

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *ATLANTIA MONOPHYLLA* (AM) EXTRACT

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ABSTRACT

Objective: Plants have long been recognised for their wide range of biological properties, including antibacterial, analgesic, anticancer, antipyretic, and antihypertensive action. They are also a significant source of several chemicals with biological activity. The Rutaceae family consists of a small shrub *Atlantia monophylla*. It is available all over India. The leaves were employed as an insect repellent and to alleviate swellings. The root bark has been found to contain atalaphyllinine, atalantin, dehydroatalantin, cycloepitalantin, and atalaphylline 3, 5-dimethyl ether. Essential oil extracted from the leaves was reported to have antimicrobial properties.

Methods: To evaluate the phytochemicals Standard chemical methods for each of the compounds were used – like Tannins (0.1% ferric chloride), saponins (2 ml of water)/flavonoids (with NaOH), Alkaloids (Drangandooff reagent), protein (Million's reagent) steroids (10% sulphuric acid), anthraquinones (aqueous ammonia), Phenols (lead acetate), terpenoids, (3% sulphuric acids) Carbohydrates (Benedict's reagent). Disc plate method was used to evaluate the antimicrobial activity of the extract

Results: The results of this study identified the presence of tannins, flavonoids, alkaloids, proteins, steroids, phenols, terpenoids, and carbohydrates in the AM extract. The microbiological studies revealed better inhibitions of microbes compared to standard drugs.

Conclusion: Tannins, flavonoids, proteins, steroids, phenols, terpenoids, carbohydrates and alkaloids were extracted from the AM extract. The extract has significant microbiological action.

Keywords: *Atlantia monophylla*, Aqueous extract, Antibacterial, Antifungal, Phytochemical analysis

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INTRODUCTION

Plants have long been recognised as having a wide range of biological properties, including antibacterial, analgesic, anticancer, antipyretic, and antihypertensive action [1-5]. They are also a significant source of several chemicals with biological activity. Over the past 2000 years, a substantial percentage of the world has utilised plants extensively for health care and disease treatment, and these data have shown a strong association among conventional therapeutic plant usages and analyses in laboratories [6, 7]. The foundation of phytotherapy is the utilisation of biologically active substances found in plants [8, 9]. The application of plant extracts to slow the growth and decrease the population of the more dangerous infections is the most intriguing [10, 11]. Recent research has centred on the expanding need for plants as essential medications [12, 13]. Moreover, in view of rising antimicrobial resistance, as directed by the World Health Organisation, there is a need to discover novel and effective drugs [14].

The Rutaceae family consists of a small shrub, *Atlantia monophylla*. It is available all over India. The leaves were employed as an insect repellent and to alleviate swellings. The root has been found to contain atalaphyllinine, atalantin, dehydroatalantin, cycloepitalantin, and atalaphylline 3, 5-dimethyl ether [15-17]. Essential oil extracted from the leaves was reported to have antimicrobial properties [18]. Pyropheophorbide, which was isolated from leaves using bioactivity as a guide, exhibited antiviral activity against type 2 of the herpes simplex virus [19]. Roots have been shown to contain antiallergic acridine alkaloids like cycloatalaphylline-A, citrusinine-I, buxifoliadine-E, junosine, and yucocitrine [20]. The essential oil that was extracted from the leaves was documented in the literature [21]. The objective of the current study was to investigate the antimicrobial properties of the aqueous extract of *A. monophylla* obtained from the leaves gathered from AP, India, along with the phytochemical characterisation.

MATERIALS AND METHODS

Plant material

Fresh leaves of *A. monophylla* were collected from the Tirumala Hills region of the Eastern Ghats of Andhra Pradesh, India. It was authenticated and approved by a Botanist. leaves were shade-dried, powdered, sieved, and aqueous extract was prepared and carried out for further studies.

Extract preparation

100 g of powdered powder was suspended in 1000 ml of distilled water to synthesize the aqueous extract. After centrifuging the contents at 2000 x g for fifteen minutes, the supernatant was recovered after the contents had been autoclaved (121 °C, 15 min). After that, dilutions in sterile PBS (phosphate buffer saline) were prepared [22]. The extract was further used for phytochemical analysis and antimicrobial activity. All the chemicals used were of analytical grade, and cell-culture supplies were obtained from HiMedia laboratories Chennai, India.

Phytochemical analysis

The qualitative analysis was performed using standard methods [23].

Test for tannins

1 ml of sample was taken, and two drops of 0.1 percent ferric chloride were added to the sample and observed for brownish green or blue-black colouration, indicating the presence of tannins.

Test for saponins

A sample of 1 ml was taken, and 2 ml of water was added to it. The suspension was shaken in a graduated cylinder for fifteen minutes. A layer of foam indicates the presence of saponins.

Test for flavonoids

1 ml of the sample was taken, and NaOH was added to the sample, observed yellow colour. In subsequent addition, Concentrated hydrochloric acid was added, observed white colour, indicating the presence of flavonoids.

Test for alkaloids

A sample of 1 ml was taken, and two drops of Drangandoff reagent were added. A prominent yellow precipitate indicates the presence of alkaloids.

Test for protein

1 ml of sample was taken, and two drops of Millon's reagent were added. A white precipitate indicates the presence of Protein.

Test for steroids

1 ml of sample was taken, two drops of 10% concentrated sulphuric acid was added and observed for brown colour, indicating the presence of steroids.

Test for anthraquinones

1 ml of sample was taken, and two drops of 10 % aqueous ammonia were added and observed for change in colour. Pink, red, or violet colour in the aqueous layer indicates the presence of anthraquinones.

Test for phenols

1 ml of sample was taken; to that, 3 ml of 10% lead acetate solution was added. A bulk white precipitate formed at the surface indicates the presence of phenolic compounds.

Test for terpenoids

2 ml of chloroform, followed by 3 ml of concentrated sulphuric acid, was added to 0.5 ml of the extract. The formation of red red-brown colour at the interface confirms the presence of terpenoids.

Test for carbohydrates

1 ml of the sample was taken; two drops of Molisch's reagent were added. Carefully layer 1 ml of concentrated sulfuric acid down the side of the test tube, tilting to avoid immediate mixing. A distinct violet-red ring forms within 2 minutes; this indicates the presence of carbohydrates.

Antimicrobial activity**Agar disc diffusion method [24]**

The disc diffusion method on Muller Hinton agar (MHA) medium determined the antibacterial extracts. MHA medium is poured into the

petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swabs moistened with the bacterial suspension. The discs were placed in MHA plates, and 20 µl of sample (Concentration: 1000 µg, 750 µg and 500 µg) were placed in the disc. Gentamicin 20 µl/disc is taken as a positive control. The plates were incubated at 37 °C for 24 h. Then, the antimicrobial activity was determined by measuring the diameter of the zone of inhibition.

The antifungal activity of the Sample was determined by the disc diffusion method on the Sabouraud Dextrose agar (SDA) medium. Sabouraud Dextrose agar (SDA) medium is poured into the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swabs moistened with the fungal suspension. Nystatin 20 µl/disc is taken as a positive control. Samples and positive control of 20 µl (Concentration: 1000 µg, 750 µg and 500 µg) each were added in sterile discs and placed in SDA plates. The plates were incubated at 28 °C for 24 h. Then, antifungal activity was determined by measuring the diameter of the zone of inhibition.

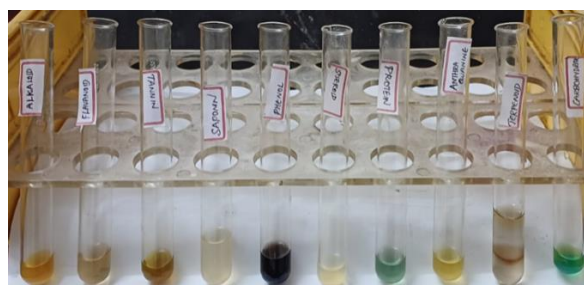
To evaluate Minimum Inhibitory concentration (MIC), 1 ml of sterile IB broth and PDA broth (Luria-bertaini broth for bacterial and potato dextrose agar for fungal) was distributed for every tube and was submitted to autoclave under constant pressure at the temperature of 121 °C. After the broth reaches room temperature add 1 ml of diluted sample in tube 1. Transferred 1 ml from tube 1 to tube 2. The transfer was repeated until tube 8. 100 µl of microbial cultures were added to all the tubes from 1 to 8. Incubation was done at 37 °C for 24 h. After incubation, the turbidity was observed. MIC was determined as the concentration of higher dilution tubes in which the absence of bacterial growth occurs

RESULTS

The qualitative analysis information has been depicted in table 1 and fig. 1. The extract contained tannins, flavonoids, alkaloids, proteins, steroids, phenols, terpenoids, and carbohydrates.

Table 1: Qualitative analysis of *A. monophylla* leaf aqueous extract

Test	Inference
Test for Tannins	Positive
Test for Saponins	Negative
Test for Flavonoids	Positive
Test for Alkaloids	Positive
Test for Proteins	Positive
Test for Steroids	Positive
Test for Anthraquinones	Negative
Test for Phenols	Positive
Test for Terpenoids	Positive
Test for Carbohydrates	Positive

**Fig. 1: Depiction of qualitative phytochemical analysis of *A. monophylla* leaf aqueous extract****Table 2: Minimum inhibitory concentration determination of aqueous extract of the *A. monophylla* leaves against bacterial cultures**

Organisms	Concentration (µg/ml)							
	1000	750	500	250	125	62.5	31.2	15
<i>Staphylococcus aureus</i>	0.092	0.115	0.158	0.201	0.267	0.315	0.384	0.435
<i>Escherichia coli</i>	0.101	0.135	0.178	0.235	0.291	0.364	0.403	0.465
<i>Salmonella</i>	0.113	0.152	0.201	0.265	0.301	0.358	0.399	0.452
<i>Bacillus cereus</i>	0.089	0.145	0.201	0.265	0.301	0.358	0.399	0.452
<i>Klebsiella</i>	0.084	0.124	0.156	0.213	0.278	0.326	0.381	0.446

Table 3: Zone of inhibition of aqueous extract of the *A. monophylla* leaves against bacterial cultures

Organisms	Zone of inhibition (mm)			Gentamicin (20 µl/disc)
	Extract (µg/ml)			
	1000	750	500	
<i>Staphylococcus aureus</i>	15	15	11	20
<i>Escherichia coli</i>	17	14	14	24
<i>Salmonella</i>	22	15	13	30
<i>Bacillus cereus</i>	14	14	11	32
<i>Klebsiella</i>	20	17	12	25

Antibacterial activity

The minimum inhibitory concentration was seen at 1000µg/ml and effective for *Staphylococcus aureus*, *Bacillus cereus* and *Klebsiella*

(table 2). The antibacterial activity of the aqueous extract of the *A. monophylla* leaves showed dose-dependent actions on the bacterial culture zone of inhibition compared to the standard drug Gentamicin (table 3).

Table 4: Minimum inhibitory concentration determination of aqueous extract of the *A. monophylla* leaves against fungal cultures

Organisms	Concentration (µg/ml)							
	1000	750	500	250	125	62.5	31.2	15
<i>Trichoderma viride</i>	0.126	0.143	0.198	0.261	0.304	0.365	0.418	0.483
<i>Penicillium Marneffeii</i>	0.129	0.135	0.169	0.254	0.298	0.365	0.417	0.487
<i>Candida albicans</i>	0.143	0.156	0.201	0.268	0.321	0.386	0.454	0.510

Table 5: Zone of inhibition of aqueous extract of the *A. monophylla* leaves against fungal cultures

Organisms	Zone of inhibition (mm)			Nystatin (20 µl/disc)
	Sample (µg/ml)			
	1000	750	500	
<i>Candida albicans</i>	12	12	10	28
<i>Trichoderma viride</i>	20	18	13	22
<i>Penicillium Marneffeii</i>	15	15	10	23

Antifungal activity

The MIC for aqueous extract of the *A. monophylla* leaves against *Candida albicans*, *Trichoderma viride*, and *Penicillium Marneffeii* has been depicted in table 4. The extract exhibited effective MIC above 1000µg/ml for the microbes as mentioned above. The antibacterial activity of the aqueous extract of the *A. monophylla* leaves showed dose-dependent actions on fungal cultures zone of inhibition, compared to standard drug nystatin (table 5).

DISCUSSION

Due to the large range of phytochemicals, plant extracts have shown remarkable action against infections. There have been few in-depth analyses of these plants' potential as antibacterial agents and phytochemical entities [25-27]. The emphasis is turning to phytomedicines due to antibiotic resistance, negative side effects, and the expensive development costs of synthetic drugs [28-30]. This investigation discovered probable plant species that have historically been used to treat various medical conditions. According to qualitative phytochemical analysis, this plant under investigation contained several phytochemical classes of chemicals, such as flavonoids, tannins, alkaloids, phenols, and steroids. The phytochemicals with the most noticeable visual colour changes in this screening were flavonoids, alkaloids, tannins, and phenols.

Some of the identified compounds, particularly certain flavonoids that were found, have been credited with various ethno-medicinal plants with antibacterial properties. Additionally, the antibacterial properties of certain alkaloids and tannins were widely recognized [31, 32].

Most plant extracts exhibited MIC ranging from 0.6 µg/ml to 5000 µg/ml [33]. In the present study, the zone of inhibition of plant extract against various pathogens selected in this study was nearly comparable to the standard drug Gentamicin 20 µl/disc. The MIC was effective at 1000 µg/ml against *Staphylococcus aureus*, *Bacillus cereus* and *Klebsiella*, but not sensitive to *Escherichia coli* and *Salmonella*.

Many different chemicals with recognized therapeutic qualities are found in medicinal plants. Therefore, significant research was

dedicated to plant-derived antifungals based on the understanding of plants possessing an inbuilt defence system. Another approach to stop the spread of diseases is the medicinal use of such plant products. Several plant extracts have shown strong antifungal properties. The aqueous extract of *Atlantia monophylla* displayed a greater range of antifungal activity on the fungi tested in the current investigation. The plant extract showed antifungal efficacy at 1000 µg/ml compared to nystatin 20 µl/disc. The test to determine fungi's susceptibility with the extract at 1000 µg/ml revealed the following: *Trichoderma viride*>*Penicillium Marneffeii*>*Candida albicans*.

CONCLUSION

The present study demonstrated the antifungal and antibacterial activity of *Atlantia monophylla* aqueous extract along with phytochemical analysis with substantial evidence for its therapeutic potential. There is plenty of potential for investigating how plants alleviate diseases with a more scientific basis as these substances are employed in traditional medicine. Therefore, the chemicals must be isolated, identified, and used in contemporary medicine.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICTS OF INTERESTS

Declared none

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