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PHYTOCHEMICAL EVALUATION OF A MEDICINAL PLANT *ALPHONSEA SCLEROCARPA*, AND ITS ANTIBACTERIAL, AND ANTIOXIDANT ACTIVITY

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ABSTRACT

Plants can be considered as the natural gift to humans with diverse abilities and capabilities incombating and curing diseases apart from being the main source of basic needs. For millennia, man started using various parts of plants such as roots, stems, bark, leaves, flowers, and fruits to curb diseases associated with nutritional defects or bacterial, fungal, and viral infections. The traditional uses of plants and natural preparations derived from them over the centuries have paved the way for investigations of these materials as sustainable medicinal agents.Phytochemicals can be classified into several major groups based on their chemical structure, function, or by the signaling pathway through which they act (1).Based on chemical structure, phytochemicals are classified as Alkaloids, glycosides, Flavonoids, Terpenoids, Tannins, Saponins, Steroids, etc.Various plants have been found beneficial in mitigating various diseases such as arthritis, atherosclerosis, aging, cancer, Alzheimer's disease, Parkinsonism, and other inflammatory disorders. The present research evaluates to screen for preliminary phytochemicals of secondary metabolites in *Alphon seasclerocarpa* leaf extracts.To evaluate the antimicrobial activity using microorganisms such as *S. aureus*, *E. coli*, *S. typhi*, and *B. subtilis,* and the antioxidant activity using the DPPH method of radical scavenging activity of the leaves extract.

INTRODUCTION

Ayurveda is an ancient and age-old practice available even today mainly because of the side effect-free, cost-efficient, natural mode of treatment treating ~65% of the population in developing and underdeveloped countries as of now as per World Health Organization (WHO) estimates more than 20,000 plants were screened globally for identifying indispensable compounds or compositions for identifying the active principles with notable bioactivity. The disease-curing capabilities of plants can be attributed to the presence of a plethora of compounds synthesized as secondary metabolites in lower concentrations as part of their defensive mechanism against various biotic and abiotic stresses. These compounds are commonly called phytochemicals. The present study evaluates the Preliminary phytochemicals of secondary metabolites of leaf extracts and assess the antimicrobial activity by using microorganisms and the antioxidant activity by using the DPPH method of radical scavenging activity of leaf extract.

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Taxonomy of *Alphonsea sclerocarpa*

Kingdom: Plantae
Division : Magnoliophyta
Class: Magnolids
Order: Magnoliales
Family: Annonaceae
Genus: *Alphonsea*
Species: *Sclerocarpa*

MATERIALS AND METHODS

Collection of plant material: The medicinal plant *A. sclerocarpa* leaves were collected from the Srikakulam forest area. The collected plant material was shade-dried for two weeks, powdered, and stored in a dry, cool place for further studies.

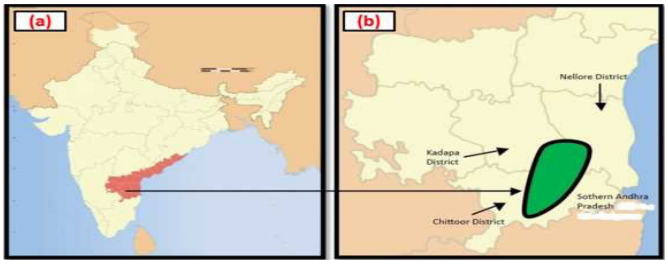


Figure 1. Image (a) corresponds to the map of India highlighted with the state of Andhra Pradesh, and image (b) corresponds to

the Srikakulam forest region along with the location selected for the collection of the plant material.

Soxhlet extraction: The dried plant material was packed into the Soxhlet apparatus, subjected to all the chemicals, solvents, and reagents used were of analytical grade and procured from Merck, Sigma, Sd-fine, and SRL. The successive extraction of solvents, such as hexane, chloroform, ethyl acetate, methanol, and aqueous (Water), is used. For this purpose, the plant material of 300 grams was packed into the extractor and fitted with the apparatus. The respective solvent was filled and the apparatus was operated at 45°C until 35 cycles were run. The experiment was repeated several times with fresh dry powder until an Sample amount of crude extract was collected. After the collection of extract, the remaining plant powder was removed, dried, and once again loaded with another successive solvent, *i.e.*, from non-polar to polar. The isolated fraction of each extract was labeled with their respective solvent names and subjected to further studies.

Qualitative Analysis of Phytochemicals: The extracts of *A. sclerocarpa* were screened to analyze for the presence of the following phytoconstituents like alkaloids, proteins, carbohydrates, phenols, flavonoids, saponins, glycosides, steroids, terpenoids, and tannins.

- **Test for Alkaloids:** The plant extracts were treated with Dragendroff's reagent. The formation of a red precipitate indicates the presence of alkaloids.
- **Test for Proteins (Ninhydrin test):** The plant extracts were treated with 2 ml of 0.2% ninhydrin solution. The presence ofviolet coloration indicates amino acids and proteins.
- **Test for Carbohydrates:** 2 ml of Benedict's solution was added to each extract and boiled. The formation
- **Test for Phenols:**2 ml of 2% ferric chloride solution was added to each extract. Blue-green or purple color formation indicates the presence of phenols.
- **Test for Flavonoids (Alkaline reagent test):** Each plant extract was mixed with 2 ml of 2% NaOH solution. The intense yellow color formed turns colourless-with the addition of a few drops of dilute hydrochloric acid indicating the presence of flavonoids.
- **Test for Saponins (Foam test):** The plant extracts were mixed with 5 ml of distilled water and shaken vigorously. The formation of stable foam showed the presence of saponins.
- **Test for Glycosides (Salkowski's test):** 2 ml of chloroform was mixed with each plant extract. 2 ml of concentrated Sulphuric acid was then added and shaken gently. A reddish-brown color showed the presence of glycosides.
- **Test for Steroids:** Plant extracts were mixed with 2 ml chloroform and concentrated sulphuric acid. A red color formed at the chloroform layer indicates the presence of steroids.
- **Test for Terpenoids:** 2 ml of chloroform was added to each extract and mixed well. 3 ml of concentrated sulphuric acid was added to each tube. The formation of a reddish-brown color indicates the presence of terpenoids.
- **Test for Tannins (Gelatine test):** 1% gelatine containing sodium chloride was treated with each plant extract. The formation of a white precipitate showed the presence of tannins.

Antimicrobial activity: Antimicrobial activity was assessed for different extracts such as Chloroform, Methanol, and Aqueous (Water) extracts. The antimicrobial activity includes testing inhibiting efficacy against both Gram-negative and Gram-positive bacteria.

Antibacterial activity: Antibacterial activity was analyzed by agar well diffusion method. The antibacterial activity was carried out against different Gram-positive bacteria such as *Bacillus subtilis* (MTCC No.-10407), *Lactobacillus acidophilus* (MTCC No.-10307), *Staphylococcus aureus* (MTCC No.-6908), and *Streptococcus mutans* (MTCC No.-890). Similarly, antibacterial activity against different Gram-negative bacteria was carried out, which includes *Escherichia coli* (MTCC No.-44), *Pseudomonas aeruginosa* (MTCC No.-1034), *Klebsiella pneumonia* (MTCC No.-9024) and *Proteus vulgaris* (MTCC No.-744). Nutrient agar (NA) media was prepared, sterilized, poured into the Petri plates, and allowed to solidify. 24-hour cultures were spread on the plates with the help of L-shaped rods and wells were made by using a 6 mm cork borer on each plate. The wells were filled with 100µL of 50mg concentrated extract (Chloroform, Methanol, and Aqueous) separately. DMSO alone was used as the control. Streptomycin was used as a positive control. The plates were kept in an incubator at 37°C. After 24 hours of incubation, each plate was examined for inhibition zones (6).

Antioxidant activity: The antioxidant activity of the extracts was determined using the free radical scavenging method, using DPPH (1, 1-diphenyl-2-picrylhydrazyl), and estimating the ion-reducing power through the FRAP assay (ferric-reducing antioxidant power).

DPPH method: For estimating the antioxidant activity using DPPH, different concentrations of the extract such as 100µg/mL, 200µg/mL, 300µg/mL, 400µg/mL, and 500µg/mL were dissolved in DMSO followed by addition of 4mL of the 0.004% (w/v) DPPH dissolved in Methanol. The reaction mixture was incubated in the dark for 30 minutes. Ascorbic acid was used as a standard. The absorbance was measured at 517nm using a Thermo scientific UV-visible spectrophotometer (7). The DPPH scavenging activity (%) was calculated as per the formula.

RESULT AND DISCUSSION

Phytochemical screening: The leaves of *A. sclerocarpa* are the richest source of various phytochemicals. Out of different phytochemicals screened from the Chloroform extract, Methanol extract, and aqueous extract, the Methanol and aqueous extract showed a notable large variety of phytochemicals. Alkaloids and flavonoids were present in all the extracts, and Carbohydrates were confined to the aqueous extract. Saponins are found in the Methanol and Aqueous fractions. Steroids are present in the two extracts used for analysis, but not in the Aqueous extract. Tannins were present in the Methanol and Aqueous extracts. Terpenoids were found in the Methanol and Aqueous extract. There are no results for the anthra quinones. The detailed identification is reported in Table 1

Table 1. Preliminary phytochemical screening from the leaves of *A. sclerocarpa* using different solvents. *[(+) = Presence/Detected, (--) = Absent/ Not detected].

Sl. No	Phytochemical screened	Chloroform extract	Methanol extract	Aqueous extract
1	Alkaloids	+	+	+
2	Carbohydrates	-	-	+
3	Flavanoids	+	+	+
4	Saponins	-	+	+
5	Soluble starch	-	-	+
6	Steroids	+	+	-
7	Tannins	-	+	+
8	Terpenoids	-	+	+

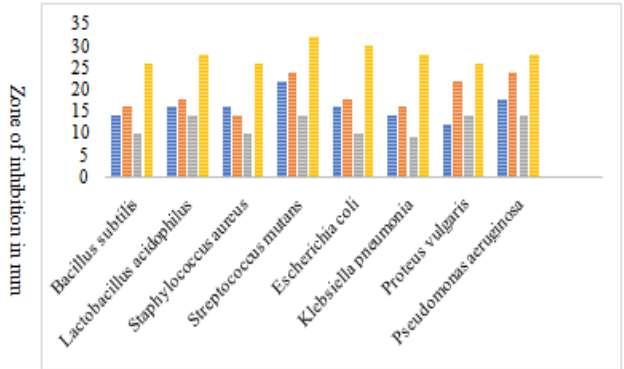
Antimicrobial activity: To identify the potential antimicrobial activity of *A. sclerocarpa,* the crude extracts obtained from different solvents, such as Chloroform, Methanol, and Aqueous (water) extracts, were subjected to test the inhibition activity against Gram-positive, Gram-negative bacteria.

Antibacterial activity: The antibacterial activity was carried out against both Gram-positive and Gram-negative bacteria. The bacteria used for screening antibacterial activity include *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, *Streptococcus mutans.* The highest inhibiting activity was observed for chloroform extract against *Streptococcus mutans* with an inhibition zone of 22mm. The Methanol extract also showed good potential antibacterial activity against *Streptococcus mutans* with an inhibition zone of 24mm. The *Bacillus subtilis* was inhibited more by the Chloroform crude with an inhibition zone of 18mm. *Lactobacillus acidophilus* was inhibited more by the Methanol extract with an inhibition zone of 18mm. *Staphylococcus aureus* was inhibited more by the Chloroform fraction with an inhibition of 20mm. *Escherichia coli* was inhibited more with the extract obtained from Chloroform with a zone of inhibition of 18mm. *Klebsiella pneumonia* was inhibited with the Chloroform extract with a zone of inhibition of 14mm. *Proteus vulgaris* was inhibited more by the Methanol extract with a zone of inhibition of 22mm. The Aqueous extract did not show much activity when compared with the other solvents. *Streptococcus mutans* was inhibited more when compared to other bacteria with a zone of inhibition of 22mm. *E. coli* and *Klebsiella pneumonia* showed the least inhibition with the Aqueous extract with a zone of inhibition of 9mm.

The antibacterial activity images of three strains used in the present study were represented, which comprises *Streptococcus mutans* (a, b, c, d), *Proteus vulgaris* (e, f, g, h), *Pseudomonas aeruginosa* (i, j, k, l). All two extracts, i.e., the Chloroform and the Methanol extract, the antibacterial activity images were represented. Table 2 illustrates the zone of inhibition of different bacterial strains used with their respective solvents and comparison with the standard drug Streptomycin.

Table 2. Antibacterial activity of *A. sclerocarpa* different extracts

Sl.No	Name of the bacteria	Zone of Inhibition in mm			
		Chloroform extract	Methanol extract	Aqueous extract	Control Streptomycin
1	*Bacillus subtilis*	14	16	10	26
2	*Lactobacillus acidophilus*	18	18	14	28
3	*Staphylococcus aureus*	20	14	10	26
4	*Streptococcus mutans*	22	24	14	32
5	*Escherichia coli*	18	18	10	30
6	*Klebsiella pneumonia*	14	16	9	28
7	*Proteus vulgaris*	12	22	14	26
8	*Pseudomonas aeruginosa*	18	24	14	28



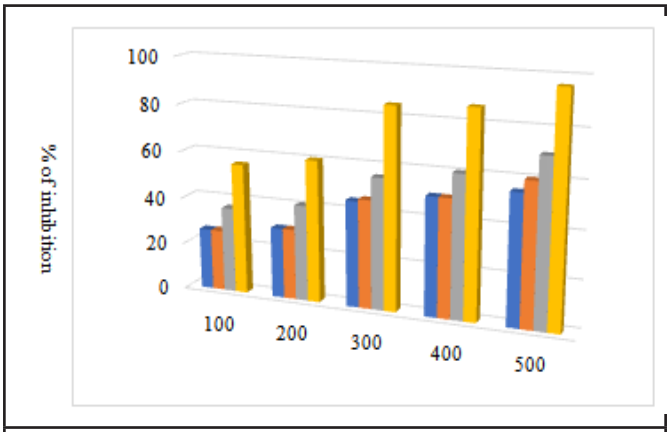
Graph 1. Antibacterial activity of *A. sclerocarpa*

Antioxidant activity: The antioxidant activity of *A. sclerocarpain* different extracts such as Hexane extract, Chloroform extract, Ethyl acetate extract, Methanol, and aqueous extract was evaluated. The free radical generated was scavenged by the antioxidants present in the different extracts. At 500 µg/mL concentration, the Methanol extract showed the highest % of scavenging which is noted as 68.12%. The least antioxidant activity is observed in the Hexane extract. The variation in different extracts of plants is due to dissolving the different

phytochemicals in the solvents, which varies from one solvent to the other. Hence, from the results of antioxidant activity, it is confirmed that the Methanol extract of *A. sclerocarpa* has potent antioxidant activity and the fraction is the richest source of diverse phytochemicals. The results of antioxidant activity are represented in Table 4 and Graph 3.

Table 3. DPPH radical scavenging antioxidant activity of *A. sclerocarpa* leaves different solvent extracts

Parameter	% of Scavenging			
Conc. (µgl /mL)	Chloroform extract	Aqueous extract	Methanol extract	Standard Ascorbic acid
100	26.03	26.08	36.40	55.67
200	30.02	30.02	40.80	60.25
300	45.00	46.00	55.60	85.08
400	50.07	50.08	60.30	86.51
500	55.03	60.08	70.12	96.26



Graph 2. Antioxidant activity (DPPH method) of different extracts of *A. sclerocarpa*

CONCLUSION

The therapeutic applications and notable evidence for the plethora of phytochemicals of the plant *A. sclerocarpa***.** The present research evaluates to screen for preliminary phytochemicals of secondary metabolites in *A. sclerocarpa* leaf extracts such as alkaloids, flavonoids, saponins, tannins, and phenolic compounds. The phytochemicals can be extracted from plants using different conventional and nonconventional methods. The conventional methods include soxhlet extraction, percolation, maceration, decoction, etc. To evaluate the antibacterial activity by using microorganisms such as *S. aureus, E. coli, S. typhi,* and *B. subtilis.* The antioxidant activity by using the DPPH method of radical scavenging activity of the leaf extract.

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