Research Article **Phytopharmacology Research Journal (PRJ) Open Access**

Received: 17 Jan 2024 | Revised: 30 Jan 2024 | Accepted: 31 Jan 2024 | Published online : Date 31 Jan 2024 | ISSN : 2959-9687

Qualitative Phytochemical Analysis and *In Vitro* Antibacterial Activity of *Punica Granatum*

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Funding information
None

Abstract

Plant materials remain an essential tool in the global battle against serious diseases. In underdeveloped nations, conventional medical practices particularly the use of medicinal plants remain essential for meeting basic health needs. Certain bioactive compounds that have different physiological effects on the human body are thought to be responsible for these plants' medicinal value. Notable bioactive components found in plants include tannins, alkaloids, flavonoids, and phenolic compounds. Recent years have seen a marked increase in antibiotic resistance due to the rising incidence of infections, which presents a more difficult therapeutic problem. Higher plant-derived natural products may contain antimicrobial agents with distinct modes of action. Plant-derived compounds have demonstrated efficiency in treating infectious diseases, with minimal side effects frequently associated with alongside. The current investigation aimed to explore the phytochemical profile and in vitro antibacterial efficacy of *Punica granatum*. Phytochemical analysis revealed the presence of flavonoids, tannins, saponins, quinones, proteins, phenols, alkaloids, and carbohydrates. *In vitro*, antibacterial assessments were conducted using the agar well diffusion methods. The findings from this study suggest the potential utility of *Punica granatum* in the treatment of diseases caused by the tested organisms.

KEYWORDS

Punica granatum, phytochemistry, phytochemical screening, *in vitro* antibacterial activity

1.0 INTRODUCTION

Punica represents a genus of deciduous shrubs or trees known for bearing fruits. Among its recognized species, the pomegranate (Punica granum L.) stands out as the most historically significant fruit tree, a member of the Punicaceae family, and locally referred to as Anar [1]. Cultivation of pomegranate has a longstanding history in the Middle East and has been practiced for numerous years in regions such as Iran, Afghanistan, India, and other Mediterranean countries [2].

The plant is either a tree or shrub, reaching a height of 5 meters. Its branches are cylindrical, arranged oppositely, and often terminate in spines. The leaves, which are glossy and hairless [3]. They are somewhat stalked, with a smooth margin, and a slightly pointed to obtuse tip. The flowers are striking, either scarlet red or white, and are substantial, exceeding 3 cm in length [4]. The calyx is reddish, slightly indented above the middle, and fleshy, with 5-7 lobes

measuring approximately 8 mm. Petals and stamens are inserted at the calyx throat, with petals and wrinkled. Filaments are about 7 mm long and persistently multiseriate [5]. The ovary is roughly globose, with a thick, reddish style around 1 cm long and a simple, slightly bilobed stigma. The fruit is spherical, ranging from 2-8 cm in diameter, sometimes persistent, and can be pale red, scarlet, or brownish [6]. The thick and leathery rind is partitioned by thin, yellow septa. Seeds are red or pink, approximately 10 mm long, angular, with a thick, fleshy, and juicy testa [7].

Pomegranate (*Punica granatum*) stands as a deciduous shrub extensively cultivated in the Mediterranean and South Asian regions, Afghanistan, and Baluchistan, where it is found growing in the warm valleys and outer hills of the Himalayan mountain range at elevations between 900 and 1,800 meters, with India leading in its production [8]. Its popularity in semiarid and drought-prone regions is attributed to low maintenance costs, high yields, resilience to low irrigation potential, and robust keeping quality. Over centuries, various plant parts have found applications in traditional medicine, particularly in Ayurvedic and Unani systems, addressing inflammatory diseases and digestive tract disorders. Pomegranate, known for its therapeutic attributes, has been recognized for its juice, peel, and oil, demonstrating anticancer and cardiovascular preventive properties [9]. Additionally, the delectable taste of pomegranate fruit arils has led to their widespread use in producing juice, squash, jelly, jam, wine, anardhana, etc. The rich color, sweet-sour flavor, and high antioxidant content make pomegranate a sought-after ingredient in various products like jelly, ice creams, truffles, and chewing gum [10].

The desiccated bark derived from both the stem and roots has been a traditional remedy recognized for its anthelmintic properties. A combination of the bark and fruit, when compounded with other medicinal substances, is recommended for treating snake bites [11]. Additionally, the bark finds application in addressing scorpion bites. Orally consuming a concoction made by stewing the bark, followed by a purgative medicine, is acknowledged for its anthelmintic effects [12]. The stewing of root bark is specifically employed in cases related to tapeworm infestations. In India, the ingestion of a hot water extract derived from the dried bark and fruit is practiced for conditions such as Hansen's disease, leucorrhoea, menorrahgia, and is valued for its anthelmintic properties [13].

The combination of pomegranate peel or rind, opium, and aromatic, such as cloves, is recognized as a potential remedy for chronic diarrhea and dysentery [14]. A decoction of the peel is recommended for abdominal pain and infectious diseases. An infusion comprising fine fruit, rind, and rice flour serves as a treatment for diarrhea and dysenteries, functioning as an anthelmintic [15]. In Europe, a hot water extract of pomegranate peel is employed to alleviate inflammation [16]. The oral intake of a water extract from pomegranate peel is practiced in India to treat diarrhea and dysentery [17].

The fine powder derived from the flowers is utilized for respiratory disorders. An infusion of the flowers serves as a vermifuge. The stewing of flowers is employed as a gargle to address throat inflammation [18]. Flowers are used as a remedy for cut wounds, bronchitis, diarrhea, and systemic issues. Stewing tender leaves serves as a gargle for buccal afflictions. In India, a hot water extract of leaves is consumed orally to address irregular menstruation. In Brazil, a hot water extract of dried leaves is applied externally for women's issues. In African countries, leaves crushed in water are ingested orally to expel tapeworms [19].

Dried roots are utilized as an abortifacient. A mixture of three parts Allium cepa seeds, three parts *Punica granatum*, two parts Cajanus cajan, and red lead oxide, taken with honey, is consumed orally [20].

A substantial quantity of pomegranate peel waste is generated, posing a challenge for proper disposal and contributing to environmental concerns. Studies suggest that the peel and seed fractions of certain fruits exhibit higher bioactivities compared to the pulp fractions. Notably, Pomegranate peel extract (PE) has been investigated for its antioxidant potential, as documented in various studies [21, 22].

2.0 MATERIALS AND METHODS

2.1. Plant material

The pomegranate fruits were purchased from the local market in Abbottabad. Care was taken to select healthy

fruits. The fruits were acquired during the period from October to December 2021. The peels of the fruit were carefully chosen and used for further investigation. The peels were thoroughly cleaned after being removed from the fruit. The seeds were separated from the peels, and the cleaned peels were left to dry after cleaning. The peels of the pomegranate were shade-dried, collected until all the water molecules evaporated. The main purpose of drying was to eliminate the water content from the peels so that they could be easily stored. If the peels were not dried immediately, they might have spoiled. The dried plant was finely powdered using an electrical grinder and subsequently stored in a sealed container for future use.

2.2. Preparation of extracts

A quantity of 15 grams of dried pomegranate peel powder was immersed separately in methanol, acetone, and distilled water. Each solvent was mixed with the powder in individual beakers and left at room temperature for 7 days, ensuring the soluble components dissolved through regular stirring. Subsequently, the extracts underwent filtration using Whatman filter paper, and the resulting filtrates were gathered and preserved at 4°C until subsequent utilization **(Fig. 1)**.

Fig. 1: Different extracts of *Punica granatum* L

2.3 Soxhlet Extraction

The solid dried sample of seeds was poured into the thimble. The apparatus had been arranged, and methanol, acetone, and distilled water had already been added to the round-bottomed flask. The flask was then placed on the heating mantle, and the solvent was brought to a boil. The boiling solvent's vapors ascended through the condenser, where they condensed and flowed down the inner tube into the chamber containing the sample. A sufficient quantity of the solvent was added to fill the chamber, and after filling, the solvent flowed down into the round bottom flask simultaneously. This process was repeated multiple times until the extraction was completed **(Fig. 2)**.

Fig. 2: Soxhlet extraction apparatus

2.4. Qualitative phytochemical analysis

The characterization of phytochemicals in the methanol, distilled water, and acetone extracts of *Punica granatum* was carried out using established standard procedures.

2.5. Alkaloids Detection Test

2.5.1. Mayer's test

A small sample of the filtrates underwent subsequent treatment with a single drop of Mayer's reagent, carefully introduced along the test tube's inner wall. The formation of a creamy or white precipitate, observed upon inspection, signified a positive test outcome [23].

2.5.2. Wagner's test

A reddish-brown precipitate resulted when Wagner's reagent was introduced to a few drops of the plant extract, suggesting the presence of alkaloids [24].

2.6. Flavonoids Detection Test:

The analysis of the plant extract for flavonoids followed the established methodology. A 2 ml solution of the extract was treated with 1 ml of sodium hydroxide solution. The appearance of yellow to red precipitates during this treatment served as a positive indication for the presence of these phenolic compounds [25].

2.7. Carbohydrates Detection Tests

2.7.1. Molisch's Test

During experimentation, the plant extract was treated with

alcoholic α-naphthol drops. Subsequently, 0.2 ml of concentrated H_2SO_4 was carefully introduced down the side of the test tube. The formation of a purple to violet ring at the interface between the two liquids confirmed the presence of carbohydrates [26].

2.7.2. Benedict's Test

The filtrate was subjected to Benedict's reagent, a concoction of sodium carbonate, sodium citrate, and copper sulfate solution. Upon reaching boiling point and simmering for five minutes, the mixture was cooled. The formation of an orange-red precipitate served as a clear indication that there are carbohydrates in the sample[27].

2.7.3. Fehling's Test

Fehling's A and Fehling's B were two solutions that were carefully mixed in equal amounts inside a test tube in a systematic experiment. Fehling's A was a cobalt-colored mixture that contained suspended copper sulfate in distilled water that was crystal clear. Fehling's B, on the other hand, contained a powerful combination of potassium tartrate and sodium hydroxide in the same pure watery safety. After adding a tiny amount of the plant extract and giving the mixture a gentle heat treatment, determined presence of reducing sugars as brick-red precipitates—the evident indicators of cuprous oxide—became visible [28].

2.8. Saponins Detection Test

2.8.1. Foam test

A 2 ml sample of the botanical specimen was homogenized with an equal volume of distilled water in a previous evaluation for saponin presence. The resultant mixture was then centrifuged for fifteen minutes. The presence of saponin was accurately indicated by the observation of a measurable foamy layer on the upper surface of the centrifuged mixture, per with the established methodology [29].

2.9. Tannins Detection Test

2.9.1. Lead Acetate Test

A yellow precipitate formed when a few drops of 10% lead acetate solution were subjected to the test solution during the qualitative analysis. This observation functioned as a necessary indicator for the presence of particular chemical components in the solution. An effective reagent for qualitative analysis is lead acetate, which quickly forms precipitates of insoluble yellow lead sulfide and is particularly useful at determining sulfide ions. Therefore, the specific reaction between lead acetate and the sulfide ions in the test solution was definitively confirmed by the yellow precipitate that was observed [30].

2.10.Phenol Detection Test

2.10.1. Ferric Chloride Test

10 mg of the extracted material was subjected to a few drops of ferric chloride solution. The subsequent development of a bluish-black coloration confirmed the phenolic compounds within the tested sample [31].

2.11. Quinone Detection Test

2.11.1. Sulphuric acid test

Two milliliters (mL) of concentrated sulfuric acid were added to two milliliters (mL) of the plant extract to test for the presence of quinone. The presence of quinone was confirmed by the distinct red coloration observed[32].

2.12. Proteins Detection Tests

2.12.1. Xanthoproteic Test

A small amount of concentrated nitric acid solution was added to the extracted samples throughout the investigation. The appearance of a yellow tint subsequently served as a qualitative indicator of the presence of proteins. The basis for this test is the historical finding that protein components react with nitric acid to produce a distinctive yellow product [33].

2.12.2. Millon's Test

A few drops of Millon's reagent were added to the sample extract before it was heated gently in order to conduct a protein analysis. The presence of protein residues was confirmed by the later discovery of a reddish-brown coloration or precipitate. The reactivity of tyrosine residues found in the protein backbone causes this colorimetric response, more especially the creation of a mercury-tyrosine complex, which is typical of the Millon's test [34].

2.13. Anthocyanin Test:

When concentrated sulfuric acid was added to a 2 mL extract during a qualitative analysis of anthocyanin presence, the result was a yellowish-orange coloration that suggested a positive reaction [35].

2.14 Antimicrobial activity

Antimicrobial activity encompasses the process of eradicating or inhibiting disease-causing microorganisms.

Various antimicrobial agents, including antibacterial, antifungal, and antiviral substances, are employed for this purpose. Despite significant advancements in human medicine, infectious diseases caused by bacteria, fungi, parasites, and viruses continue to pose a substantial threat to public health. This impact is particularly pronounced in developing countries due to limited access to medications and the escalating problem of widespread drug resistance [36].

Natural products, especially those utilized in ethnomedicine, constitute a crucial reservoir of innovative therapeutic agents for diverse conditions, including infectious diseases. Numerous studies have reported the antimicrobial activity of various plant extracts [37]. While different classes of antibiotics have historically been employed to combat bacterial infections, the efficacy of existing antimicrobial agents is diminishing rapidly. This shift is favoring multidrug-resistant pathogens, such as MRSA, and the current arsenal lacks new drug classes to counteract these challenges. Multidrug resistance (MDR) exhibited by several bacterial species poses a significant obstacle in treating both hospital and community-acquired infections [38].

2.15 Media preparation

The chosen growing medium for bacteria was nutrient agar. 3.0g of beef extract, 15.0g of agar, and 5.0g of peptone made up the medium. 40g of nutritional agar were dissolved in 700ml of distilled water to create one litre of media. More distilled water was added to the media to bring its ultimate volume to 1000ml once it had completely dissolved. A heated plate was used to boil the medium. At 25ºC, the pH was brought to 7.0 by adding 0.1M NaOH and 0.1M HCl. All glassware and necessary media were autoclaved for 20 minutes at 121°C and 15 pressure to achieve sterilization.

2.16 Autoclaving of Apparatus

The media plates were carefully washed, and other apparatus used for antimicrobial activity were autoclaved for 15-20 minutes at 121 degrees Celsius at a pressure of 15 psi. After autoclaving, all the apparatus were carefully taken into the laminar airflow, which was already free from contamination due to exposure to UV light.

2.17 Media Pouring and Streaking Of Microbes

Nutrient media was poured into media plates carefully, one by one, and left to solidify for 15-20 minutes. After the media became gel, microbial strains were streaked into media plates using a glass spreader. Four wells of equal size were made in plates, spaced 8 cm apart from each other, using a sterile cork borer. After well formation, solvent extracts of different concentrations were placed into different plates using a micropipette. Ethanol was used as the negative control in all plates for both microbes. After the careful transfer of extracts into wells, media plates were placed at 37°C for 16 hours of observation, after which microbial activity was assessed (**Fig. 3**).

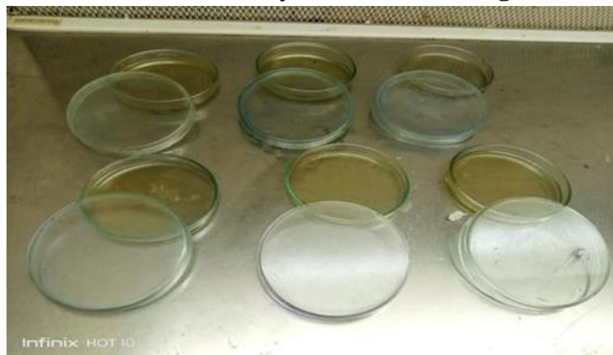

Fig. 3: showing media plates inside laminar flow

2.18 Agar well diffusion method

Around 20ml of nutrient agar was spread onto Petri dishes and allowed to solidify for half an hour. Using sterile cork borers, wells with a diameter of 6mm and spaced approximately 2 cm apart were created in the agar, resulting in three to five uniform wells per Petri dish. Each well's base was sealed with a drop of molten nutrient agar. The wells were then filled with the test solution using a micropipette. Samples of extracts, along with a positive control (Cefotaxime) and a negative control (DMSO), were administered to each Petri plate. Subsequently, the plates were placed in an incubator set at 37°C. After 24 hours of incubation, the diameter of the clear zones surrounding each well, referred to as the zone of inhibition, was measured. The diameter of the inhibition zone caused by the extract was then measured and compared to the standard. Each sample was tested in triplicate to assess antibacterial activity. The experiments were conducted in a laminar flow setting to maintain sterility.

3.0. RESULTS AND DISCUSSION

A preliminary phytochemical screening test was conducted to detect the presence of active chemical compounds in methanolic, acetonic, and distilled water extracts, such as

alkaloids, flavonoids, saponins, phenols, quinones, tannins, anthocyanin, and carbohydrates. The different extracts underwent qualitative analysis to identify various constituents. Generally, the test for the presence of phytochemical compounds involved adding appropriate chemical reagents to the extracts in test tubes. The mixture was shaken and heated as needed.

The three solvent extracts, i.e., acetone, methanol, and distilled water, were tested for nine phytochemicals. The procedure for phytochemical analysis was carried out using standardized protocols. Carbohydrates were tested using Molisch's test and Fehling's test. Saponins were tested using the foam test. Tannins were tested using the lead acetate test. Quinones were tested using the hydrochloric acid test. Alkaloids were tested using Wagner's test and Mayer's test. Flavonoids were tested using the acid test. Anthocyanin was tested using the sulfuric acid test. Proteins were tested using the Xanthoproteic Test. Phenols were tested using the ferric chloride test.

Wagner's test for alkaloids revealed positive findings in the distilled water extract, whereas Mayer's test for alkaloids yielded negative results. The saponin foam test yielded a positive outcome. The flavonoid acid test yielded a positive result. The test for phenols using ferric chloride produced a positive result. Positive findings were obtained from the lead acetate test for tannins. Quinones tested positive in the H_2SO_4 test. For carbohydrates, the findings of Fehling's and Molisch's tests were positive. The anthocyanin test with sulfuric acid produced a positive result. Positive findings were obtained from the Xanthoproteic test for proteins.

In the methanolic extract, Mayer's test for alkaloids showed negative results, while Wagner's test for alkaloids showed positive results. Fehling test and Molisch test for carbohydrates were found positive. The foam test for saponins showed a positive result. The tannins test showed a positive result. Molisch's test and Fehling's test for carbohydrates showed positive results. The acid test for flavonoids showed positive results. The ferric chloride test for phenols showed a positive result. The H_2SO^4 test for quinones showed a positive result. The sulfuric acid test for anthocyanin showed negative results. The Xanthoproteic test for protein showed a positive result.

Wagner's test for alkaloids yielded positive findings in the acetonic extract, however Mayer's test for alkaloids produced negative results. The flavonoid acid test was positive. Positive findings were obtained from the lead acetate test for tannins. The saponin foam test yielded a positive result. The test for phenols using ferric chloride produced a positive result. Quinones tested positive in the H_2SO_4 test. For carbohydrates, the findings of Fehling's and Molisch's tests were positive. The protein Xanthoproteic test yielded a positive result. The anthocyanin test with sulfuric acid yielded negative findings. The results are summarized in **Table-1.**

Table-1: phytochemical analysis of of *Punica granatum* in selected Solvents

Phytochemicals	Tests	Distilled water	Methanol	Acetone
Carbohydrates	Molisch's test Fehling's test	+ +	+ -	+ +
Alkaloids	Mayer's test Wagner's test	- +	- +	- +
Tannins	Lead acetate test	+	+	+
Saponins	Foam test	+	+	+
Flavonoids	Acid test	+	+	+
Proteins	Xanthoproteic	+	+	+
Phenol	$FeCl_2$ test	+	+	+
Quinone	H_2SO^4 test	+	+	+
Anthocyanin	Sulphuric acid test	-	-	-

The antibacterial potential of *Punica granatum* extracts was investigated against Pseudomonas

aeruginosa and Bacillus subtilis in a previous study. Utilizing two concentrations (5mg/ml and 10mg/ml), the efficacy of these extracts was assessed, revealing encouraging results. Notably, the methanolic extract displayed a superior inhibitory effect against Pseudomonas aeruginosa, while the acetonic extract exhibited greater potency against Bacillus subtilis. These observations suggest the presence of active phytochemical constituents within the extracts that contribute to their antibacterial properties.

Zone Of Inhibition Against *Pseudomonas aurgenosa*

The well diffusion method was performed to check the antibacterial activity of *Punica granatum* L. against Pseudomonas aeruginosa. The result showed that at a concentration of 10mg/ml, the acetonic extract showed a maximum zone of inhibition of 20mm, acetonic methanolic extract showed a zone of inhibition of 18mm, while the distilled water extract formed a zone of inhibition of 16mm. Similarly, at a concentration of 5mg/ml, methanol extract showed a maximum zone of inhibition of 16mm, acetonic extract showed a zone of inhibition of 15mm, while distilled water showed a zone of inhibition of 10mm.

Zone Of Inhibition Against *Bacillus subtilis*

The antibacterial activity of peels extract of *Punica granatum* L. was carried out by using the well diffusion method also against Bacillus subtilis. The result showed that at a 10mg/ml concentration, the methanolic extract of *Punica granatum* L. formed the maximum zone of inhibition of 17mm, acetonic extract formed a zone of inhibition of 14mm, while distilled water formed a zone of inhibition of 10mm. Similarly, at a concentration of 5mg/ml, methanolic extract showed a zone of inhibition of 14mm, acetonic extract formed a zone of inhibition of 12mm, while distilled water formed a zone of inhibition of 11mm. the results are summazized in **Table-2**.

Table-2: Antibacterial activity of *Punica granum L.* extracts against selected strains

Extract	Methanol		Acetone		Distilled water	
Concentration	10mg/ml	5mg/ml	10mg/ml	5mg/ml	10mg/ml	5mg/ml
B . subtilis	18mm	16mm	20mm	15mm	13mm	10mm
P.aurgonosa	17mm	14mm	14mm	12mm	14mm	11mm

Much attention was focused on finding molecules that may function as appropriate antimicrobial agents to replace synthetic ones in the hunt for antimicrobials derived from natural sources. Plant-based phytochemicals functioned as a model for the development of less hazardous and more potent medications that inhibited the growth of microorganism.

4.0. CONCLUSION

The present study concluded that the selected medicinal plant *Punica granatum* L. contained different biologically active compounds called phytochemicals. Preliminary phytochemical screening tests were conducted on methanolic, acetonic, and distilled water extracts of *Punica granatum* L. According to the current study, Punica granatum has components with potential antibacterial properties that might be very helpful for the pharmaceutical industry's development as a treatment for a variety of illnesses. Punica granatum extracts in methanol and acetone showed a notable inhibitory impact on the pathogens that were tested.

ACKNOWLEDGMENTS

We express our sincere gratitude to the Department of Botany, Government Post Graduate College No.1, Abbottabad, Pakistan, for generously providing the necessary experimental facilities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Prakash, C.V.S. and I.J.I.J.R.C.E. Prakash, Bioactive chemical constituents from pomegranate (Punica granatum) juice, seed and peel-a review. 2011. **1**(1): p. 1-18.
2. Amjad, H., Pomegranate: Anatomy of a divine remedy. 2003: Lulu. com.
3. Lai, M.W., Metabolite Profiling And Dna Barcoding Analysis Of 35 Malaysian Medicinal Plants. 2022, UTAR.
4. BOOPATHI, C.A., Medicinal & Poisonous Plants of India. 2019: MJP Publisher.
5. Alamgir, A., A.J.T.U.o.M.P. Alamgir, and T.E.V. Pharmacognosy, Pharmacognostical Botany: Classification of medicinal and aromatic plants (MAPs), botanical taxonomy, morphology, and anatomy of drug plants. 2017: p. 177-293.

6. Malik, K., et al., Medicinal Plants Used for Hypertension. 2021: p. 37-103.
7. Erkan, M. and A. Kader, Pomegranate (Punica granatum L.), in Postharvest biology and technology of tropical and subtropical fruits. 2011, Elsevier. p. 287-313e.
8. Nair, K.P., Herbal and Acidulant Tree Spices: Their Global Commercial Potential. 2022: Springer Nature.
9. Khwairakpam, A.D., et al., Possible use of Punica granatum (Pomegranate) in cancer therapy. 2018. **133**: p. 53-64.
10. Aziz, S. and S. Sharma, Fruit & vegetable juice therapy. 2001: Pustak Mahal.
11. Kabir, H., Introduction to Ilmul advia. 2002: Shamsher Publisher and Distributors.
12. Oliver, D., Official Guide to the Kew Museums: A Handbook to the Museums of Economic Botany of the Royal Gardens, Kew. 1871: Reeve.
13. Fatima, N., et al., AN INVESTIGATION OF THE PROPERTIES OF INDIAN HERBAL REMEDIES.
14. Haque, N., Evaluation of Wound Healing Activity of Post Anar (Punica granatum Linn) in Animal Models. 2011, Rajiv Gandhi University of Health Sciences (India).
15. Bhatia, H., et al., Ethnomedicinal plants used by the villagers of district Udhampur, J&K, India. 2014. **151**(2): p. 1005-1018.
16. Asprey, G. and P.J.W.I.M.J. Thornton, Medicinal plants of Jamaica. Parts 1 & 11. 1955. **2**(4): p. 69.
17. Ismail, T., P. Sestili, and S.J.J.o.e. Akhtar, Pomegranate peel and fruit extracts: a review of potential anti-inflammatory and anti-infective effects. 2012. **143**(2): p. 397-405.
18. Bakhru, H., Foods that heal: The natural way to good health. 1995: Orient Paperbacks.
19. Erichsen-Brown, C., Medicinal and other uses of North American plants: a historical survey with special reference to the eastern Indian tribes. 2013: Courier Corporation.
20. Ross, I.A., Punica granatum, in Medicinal Plants of the World: Volume 1 Chemical Constituents, Traditional and Modern Medicinal Uses. 2003, Springer. p. 431-444.
21. More, P.R., et al., Green, environment-friendly and sustainable techniques for extraction of food bioactive compounds and waste valorization. 2022.
22. Smaoui, S., et al., Pomegranate peel as phenolic compounds source: Advanced analytical strategies and practical use in meat products. 2019. **158**: p. 107914.
23. Bick, I., et al., Alkaloids of Anthocercis tasmanica (Solanaceae). 1974. **27**(11): p. 2515-2518.
24. Jha, D.K., et al., Detection and confirmation of alkaloids in leaves of Justicia adhatoda and bioinformatics approach to elicit its anti-tuberculosis activity. 2012. **168**: p. 980-990.
25. Hossain, M.A., et al., Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. 2013. **3**(9): p. 705-710.
26. Niaz, M., et al., Qualitative phytochemical analysis of stem, leaves and fruits of Withania somnifera L. in different solvents. 2023. **2**(3): p. 17-21.
27. Niaz, M., et al., Qualitative phytochemical analysis of rhizomes and roots extracts of Bergenia ciliate. 2023. **2**(3): p. 22-27.
28. Çakıroğlu, B. and M.J.J.o.T.E.S. Özacar, Photoelectrochemical and Non-Enzymatic Glucose Sensor Based on Modified Fehling's Test by Using Ti/TiO2 NTs-rGO-Cu2O Electrode. 2019. **166**(8): p. B728.
29. Mohlakoana, M. and A.J.R. Moteetee, *Southern african soap plants and screening of selected phytochemicals and quantitative analysis of saponin content.* 2021. **10**(10): p. 96.
30. Sruthi, D., G.J.J.o.P. Indira, and Phytochemistry, *Isolation of tannins from the leaves of Nephelium lappaceum L.(Sapindaceae) and the HPTLC of the isolated compound.* 2016. **5**(5): p. 395-397.
31. Syed, A.L., et al., *GC-MS analysis, antimicrobial, antioxidant, phytochemical, and phytotoxic activities of Elaeagnus angustifolia L. using different extracts.* 2023. **2**(3): p. 1-12.
32. Stom, D.J.A.h.e.h., *Use of Thin-Layer and Paper Chromatography for Detection of Ortho-and Para-Quinones Formed in the Course of Phenol Oxidation.* 1975. **3**(1): p. 39-45.
33. Abrar, H., et al., *Qualitative phytochemicals profile of five different crude solvents of Galium asperifolium Wall.* 2023. **2**(2): p. 26-37.
34. Subroto, E., et al., *The analysis techniques of amino acid and protein in food and agricultural products.* 2020. **9**(10): p. 29-36.
35. Amalia, F., G.N.J.J.o.F. Afnani, and P. Sciences, *Extraction and stability test of anthocyanin from buni fruits (Antidesma Bunius L) as an alternative natural and safe food colorants.* 2013. **1**(2).
36. Annan, K., et al., *Antibacterial and Resistant Modifying activity of Paullinia pinnata.* 2005. **57**: p. S74-S75.
37. Allen, P.Z., M.C. Connelly, and M.A.J.C.j.o.m. Apicella, *Interaction of lectins with Neisseria gonorrhoeae.* 1980. **26**(4): p. 468-474.
38. Abou EI-Souod, S. and S.J.A.J.S. Moustafa, *MNEl-Shoubagy and IMEl-Refae. Chemical and microbiological investigations of Thymelaea hirsute Endl.(L) growing in Egypt.* 1983. **3**: p. 85-93.