{s}$	$\boldsymbol{r}$	$\boldsymbol{n}$	$\boldsymbol{\mu_{r,s:n}}$	$\boldsymbol{\sigma_{r,s:n}}$	$\boldsymbol{\alpha}$	$\boldsymbol{s}$	$\boldsymbol{r}$	$\boldsymbol{n}$	$\boldsymbol{\mu_{r,s:n}}$	$\boldsymbol{\sigma_{r,s:n}}$
0.5	2	1	2	0.174081	0.048324	1.5	2	1	2	0.304379	0.070893
			3	0.062572	0.019512				3	0.124072	0.033237
			4	0.032014	0.010330				4	0.068491	0.019703
			5	0.019387	0.006345				5	0.043646	0.012760
			6	0.012981	0.004279				6	0.030312	0.009023
			7	0.009293	0.003075				7	0.022306	0.006756
			8	0.006978	0.002315				8	0.017113	0.005241
			9	0.005431	0.001805				9	0.013551	0.004186
			10	0.004347	0.001444				10	0.010999	0.003422
	3	1	3	0.121949	0.020403		3	1	3	0.004304	0.032046
			4	0.052879	0.010902				4	0.107458	0.018786
			5	0.030039	0.006632				5	0.064983	0.012397
			6	0.019434	0.004442				6	0.043919	0.008814
			7	0.013617	0.003176				7	0.031785	0.006597
			8	0.010075	0.002381				8	0.024115	0.005128
			9	0.007758	0.001851				9	0.018942	0.004103
			10	0.006155	0.001476				10	0.015283	0.003358

	2	3	0.337724	0.073320		2	3	0.564761	0.099368
		4	0.133379	0.032194			4	0.251849	0.050121
		5	0.071871	0.017846			5	0.146535	0.030673
		6	0.044965	0.011288			6	0.096706	0.020851
		7	0.030786	0.007764			7	0.068873	0.015152
		8	0.022399	0.005660			8	0.051652	0.011533
		9	0.017028	0.004306			9	0.040222	0.009085
		10	0.013381	0.003384			10	0.032233	0.007349
4	1	4	0.097198	0.115785	4	1	4	0.183050	0.018223
		5	0.046813	0.006963			5	0.096124	0.012062
		6	0.028395	0.004626			6	0.061616	0.008598
		7	0.019212	0.003288			7	0.043368	0.006449
		8	0.013905	0.002454			8	0.032342	0.005022
		9	0.010545	0.001901			9	0.025111	0.004024
		10	0.008286	0.001523			10	0.020090	0.003298
	2	4	0.240518	0.049650		2	4	0.423658	0.048678
		5	0.110693	0.018715			5	0.215076	0.029865
		6	0.065141	0.011748			6	0.134910	0.020348
		7	0.043149	0.008035			7	0.093557	0.014816
		8	0.030746	0.005832			8	0.069025	0.013484
		9	0.023040	0.004422			9	0.053158	0.008912
		10	0.017916	0.003466			10	0.042261	0.007218
	3	4	0.488499	0.088893		3	4	0.791768	0.114409
		5	0.206230	0.041159			5	0.375548	0.060208
		6	0.115594	0.023608			6	0.227090	0.037974
		7	0.074231	0.015268			7	0.153948	0.026405
		8	0.051774	0.010669			8	0.111849	0.019530
		9	0.038194	0.007869			9	0.085190	0.015079
		10	0.029347	0.006041			10	0.067164	0.012085
5	1	5	0.082217	0.007349	5	1	5	0.156806	0.011753
		6	0.042445	0.004836			6	0.087540	0.008396
		7	0.026957	0.003414			7	0.058480	0.006310
		8	0.018851	0.002535			8	0.042422	0.004922
		9	0.013987	0.001955			9	0.032376	0.003950
		10	0.010797	0.001536			10	0.025602	0.003241
	2	5	0.192530	0.019976		2	5	0.348708	0.029117
		6	0.096755	0.012273			6	0.190892	0.019879
		7	0.060255	0.008339			7	0.125768	0.014501
		8	0.041590	0.006092			8	0.090312	0.011074
		9	0.030461	0.004549			9	0.068396	0.008749
		10	0.023341	0.003554			10	0.053759	0.007094
	3	5	0.353419	0.043292		3	5	0.602898	0.058761
		6	0.170184	0.024637			6	0.319425	0.037122
		7	0.103014	0.015837			7	0.206082	0.025854

		8	0.069581	0.011014			8	0.145867	0.019150
		9	0.050299	0.008094			9	0.109320	0.014805
		10	0.038109	0.006194			10	0.085248	0.011813
	4	5	0.627615	0.099618		4	5	0.099346	0.123636
		6	0.278669	0.047919			6	0.493228	0.066875
		7	0.161149	0.028187			7	0.307235	0.043150
		8	0.105724	0.019933			8	0.212776	0.030441
		9	0.074899	0.013135			9	0.157123	0.022815
		10	0.055915	0.009784			10	0.121222	0.017810
6	1	6	0.071944	0.005076	6	1	6	0.138228	0.008209
		7	0.039058	0.003556			7	0.080680	0.006180
		8	0.025679	0.002625			8	0.055605	0.004828
		9	0.018422	0.002016			9	0.041297	0.003879
		10	0.013963	0.008094			10	0.032106	0.003187
	2	6	0.163080	0.012874		2	6	0.300386	0.019442
		7	0.086968	0.008681			7	0.173100	0.014206
		8	0.056392	0.006235			8	0.118156	0.010864
		9	0.040022	0.004688			9	0.087105	0.008594
		10	0.030027	0.003649			10	0.067327	0.006967
	3	6	0.284592	0.025813		3	6	0.500997	0.036325
		7	0.147939	0.016480			7	0.282707	0.025336
		8	0.094151	0.011098			8	0.190372	0.018792
		9	0.065891	0.008340			9	0.138953	0.014545
		10	0.048906	0.006360			10	0.106590	0.011619
	4	6	0.046038	0.050116		4	6	0.765921	0.065499
		7	0.229797	0.029300			7	0.419466	0.042226
		8	0.142357	0.019190			8	0.276771	0.029881
		9	0.097752	0.013530			9	0.199205	0.022420
		10	0.071543	0.010046			10	0.151260	0.017519
	5	6	0.756660	0.107504		5	6	1.175409	0.129852
		7	0.349647	0.053207			7	0.604665	0.071597
		8	0.207416	0.031926			8	0.385696	0.046814
		9	0.138541	0.021321			9	0.271821	0.033500
		10	0.099469	0.015257			10	0.203464	0.025367
7	1	7	0.064354	0.003717	7	1	7	0.124202	0.006058
		8	0.036312	0.002726			8	0.075015	0.004739
		9	0.024531	0.002082			9	0.052985	0.003812
		10	0.017947	0.001624			10	0.040105	0.003136
	2	7	0.142782	0.009069		2	7	0.265904	0.013927
		8	0.079545	0.006471			8	0.159158	0.010666
		9	0.053188	0.004842			9	0.111625	0.008447
		10	0.038631	0.003758			10	0.084015	0.006864
	3	7	0.241731	0.017201		3	7	0.432994	0.024848
		8	0.132388	0.011824			8	0.255922	0.018453

		9	0.087357	0.008611			9	0.177792	0.014299
		10	0.062792	0.006544			10	0.132842	0.011433
	4	7	0.373005	0.030556		4	7	0.639669	0.041434
		8	0.199339	0.019899			8	0.371050	0.029351
		9	0.129204	0.013965			9	0.254371	0.022045
		10	0.091634	0.010331			10	0.188213	0.017242
	5	7	0.561715	0.055395		5	7	0.915661	0.070308
		8	0.288477	0.033077			8	0.515022	0.046005
		9	0.182386	0.021998			9	0.346145	0.032950
		10	0.127020	0.015687			10	0.252645	0.024972
	6	7	0.877052	0.113555		6	7	1.341508	0.134318
		8	0.418720	0.057467			8	0.710182	0.075109
		9	0.253747	0.035043			9	0.461931	0.049681
7	6	10	0.172103	0.023687	7	6	10	0.330342	0.035897
8	1	8	0.058460	0.002838	8	1	8	0.113146	0.004640
		9	0.034019	0.002156			9	0.070227	0.003749
		10	0.023502	0.001701			10	0.050603	0.003887
	2	8	0.127755	0.006737		2	8	0.239720	0.010479
		9	0.073634	0.005013			9	0.147797	0.008307
		10	0.050446	0.003868			10	0.105919	0.006757
	3	8	0.211968	0.012304		3	8	0.384736	0.018132
		9	0.120682	0.008912			9	0.235096	0.014065
		10	0.081870	0.006743			10	0.167303	0.011257
	4	8	0.317862	0.020693		4	8	0.556362	0.028849
		9	0.178016	0.014448			9	0.335777	0.021689
		10	0.119239	0.010648			10	0.236727	0.016978
	5	8	0.457779	0.034365		5	8	0.769304	0.045238
		9	0.250398	0.022746			9	0.455844	0.032426
		10	0.167858	0.016158			10	0.317222	0.024594
	6	8	0.657884	0.059616		6	8	1.054331	0.073909
		9	0.346631	0.036206			9	0.606244	0.048912
		10	0.222618	0.024387			10	0.413815	0.035364
	7	8	0.989968	0.118357		7	8	1.494592	0.137676
		9	0.485725	0.060979			9	0.810255	0.077823
		10	0.299758	0.037684			10	0.535744	0.051944
9	1	9	0.053721	0.002239	9	1	9	0.104158	0.003689
		10	0.032059	0.001746			10	0.061124	0.003040
	2	9	0.116079	0.005204		2	9	0.218985	0.008175
		10	0.068764	0.003999			10	0.138281	0.006656
	3	9	0.189842	0.009248		3	9	0.347887	0.013843
		10	0.111422	0.006960			10	0.218221	0.011089
	4	9	0.279274	0.015585		4	9	0.496031	0.021350
		10	0.161982	0.010989			10	0.308416	0.016727
	5	9	0.391412	0.023578		5	9	0.671840	0.031928

		10	0.223438	0.014206			10	0.412662	0.024234
	6	9	0.539075	0.037499		6	9	0.890745	0.048178
		10	0.300789	0.025156			10	0.537202	0.034855
	7	9	0.749362	0.063068		7	9	1.183631	0.076705
		10	0.403249	0.038845			10	0.693401	0.051216
	8	9	1.096375	0.122265		8	9	1.636778	0.140294
		10	0.550641	0.063928			10	0.905380	0.079980
10	1	10	0.049809	0.001812	10	1	10	0.096676	0.002995
	2	10	0.106686	0.004140		2	10	0.202059	0.006559
	3	10	0.172609	0.007208		3	10	0.318578	0.010928
	4	10	0.250447	0.011370		4	10	0.449427	0.016487
	5	10	0.344630	0.017248		5	10	0.600815	0.023890
	6	10	0.462432	0.026004		6	10	0.780459	0.034368
	7	10	0.617109	0.040124		7	10	1.004345	0.050502
	8	10	0.836593	0.065949		8	10	1.304888	0.078937
10	9	10	1.197065	0.125510	10	9	10	1.769688	0.142389

Table 4. Coefficient of the BLUEs of the location parameter

$\boldsymbol{\alpha}$	n	$\boldsymbol{c}$	$\boldsymbol{a_i}$	$i = 1, \ldots, n - c$					
0.5	7	0	1.178745	-0.038835	-0.034618	-0.030611	-0.027018	-0.051443	0.003779
		1	1.173208	-0.037716	-0.033606	-0.029697	-0.026184	-0.046005	
		2	1.144621	0.123957	-0.129269	-0.082857	-0.056451		
	10	0	1.105527	-0.017912	-0.009782	-0.023530	-0.000331	-0.020884	-0.005418
			-0.010879	-0.008385	-0.008408				
		1	1.117465	-0.019573	-0.011321	-0.025076	-0.001561	-0.022239	-0.006540
			-0.012010	-0.019145					
		2	1.132523	-0.021891	-0.013114	-0.026957	-0.003057	-0.023908	-0.007916
			-0.035680						
		3	1.152751	-0.023946	-0.015696	-0.029671	-0.005107	-0.026217	-0.052114
		4	1.180453	-0.028958	-0.018815	-0.032911	-0.007644	-0.092124	
1.5	7	0	1.140886	-0.016730	-0.020060	-0.023010	-0.025496	-0.027366	-0.028224
		1	1.171255	-0.021116	-0.024867	-0.028158	-0.030882	-0.066233	
		2	1.148560	0.194900	-0.192092	-0.027230	-0.064139		
	10	0	1.095945	-0.006515	-0.007816	-0.009024	-0.010131	-0.011124	-0.012001
			-0.012711	-0.013223	-0.013400				
		1	1.109211	-0.007722	-0.009125	-0.010424	-0.011610	-0.012669	-0.013594
			-0.014331	-0.029738					
		2	1.126303	-0.009294	-0.010837	-0.012248	-0.013538	-0.014677	-0.015664
			-0.050047						
		3	1.148992	-0.011414	-0.013126	-0.014701	-0.016121	-0.017369	-0.076261
		4	1.180531	-0.014386	-0.016349	-0.018139	-0.019746	-0.111911	

Table 5. Coefficient of the BLUEs of the Scale parameter

α	n	c	b_i	$i = 1, \ldots, n-c$					
0.5	7	0	-3.271138	0.677667	0.611474	0.550354	0.498372	1.009043	-0.075771
		1	-3.160129	0.655242	0.591197	0.532034	0.481649	0.900079	
		2	-2.699495	-2.357091	2.426312	1.560539	1.069735		
	10	0	-2.690981	0.392263	0.349291	0.359006	0.281710	0.311082	0.255547
			0.254856	0.240932	0.246294				
		1	-3.040687	0.440926	0.394382	0.404299	0.317754	0.350783	0.288417
			0.287993	0.556133					
		2	-3.478129	0.508268	0.446466	0.458945	0.361209	0.399277	0.328374
			0.975591						
		3	-4.031221	0.564449	0.517052	0.533151	0.417257	0.462419	1.536892
		4	-4.848149	0.712265	0.609034	0.628698	0.492092	2.406060	
1.5	7	0	-1.750535	0.248308	0.272584	0.292633	0.307597	0.315949	0.313474
		1	-2.087839	0.297018	0.325974	0.349799	0.367417	0.747632	
		2	-1.759661	-2.264413	2.258791	1.020234	0.745049		
	10	0	-1.724404	0.154536	0.167786	0.179562	0.189906	0.198524	0.205306
			0.209782	0.211266	0.207738				
		1	-1.930077	0.173234	0.188076	0.201281	0.212832	0.222469	0.230002
			0.234893	0.467290					
		2	-2.198638	0.197944	0.214981	0.229934	0.243132	0.254037	0.262494
			0.796115						
		3	-2.559560	0.231674	0.251396	0.268962	0.284218	0.296845	1.226466
		4	-3.066792	0.279466	0.303224	0.324252	0.342528	1.817322	

Table 6. Variances and covariance of the BLUEs when $\mu = 0$ and $\sigma = 1$, $\beta = 1$ and $\lambda = 2$

α	n	c	$Var(\mu^*)$	$Var(\sigma^*)$	$Cov(\mu^*, \sigma^*)$
0.5	10	0	0.001555	0.129563	-0.004301
		1	0.001574	0.145946	-0.004860
		2	0.001598	0.165652	-0.005539
		3	0.001632	0.191281	-0.006476
		4	0.001671	0.225472	-0.007635
1.5	10	0	0.003920	0.101392	-0.006637
		1	0.003971	0.113679	-0.007430
		2	0.004037	0.129957	-0.008466
		3	0.004124	0.152123	-0.009859
		4	0.004246	0.183650	-0.011819

Table 7. Numerical value of Renyi entropy for different value of parameters and $\tau = 2$.

α	β	λ	n	r	$H_\tau(X_{r:n})$
0.5	1	1	5	1	-1.221920
				2	-0.365278
				3	0.128146
				4	0.592574
				5	1.199999
1.5	1	1	5	1	-0.730105
				2	0.037491
				3	0.439055
				4	0.807705
				5	1.312622
1.5	2	1	5	1	0.089153
				2	0.396875
				3	0.636541
				4	0.913351
				5	1.357258
0.5	1	1.5	5	1	-1.627385
				2	-0.770742
				3	-0.277315
				4	0.187112
				5	0.794534

Table 8. Average values of estimates (MLEs) based on Type-II censored sample

Parameters					MLEs		
α	β	λ	n	r	$\hat{\alpha}$	$\hat{\beta}$	$\hat{\lambda}$
0.5	2	2	5	1	0.569855	2.102557	2.175311
				2	0.523621	2.178077	2.194435
				3	0.534477	2.174255	2.180113
				4	0.567816	2.068224	2.133215
				5	0.512030	2.023805	2.134025
1.1	1	1	5	1	1.005099	1.000181	1.918466
				2	1.169524	1.034826	1.767857
				3	1.119852	1.153028	1.400925
				4	1.192071	0.971110	1.288186

				5	1.159180	1.223168	1.198659
1.5	2	2	20	1	1.595542	2.087557	2.364850
				2	1.470647	2.094314	2.394362
				3	1.441310	2.118301	2.308546
				4	1.467177	2.113624	2.217646
				5	1.615459	2.126588	2.194092
				6	1.497460	2.116197	2.121607
				7	1.508776	2.118124	2.115592
				8	1.594838	2.124971	2.126780
				9	1.545933	2.111903	2.097218
				10	1.588309	2.090980	2.115659
				11	1.509790	2.113737	2.108982
				12	1.554149	2.107182	2.089215
				13	1.543652	2.092056	2.090595
				14	1.558771	2.097415	2.064608
				15	1.492287	2.107501	2.058084
				16	1.456122	2.127646	2.060692
				17	1.520278	2.102482	2.065416
				18	1.414203	2.117304	2.061259
				19	1.533219	2.125553	2.044621
				20	1.547710	2.121653	2.049143

8. Real data analysis

In this section, we use the model of daily ozone measurements in New York from May to September of 1973. Nadarajah (2008) and Vilca et al. (2011) also discuss these data. By using the $APTGE$ distribution in Eq. (1) for this data, we have three values for the maximum likelihood estimate of the parameters: $\hat{\alpha} = 0.437556$, $\hat{\beta} = 1.915636$, and $\hat{\lambda} = 0.030028$. The Kolmogorov-Smirnov (K-S) tests also verify this conclusion; KS statistic is 0.07512516 and p-value is 0.9999887. Figure 3 shows the Q-Q plot of the sample. This real data demonstrates the appropriateness of the $APTGE$ distribution.

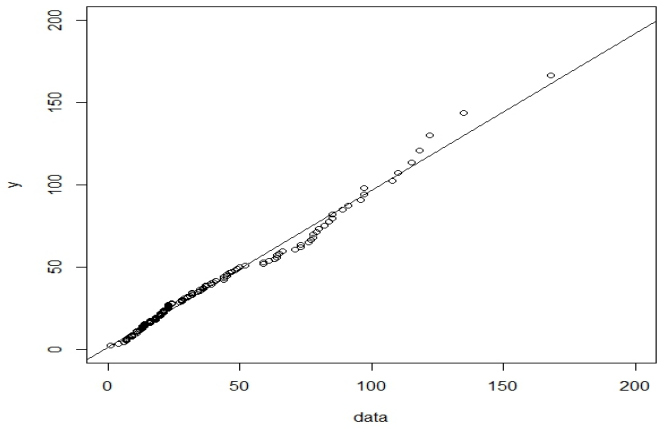

Figure 3. The QQ plot of the sample

9. Conclusion

The $APTGE$ distribution model has one scale and two shape parameters. Depending on the shape parameters, the $APTGE$ density function can take various forms. Furthermore, depending on its shape parameters, the $APTGE$ distribution failure rate function can have one of four forms: 1) rising, 2) falling, 3) bathtub and 4) upside down bathtub shaped. As a result, it may be utilised to analyse lifespan data rather well. The single and product moments are obtained in this research study. The variances and BLUEs of the position (location) and scale parameters were calculated using single and product moments of order statistics. Some of the features will be handled in the future, as our goal is to increase the reach and scope of this distribution.

Data availability

The data used to support the findings of this study are included within the manuscript.

Conflicts of interest

The author declare that there are no conflicts of interest regarding the publication of this paper.

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Ethical Statement

The author affirms that all procedures followed in this article to ethical and scientific standards.

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