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Phytochemical Screening of Pomegrante Peel and Mosambi Peel Methanol Extracts

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ABSTRACT

The phytochemical screening of pomegranate and mosambi peels reveals a rich composition of bioactive compounds that contribute to their nutritional and medicinal potential. Despite the promising bioactive profile of pomegranate and mosambi peels, the study faced several challenges. Standardizing extraction conditions to ensure consistent phytochemical yield proved difficult due to variability in peel maturity, storage conditions, and regional cultivar differences. Moreover, translating these findings into scalable applications demands further investigation into compound stability, synergistic interactions, and regulatory compliance for functional food and pharmaceutical integration. The study identified the presence of flavonoids, tannins, phenols, terpenoids and saponins in both peels, highlighting their strong antioxidant and antimicrobial properties. Notably, pomegranate peel exhibited alkaloids, reinforcing its traditional use in pharmacological applications, while mosambi peel contained higher flavonoid and fixed oil concentrations, making it an excellent source of natural antioxidants. The absence of cardiac glycosides, steroids, phlobatannins, and quinones suggests limited cardiovascular-related effects. Given their bioactive profile, these peels hold promise for utilization in functional foods, dietary supplements, pharmaceuticals, cosmetics, and eco-friendly antimicrobial agents. To maximize their therapeutic potential, efficient extraction and processing techniques are crucial for improving bioavailability. This study emphasizes the importance of valorizing fruit peels as valuable bio-resources, promoting sustainable waste management while enhancing health and industrial applications.

Keywords: *Phytochemical Screening, Bioactive Compounds, Antioxidants, Pomegranate Peel, Mosambi peel, bioavailability, Human health and Antinutrients.*

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a widely recognized medicinal plant native to the Mediterranean region and extensively used in traditional medicine across the Indian subcontinent and other countries. The name "pomegranate" originates from the Latin words "ponus" and "granatus." Global production of pomegranates is estimated at around 1.5 million tons. Among various medicinal plants, pomegranate stands out due to its vibrant appearance and numerous health benefits, primarily concentrated in its fruit[1]. Pomegranate peels, which make up approximately 60% of the fruit's total weight, are often discarded as agro-waste. However, they are rich in antioxidants, phytochemicals, and possess antibacterial and antifungal properties. The peel contains a high concentration of polyphenols and bioactive compounds like gallotannins, ellagic acid, gallic acid, punicalins, and punicalagins. These secondary metabolites contribute to the plant's medicinal properties. In addition to the edible portion, the non-edible parts such as leaves, bark, buds, flowers, and peel also contain significant nutritional and bioactive compounds[2].

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The peel has been recognized for its medicinal applications, including wound healing, immune modulation, antibacterial effects, anti-atherosclerotic, and antioxidant properties. Various parts of the plant, including seeds and juice, are used to treat ailments such as throat infections, eye diseases, gum bleeding, skin conditions, cancer, cardiovascular diseases, diabetes, infant brain ischemia, and male infertility. [3,4]

Citrus fruits form one of the most widely cultivated fruit families worldwide, with China, Brazil, and India leading global production, collectively contributing 49.22% of the total output. These fruits are rich in nutrients, phytochemicals, and bioactive polyphenols, which enhance their antioxidant properties, offering significant health benefits [5]. Sweet lime (*Citrus limetta*), commonly referred to as "Mosambi," is a highly regarded citrus fruit known for its distinctive taste, flavor, and aroma. It is widely consumed fresh or processed into juice [6]. While the edible portion of the fruit is well-utilized, the peel-accounting for approximately 50–55% of the total fruit weight considerable value due to its biologically active compounds, including phenols, flavonoids, essential oils, carotenoids, organic acids, ascorbic acid, and vitamins. Sweet lime peel is a rich source of antioxidants, pectin, and dietary fiber, aiding in blood glucose and cholesterol regulation. Its soluble-to-insoluble fiber ratio surpasses that of cereals, enhancing its nutritional value. Furthermore, the peel contains various bioactive compounds that exhibit antibacterial, antiviral, anti-inflammatory, anticancer, and free radical-scavenging properties.

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Due to its numerous health benefits, sweet lime peel holds potential for use as an ingredient in jams, jellies, beverages, dairy and bakery products, candies, and chocolates, provided effective processing techniques are employed to preserve its nutrient bioavailability [7]. However, the high moisture content of the peel presents a challenge to its optimal utilization, requiring appropriate drying and preservation methods to enhance its applicability.

A study was conducted to analyze the presence of phytochemicals in tray dried pomegranate peel and mosambi peel.

MATERIAL AND METHODS

The Pomegranate fruits of Bhagwa variety and Mosambi fruits were procured from Nalgonda district and Medak district respectively. The fruits were collected and surface disinfected by immersing gently with 200 µL/L sodium hypochlorite solution for 2 min, and then washed, peeled and the edible portion was manually separated. The peels were cut into small pieces and dried in a tray drier at 60˚C for 36 hours, cooled, powdered and stored in air tight glass bottles. From this 2.0 g of samples were used to extract by cold steeping in 100.0 ml of methanol for 24 hrs, centrifugated at 3000 rpm for 10 min and filtered with Whatman No. 41 filter paper to obtain clear extracts. The above filtrate is stored in refrigerator and used for further study.

Preliminary Phytochemical Screening: The preliminary tests of carbohydrate, alkaloids, proteins, amino acids, flavonoids, fixed oils, terpenoids, cardiac glycosides, steroids, tannins, phlobatins, phenols and quinones were carried out as per procedure given by [8].

Test for carbohydrates: To 2.0 ml of sample extracts, two drops of Molisch reagent were added and shaken vigorously. To this 2.0 ml of concentrated H_2SO_4 was added from the walls of the test tube. A reddish violet ring formed at the juncture of two layers immediately which indicated the presence of carbohydrates.

Test for alkaloids: The presence or absence of alkaloids was carried out using Mayer's, Wagner's and Hager's tests.

Mayer's test: A portion of the extract was added with 1.0% HCl and 6 drops of Mayer's reagent (1.36 g of mercuric chloride and 5.0 g of potassium iodide in 100.0 ml of water). The organic precipitate indicated the presence of alkaloids in the samples.

Wagner's test: A fraction of the extract was treated with Wagner's reagent which has 0.28 g of iodine and 0.20 g of potassium iodide in 10.0 ml water. The formation of cream-colored precipitate indicated the presence of alkaloids.

Hager's test: The extracted sample was treated with Hager's reagent containing saturated aqueous solution of picric acid and the formation of a prominent yellow coloured precipitate indicated the presence of alkaloids.

Test for proteins: To 2.0 ml of sample extract, 1.0 ml of 40.0% sodium hydroxide and 1 to 2 drops of 1.0% copper sulfate solution were added. The formation of violet color indicated the presence of peptide linkages found in proteins of samples.

Test for amino acids: To 2.0 ml of sample extract, 2.0 ml of ninhydrin reagent was added and kept in a water bath at 60°C for 20 minutes. The appearance of purple color indicated the presence of amino acids in the sample.

Test for flavonoids: Each fraction of sample extracts was added with 5.0 ml ammonia followed by a few drops of concentrated H_2SO_4. The development of a yellow colour confirmed the presence of flavonoids which disappeared on standing for a few minutes.

Test for fixed oils and fats: A few drops of 0.5 N alcoholic KOH and a drop of phenolphthalein indicator were added to the extract fractions. The mixtures were heated at 60°C in a water bath for 1½ – 2 hrs. The formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Test for terpenoids: To 5.0 ml of each extract, 2.0 ml of chloroform and 3.0 ml of concentrated sulphuric acid were added and formation of a single layer of reddish-brown color at the juncture was due to the presence of terpenoids.

Test for cardiac glycosides: About 5.0 ml of each extract was added with 2.0 ml of glacial acetic acid containing one drop of $FeCl_3$ solution and 1.0 ml of concentrated sulphuric acid. The formation of a brown ring at the interface indicated the deoxy sugar which was distinctive for cardenolides. A violet ring can appear below the brown ring in the acetic acid layer whereas a green layer can also form just gradually into a thin layer.

Test for steroids (Liebermann - Burchard test): 2.0 ml each of acetic anhydride and concentrated sulphuric acid were added to 0.5 ml of extracts in triplicates. The appearance of blue or green color from initial violet color indicated the presence of steroids.

Test for saponins: The extracts were added with 20.0 ml of distilled water and agitated vigorously in a graduated cylinder for 15 min. The formation of a centimetre layer of foam indicated the presence of saponins.

Test for tannins: A few drops of 1.0% lead acetate were added 5.0 ml of extracts and the formation of yellow precipitate indicated the presence of tannins.

Test for phlobatinins: Each extract was boiled with a few drops of 1.0% hydrochloric acid and the deposition of red precipitate indicated the presence of phlobatins.

Test for phenols: Ferric chloride and Liebermann's tests were used to determine the presence or absence of phenols in methanol extracts.

Ferric chloride test: A fraction of each of the extracts was added with a few drops of 5.0% Ferric chloride and the formation of a deep blue or black color indicated the presence of phenols.

Liebermann's test: The extracts were heated with sodium nitrite and concentrated sulphuric acid solution, diluted in water, cooled and added with an excess of dilute sodium hydroxide. The formation of deep red green or blue colours indicated the presence of phenols.

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Test for quinones: A small amount of each extract was treated with concentrated HCl and appearance of yellow colored precipitate indicated the presence of quinones. The absence of yellow coloured precipitate showed that the extracts had not undergone any form of oxidation.

Test for Anthocyanosides: 1 ml of extract was taken in a test tube and treated with 5 ml diluted HCl(aq). Pale pink color solution confirms the presence of anthocyanosides.

RESULTS AND DISCUSSION

Phytochemicals, also known as phytonutrients, are naturally occurring compounds in plants that offer significant health benefits. These bioactive substances exhibit strong antioxidant and anti-inflammatory properties, helping to protect human health. Their key function involves detoxifying the body by eliminating harmful chemicals, which can otherwise contribute to the formation of free radicals and lead to various health issues [9].

The table provides an in-depth phytochemical screening of pomegranate peel and mosambi peel highlighting the presence or absence of key bioactive compounds. The screening involved different chemical tests to identify these compounds, with the results indicating variations between the two peels.

Pomegranate and mosambi peels contain various bioactive compounds with medicinal potential. Mosambi peel exhibits higher flavonoid and fixed oil content, while pomegranate peel shows a distinct presence of alkaloids and anthocyanosides. Both peels contain saponins, tannins, phenols, proteins, amino acids and terpenoids, indicating possible antioxidant and antimicrobial properties. Similar findings of phytochemical screening of pomegranate peel and mosambi peel were reported by Karthikeyan and Vidya [10] and Shyam Jee [11] using various extracts of aqueous, acetone, hexane and ethanol.

Table 1: Phytochemical screening of punica granatum peel (pomegranate peel) and citrus limetta peel (mosambi peel) methanol extracts

S.No	Phytochemicals	Test	Pomegranate peel	Mosambi Peel
1	Carbohydrates	Molisch test	+	+
2	Alkaloids	Mayer's test	+	--
		Wagner's test	+	--
		Hager's test	+	--
3	Proteins	NaOH and $CuSO_4$	+	+
4	Amino acids	Ninhydrin solution test	+	+
5	Flavonoids	With ammonia solution	++	++
6	Fixed oils and fats	Foam test	+	++
7	Terpenoids	Chloroform and Sulphuric acid	++	+
8	Cardiac glycosides	Glacial acetic acid and $FeCl_3$ solution	-	-
9	Steroids	Liebermann's – Burchard test	-	-
10	Saponins	Foam test	++	++
11	Tannins	$FeCl_3$ test	+	+
12	Phlobatinins	With HCl	-	-
13	Phenols	$FeCl_3$ test Liebermann's test	+	+
14	Quinones	With concentrated HCl	-	-
15	Anthocyanosides	With concentrated HCl	+	-

CONCLUSION

The phytochemical composition of pomegranate and mosambi peels underscores their untapped potential beyond agricultural waste, presenting various applications in medicine, cosmetics, food, and textile industries. Rich in bioactive compounds such as flavonoids, tannins, phenols, terpenoids, and saponins, these peels possess significant antioxidant, antimicrobial, and anti-inflammatory properties, making them valuable resources for health-promoting formulations. Pomegranate peel, with its unique presence of alkaloids, suggests additional pharmacological benefits, reinforcing its traditional use in herbal medicine. Mosambi peel, on the other hand, exhibits a higher concentration of flavonoids and fixed oils, amplifying its role as a powerful antioxidant source and a potential ingredient for skin-care, functional foods, and nutraceuticals.

Incorporating these peels into commercial applications requires effective extraction and processing techniques to optimize their nutrient bioavailability. Drying methods, solvent extractions, and biotechnological advancements can improve the preservation and efficacy of these bioactive compounds, enabling their use in dietary supplements, pharmaceuticals, food additives, and even natural dyes for textiles. Additionally, their antibacterial and antifungal activities make them promising candidates for developing eco-friendly antimicrobial coatings, herbal medicines, and organic preservatives.

With the rising demand for sustainable and natural alternatives, harnessing the potential of these fruit peels aligns with environmentally conscious initiatives, minimizing agricultural waste while promoting holistic health benefits. Future research should explore advanced extraction techniques, synergistic formulations, and industrial scalability, ensuring these peels transition from discarded byproducts to valuable, functional bio-resources.

FUTURE SCOPE OF STUDY

Future studies on phytochemical screening of pomegranate and mosambi peels could delve deeper into quantifying individual bioactive compounds using high-resolution analytical techniques such as HPLC, LC-MS/MS, and NMR spectroscopy. Expanding the screening to include peels from different cultivars, growth conditions, and post-harvest treatments may reveal variations in phytochemical richness and stability. Additionally, longitudinal studies on seasonal and geographical influences can provide insight into optimizing harvest times for maximum therapeutic potential. Standardizing qualitative and quantitative screening protocols will be essential to ensure reproducibility and facilitate cross-study comparisons, ultimately supporting the development of peel-based formulations in nutraceutical and pharmaceutical applications.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this study. All experimental procedures and interpretations were conducted objectively, without any financial or personal influence.

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