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SHORT COMMUNICATION

A Study on Phytochemical Screening and Antimicrobial Activity of Water Apple

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Abstract:

Background: Natural herbs have been ascribed many healing attributes, and medicinal plants serve as the primary wellspring for innovative pharma and health supplement products. The utilization of plants for medicinal purposes likely dates back as far as human history itself. **Aim:** To perform phytochemical screening and antimicrobial activity of water apple. **Methods:** The extraction and identification of numerous potent phytochemical compounds from these botanical resources have yielded a range of highly effective drugs. In the present study, water apple fruits were collected from the garden in Guntur. The fresh fruits were washed with tap water and cut into small pieces for maceration. Based on the polarity of solvents, the bioactive compounds were extracted from water apple fruits. The solvent extraction was used for the screening of phytochemical reactions and antimicrobial activity. The pathogenic organisms were sensitive to the bioactive extracts of water apple. The zone of inhibition around the colonies was measured. **Results:** Phytochemical screening revealed the presence of Flavonoids, phenols, Terpenoids, and Carbohydrates. Further, ethyl acetate fruit extract showed better antibacterial activity when compared with the standard antibiotic Ampicillin and showed potent antimicrobial activity. **Conclusion:** Overall results concluded that water apple showed potent antimicrobial activity due to the presence of important bioactive compounds, which were identified by phytochemical screening.

Major Findings: The phytochemical analysis revealed the presence of bioactive phytochemicals and contained bioactive secondary metabolites, including tannins, phenols, flavonoids, steroids, terpenoids, and carbohydrates were present in good amounts. Fruit extract showed good antibacterial activity because of the presence of bioactive components in *S. samarangense* fruit extract.

Keywords: Antimicrobial, Bioactive Compounds, Phytochemicals, Water Apple

1. Introduction

India boasts a rich array of plants with medicinal properties. These plants and their components find use among people, both directly as traditional remedies and indirectly as ingredients in modern pharmaceutical preparations[1]. Natural herbs are attributed with various medicinal virtues, making them a primary source of novel pharmaceuticals and health supplement products[2]. The practice of utilizing plants for medicinal purposes likely stretches back as far as human history itself. The extraction and characterization of numerous active phytochemical compounds from these botanical sources have given rise to a host of highly effective drugs[3]. A mounting body of evidence underscores the pivotal roles that secondary plant metabolites play in human health, possibly holding significant nutritional importance[4].

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Phytochemical screening of plants has uncovered a plethora of chemicals, including alkaloids, tannins, flavonoids, steroids, glycosides, saponins, and more. Many plant extracts and phytochemicals exhibit potent antioxidant and free radical scavenging properties[5]. These secondary metabolites serve as the plants' defense mechanisms against predation by microorganisms, insects, and herbivores[6,7].

The water apple is a small, bell-shaped fruit characterized by its glossy pink to red skin, sweet and mildly acidic flavor, and white flesh that exudes a delightful rose-like scent. Scientifically known as *Syzygium samarangense*, it belongs to the botanical family Myrtaceae. This delectable fruit is native to Indonesia and Malaysia and thrives in tropical regions across Africa and Southern Asia, including India and Thailand. Water apples fruits have a long history of use in traditional Indian folk medicine to treat various ailments, inflammations, and ulcers. While there is documentation of their pharmacological effects, there is limited clinical data available to substantiate these traditional uses. Including a variety of fruits in one's diet can offer protection against common diseases such as cancer, cardiovascular diseases, and age-related degenerative conditions[8]. Research suggests that free radicals, which can damage lipids, proteins, and nucleic acids within cells, are implicated in the development of these prevalent diseases[9].

Recent studies have demonstrated that regular consumption of fruits and vegetables can lower the risk of cancer and stroke. This protective effect is attributed to the presence of various dietary phytochemicals found in plant parts. Different fruits possess different capacities to combat oxidative stress due to the diversity of phytochemicals they contain, including vitamin C and E, carotenoids, flavonoids, and other phenolic compounds. Incorporating a wide range of fruits into one's diet can provide a valuable source of these protective compounds, potentially contributing to overall health and reducing the risk of chronic diseases[10].

Plants can have active components in various parts such as the bark, leaves, flowers, roots, fruits, seeds, and more[11]. Understanding the chemical constituents of plants is valuable because it can aid in the synthesis of complex chemical compounds. Many researchers have conducted phytochemical screening of various plants to uncover their chemical makeup and potential medicinal properties[5,12].

In our current study, we have endeavored to identify the diverse phytochemical compounds present in water apple and assess their antimicrobial activities. This research aims to shed light on the chemical composition of water apple and its potential benefits in terms of inhibiting the growth of microorganisms.

2. Materials and Methods

2.1 Plant Collection and Extraction

2.1.1 Plant Materials

Fresh fruits were collected randomly from the plants. The locally available wild fruits, such as water apple (*S. samarangense*), were collected for the analysis of phytochemical and antimicrobial properties. Fresh fruits were washed in clean water, air dried, and chopped into small pieces and used for solvent extraction.

2.1.2 Extraction

Hexane, benzene, ethyl acetate, and methanol solvents were added to small pieces of fruits until all the pieces were immersed in the solvent and subjected to cold maceration for a week, then the solvents were filtered using Whatman no 1 filter paper. The extracts were concentrated under reduced pressure at 40°C using rotatory evaporator and the filterate was condensed and stored in a bottle.

2.1.3 Cold Maceration

The cold maceration method was done by soaking the chopped plant materials (fruits) in a solvent and then sealing it with a stopper. To obtain the plant extract, incubate the container at room temperature for a week with frequent stirring. The cold maceration method softens the chopped plant material (fruits), breaks the cell walls, releases, and dissolves the phytochemical constituents. The mixture is then pressed or strained by filtration or decantation after a week[13]. Generally speaking, maceration is a straightforward and popular process. The idea of molecular diffusion underpins this extraction procedure. Maceration guarantees that the concentrated solution buildup around the surface of the particles is dispersed and introduces new solvent to the surface of the particles for additional extraction[14].

2.2 Preliminary Qualitative Phytochemical Screening

The standard procedures were followed for the following tests as given by Harborne[13]. The phytochemical screening was carried out for fruits extracts of water apple using four different solvents.

2.3 Antimicrobial Activity

Antimicrobial activity of four solvent extracts was carried out against the Gram-positive and Gram-negative bacterial cultures and yeast-like fungi, *Candida albicans*. The test organisms used include Gram-positive test bacterial cultures, viz *Staphylococcus aureus,* and *Bacillus subtilis*; and Gram-negative test bacterial cultures, viz *Escherichia coli,* and *Proteus vulgaris.* A yeast-like fungal test culture, *C. albicans* was also used for the test.

Freshly inoculated bacterial strains were kept overnight at 37°C in Nutrient Agar Medium (NAM), whereas Sabouraud Dextrose Agar (SDA) medium was used for subculturing of *C. albicans*. The agar well assay method was used for the determination of antimicrobial activity of extracts. Separately dissolved these four solvent extracts in Dimethyl Sulfoxide (DMSO)[15]. 100 µl of suspension containing 10^8 colony-forming units per milliliter (CFU/ml) of bacteria and 10^6 CFU/ml of *C. albicans* was spread on the NAM.

The well (6 mm in diameter) was bored using a metal cork borer on the solidified agar media, which were already inoculated using test bacterial cultures and yeast culture by the pour plate method. 300 mg of standard antibiotics, ciprofloxacin and ampicillin, were tested against test bacterial cultures and test fungal cultures. Four different solvent extracts were placed in wells for each of the test cultures. The test cultures were inoculated on plates and incubated for 24 h at 37°C. After the incubation period, the zone of inhibition was measured and recorded.

3. Results

3.1 Collection of Water Apple Fruits

Water apple fruits were collected in the garden located in Vidya Nagar, Guntur. The area lies at 16*24* to 16* 25* N latitude and 80* 33* to 80* 34* E longitude (Figure 1).

3.2 Qualitative Phytochemical Screening of Water Apple Fruit Extract

The phytochemical analysis of the hexane, benzene, ethyl acetate, and methanol extract of the fruits of water apple revealed the presence of terpenoids, flavonoids, Phenols, and carbohydrates. Alkaloids, saponins, steroids, proteins, and photobatonins were shown to be negative for all four different solvent extracts. Flavonoids tested positive for hexane, ethyl acetate, and methanol, whereas benzene tested negative. Methanol solvent extract showed positive for the tannins test, whereas the other solvent extracts showed negative.

All four solvent extracts were negative for the saponin test. Carbohydrate test showed positive results for benzene, ethyl acetate, and methanol extract where whereas hexane solvent showed negative results for the carbohydrate test. The anthraquinone test was negative for hexane, methanol, and benzene, whereas the ethyl acetate extract showed positive (Table 1).

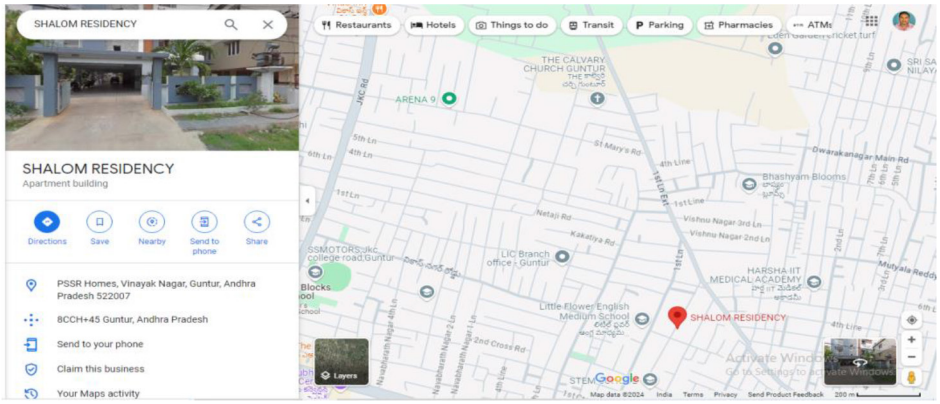


Figure 1. Region of the sample collection area.

3.2.1 Phytochemical Screening of Water Apple

The qualitative analysis of the extract from the fruits of water apple showed the presence of phytochemical constituents such as terpenoids, flavonoids, and carbohydrates. At the same time, alkaloids, saponins, steroids proteins and photobatonins were absent.

3.3 Antimicrobial Activity of Water Apple Fruits of Four Different Solvent Extracts

In the present work, the antimicrobial effect of water apple fruit extracts was studied. After studying the antibacterial activity of four solvent extracts, it was found that benzene extract, ethyl acetate extract, and methanol extract showed antimicrobial activity against the microbial cultures of Gram-positive, Gram-negative bacteria, and fungal test cultures, whereas the test cultures were not sensitive to the hexane solvent extract. The ethyl acetate fruit extracts showed the highest antimicrobial activity against *E. coli* (26mm), while, hexane extract as no antibacterial activity against both Gram-positive and Gram-negative bacteria. Hexane extract has shown the least zone of inhibition around *C. albicans*. Ethyl acetate solvent extract of water apple fruits exhibits the zone of inhibition *P. vulgaris* (12mm), *B. subtilis* (14mm), and *E. coli* (26mm) zone of inhibition around the colonies. The order of effective antibacterial activity of four different solvent extracts is as follows.

The highest antibacterial activity was shown by ethyl acetate extract against *E. coli* (26mm) followed by *C. albicans* (18mm), then by *B. subtilis* (14mm), and the least antibacterial activity was recorded for *S. aureus* (6mm) (Table 2). When compared with the standard antibiotic discs, it was noted that all the ethyl acetate fruit extracts showed better antibacterial activity than the standard antibiotic disc, Ampicillin. But these isolates were not effective, as ciprofloxacin showed the highest zone of inhibition (Figure 2).

Table 1. Phytochemical screening of water apple

Sl. No	Name of the phytochemical test	Hexane fruit extract	Benzene fruit extract	Ethyl acetate fruit extract	Methanol fruit extract
1	Alkaloids	_	_	_	_
2	Flavonoids	+	_	+	+
3	Tannins	_	_	_	+
4	Saponins	_	_	_	_
5	Terpenoids	+	+	+	+
6	Steroids	_	_	_	_
7	Proteins	_	_	_	_
8	Photobatonins	_	_	_	_
9	Carbohydrates	_	+	+	+
10	Anthraquanines	_	_	+	_
11	Phenols	+	+	+	+

Table 2. Antibacterial activity

Name of the organism	Name of the solvent extract				Name of the standard antibiotics	
	Hexane extract	Benzene extract	Ethyl acetate extract	Methanol extract	CF	AMP
C. albicans	4mm	12mm	18mm	10mm	36	9
S. aureus	--	2mm	6mm	--	34	9
E. coli	--	6mm	26mm	4mm	26	--
B. subtilis	--	4mm	14mm	--	16	6
P. vulgaris	--	6 mm	12 mm	2 mm	16	-

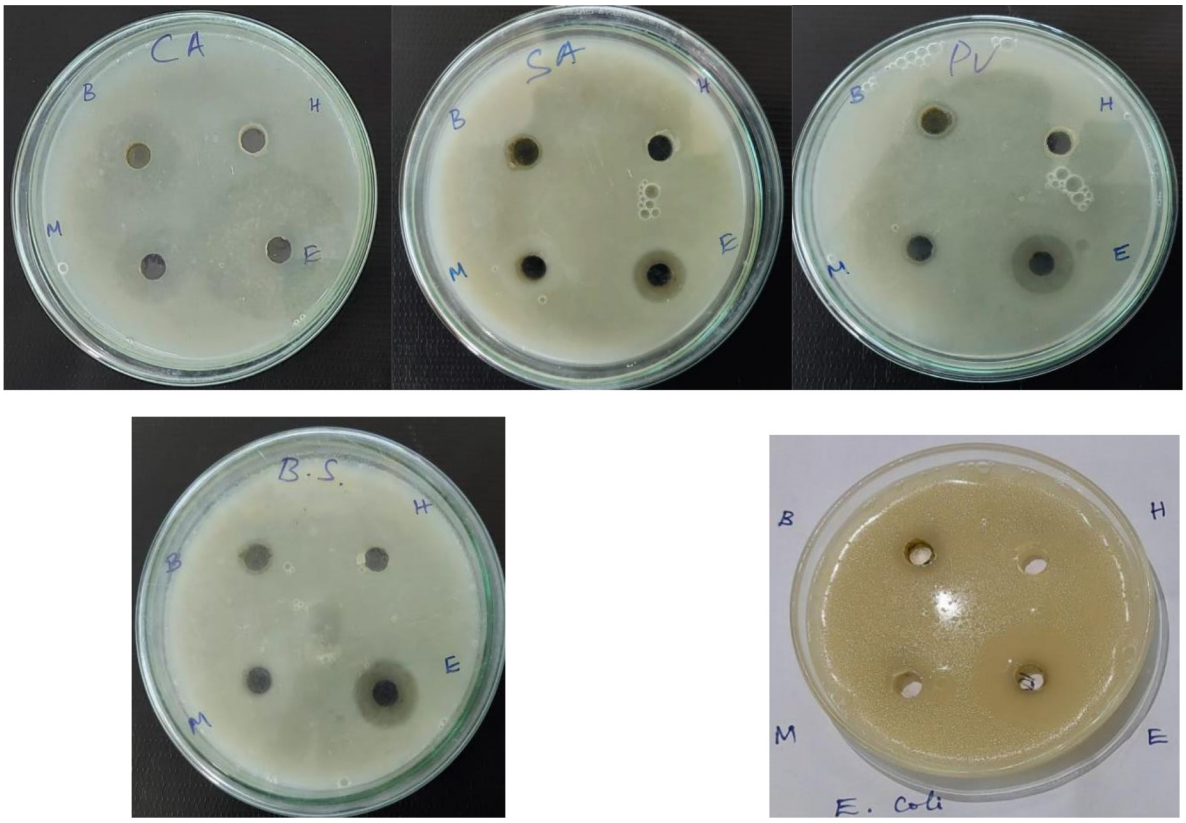


Figure 2. Antibacterial activity.

4. Discussion

Plants include bioactive phytochemicals that can help humans treat a variety of illnesses brought on by malfunctioning biochemical events that normally occur in our bodies as part of metabolism. Herbal medicines play a vital and expanding role in the global pharmacopeia. Ongoing research and testing are enhancing our understanding of their medicinal properties, positioning them as increasingly secure alternatives or preferable choices to allopathic medicine. Presently, there is a revived enthusiasm for traditional medicine, accompanied by a growing demand for pharmaceuticals derived from plant sources. The resurgence of interest in plant-based drugs is primarily attributed to the prevailing belief that "green medicine" is safer and more reliable than expensive synthetic drugs, many of which entail adverse side effects[16]. Plants and plant-based drugs are comparatively less toxic and come with acceptable side effects. Therefore, it is imperative to integrate the use of these remedies into an established framework or a rational, scientifically guided approach to medicine.

Phytochemical screening test showed maximum solubility of flavonoids, including tannins, in methanol solvent. Earlier reports also showed a similar kind of result[17]. Ethyl acetate solvent showed also the presence of flavonoids which was similar with the earlier scientific report[18].

When antimicrobial activity was carried out, it was observed that broad-spectrum activity was observed with the extract when compared with the standards. A similar report was observed with the earlier literatures[19]. The study showed, ethyl acetate extract gave a higher zone of inhibition than methanol extract, and this may be due to the presence of flavonoids, especially and flavonoids play an immense role in the antibacterial effect. A similar result was correlated with the earlier reports[20,21].

5. Conclusion

In the present study, the phytochemical analysis of the hexane, benzene, ethyl acetate, and methanol extract of the fruits of water apple revealed the presence of bioactive phytochemicals. *S. samarangense* fruit extract

was shown to contain bioactive secondary metabolites, including tannins, phenols, flavonoids, steroids, and terpenoids. The fruit extract from *S. samarangense* was quantified, and the results indicated that phenolic chemicals, terpenoids, flavonoids, and carbohydrates were present in good amounts. Good antibacterial activity is provided by the bioactive components found in *S. samarangense* fruit extract.

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7. Author Contribution

Swapna Kethipally: worked on the experimental part and drafted manuscript; Babitha Bobba: conceptulisation and analysis; Ravi Ketipally: sample collection and processing of the method; Samuel John Konatham: extraction process; Rahamtulla: conducted chemical tests and antimicrobial study. All authors have read and agreed to the published version of the manuscript.

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