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Ethnopharmacology, Antioxidant Activities, and Phytochemical Screening of Bioactive Extracts from the Seeds of *Alangium Salvifolium*, A Medicinal Plant

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ABSTRACT

Phytochemical with antioxidant properties have great potential to combat plant and human diseases. This study aims to analyze the effects of different phytochemical constituents on different solvents on their extraction yield and also to determine the bioactive phytochemical and the amount of antioxidant activity of *Alangium salvifolium* seeds. For this study, the seeds of *Alangium salvifolium* were extracted, analyzed for phytochemical constituents, and evaluated for their antioxidant activity. Chemical analysis of phytochemical constituents was performed according to standard protocols. The antioxidant capacity of the plant extracts was measured using several in vitro techniques, including the DPPH radical scavenging assay and SOS activity. The plant extracts were subjected to phytochemical screening to determine their potential for antioxidant activity and the presence of bioactive components. The results of the current study show the ethanolic extract of Alangium salvifolium seeds showed remarkable antioxidant properties. *Alangium salvifolium* is a remarkable medicinal plant that contains a variety of bioactive secondary plant compounds and can be used to prevent several diseases. Recent research suggests that plant-derived antioxidants that scavenge free radicals may be useful in treating chronic diseases caused by free radicals.

Keywords: Phytochemical screening; Antioxidant Activity; Bioactive Extracts; medicinal plants; free radicals

1. INTRODUCTION

Plants can produce a wide range of compounds with important biological functions1. These bioactive components and natural antioxidants are critical for disease prevention and treatment2, and medicinal plants may be a good source of these compounds. Many diseases are treated with the help of medicinal plants, and many of these plants are included in traditional remedies and country-specific systems of medicine such as Ayurveda used by the Indian people.

The medicinal plant *Alangium salvifolium* belongs to the Alangiaceae family. In India, China, and the Philippines, Alangium salvifolium is widely used as a medicinal plant. With a variety of biological activities, it is one of the most versatile medicinal plants. Antioxidant, anti-inflammatory, antibacterial and anti-cancer properties have been demonstrated for *Alangium salvifolium*[3]. It is one of the most active and adaptable medicinal plants[4]. Almost all parts of *Alangium salvifolium* are studied to have essential therapeutic and significant applications for various purposes in ancient herbal medicine[5]. The plant *Alangium salvifolium* is used as a reliable, consistent, naturally occurring antioxidant, especially phytochemical such as phenolic compounds, flavonoids, tannins, etc. According to preliminary phytochemical analyzes, various phyto chemical are present in plants[6]. They impart organoleptic properties and colour to the plant.Plant produce many antioxidants to control reactive oxygen species build-up in cells and the imbalance in their production lead to oxidative stress[7]. Cancer, diabetes, oxidative stress, and other diseases could be treated with this combination of herbs. The study's goal was to evaluate the phytochemical and antioxidant properties of *Alangium salvifolium* seed.

2 MATERIAL AND METHODS

2.1 Plant Material

Seed Sample collected Between February -March 2021 from the garden of Govt. Autonomous Ashtang Ayurveda College Department of Education Dravyaguna Indore, India. plant identified and authenticated by an expert Dr. Hariom Parihar Assistant Professor & Head of Department of Dravyaguna Autonomous Ashtang Ayurveda College Indore, India. A portion of the gathered seeds of the plant *Alangium salvifolium* was dried, cleaned, and ground to a fine powder using a fine grinding machine to a powdery consistency. In an airtight container, the powder was stored until further study.

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2.2 Extract Preparation

powdered seeds of *Alangium salvifolium* were placed in a thimble of a Soxhlet apparatus. The extraction was carried out using various organic solvents, ethanol, ethyl acetate, and Petroleum ether. The extraction time was 8-10 hours, and the temperature of the heater was set at 40-60°C. The sample extract was filtered and concentrated to dry the time after extraction. The extracted materials were stored in an airtight container[8]. The extraction yields of all extracts were calculated.

2.3 Phytochemical Screening

Standard techniques were used to conduct phytochemical analysis on both extracts to determine the presence or absence of different bioactive components (phytochemical) in extracts of *Alangium salvifolium* seeds using standard procedures[9].

2.4 Antioxidant Activity

2.4.1 DPPH free radical scavenging assay

According to a standard approach10,"we were able to calculate the free radical scavenging capability of each extract. There was made a DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in methanol at a concentration of 0.1 mM. All Alangium salvifolium seed/standard extracts were freshly diluted in a 1 mg/mL methanol solution. A 1 mg/mL stock solution was prepared by diluting 1 mg of the extract of seeds/standard ascorbic acid solution with methanol. Several test tubes were filled with methanol to a volume of 1 mL, and then various amounts of the extract/standard solution (20-100 µl) were added. 2 millilitres of a 0.1mM DPPH reagent solution was added and properly blended with the sample. After thirty-minute incubation at room temperature in the dark, the absorbance was measured at 517 nm. As a reference, we gathered a 0.1 mM DPPH solution and left it to incubate in the dark at ambient temperature for 30 minutes. Methanol was used as a blank in an absorbance measurement at 517 nm to establish a standard". With the help of the methodology, we determined the percentage of antioxidant activity and the 50% inhibitory concentration of the sample/standard solution (IC50).

2.4.2 Superoxide Anion Radical Scavenging Activity

The radical scavenging activity of superoxide anions may be computed using a tried-and-true approach11One millilitre of a nitroblue tetrazolium (NBT) solution (100 µl of NBT in 100 mM phosphate buffer, pH 7.4) was added to methanol-containing extracts of Alangium salvifolium seeds (sample) at concentrations of 20, 40, 60, 80, and 100 µg/mL. After adding 1 mL of phenazine methosulfate (PMS) (60 µl /100 mM phosphate buffer, pH 7.4), the reaction was set in motion. We let the reaction mixture settle at 30 degrees Celsius for 15 minutes after adding the catalyst. Absorbance was measured at 560 nm using a spectrophotometer. The incubation process without the sample (extract) was used as a comparison. Ascorbic acid was used as a standard against which the other samples could be evaluated. The superoxide anion's absorption activity increases as the reaction mixture absorbs less light.

3. STATISTICAL ANALYSIS

To ensure accuracy, analyses were performed on triplicate samples. Statistics were presented in mean and standard deviation form. Significant connections between experimental parameters and IC50 value were determined using linear regression analysis. All of the statistical calculations and charts were created in Microsoft Excel. As seen in the figure, the concentration of the extracts is a direct function of their ability to prevent growth.

4. RESULTS

When studying plant materials for potential medicinal chemicals, the initial step is extraction. The correct solvent may be used to extract plant components with varying polarity. Traditional solvent extraction techniques for obtaining bioactive chemicals from plants have been the subject of much research. The extracted information looks like this:

4.1 Percentage Yield

There were 354 g of *Alangium salvifolium* seeds utilized. *Alangium salvifolium* seed extract yielded 0.22% (0.765 g) and 1.75% (5.68 g) in ethanol and ethyl acetate, respectively, after being extracted from the seeds using these two solvents.

Table 1. percentage yield of *Alangium salvifolium*

S.No.	Solvent	Color of extract	Theoretical weight(gm)	Yield in gms	% Yield
1.	Pet.Ether	Transparent	354	No yield	-
2.	Ethyl acetate	Brown	335.76	0.765	0.22
3.	Ethanol	Brown	324.8	5.68	1.75

4.2 Solubility Determination

Table 2. Solubility Determination of *Alangium salvifolium* Extract

S.No.	Solvent	Ethyl acetate	Ethanol
1	Water	Insoluble	Slightly soluble
2	Ethanol	Insoluble	Soluble
3	Chloroform	Soluble	Slightly soluble
4	DMSO	Soluble	Soluble
5	Petroleum Ether	Slightly soluble	Insoluble

4.3 Phytochemical Analysis

Qualitative phytochemical analysis findings of both (ethyl acetate and ethanol) extracts of *Alangium Salvifolium* seeds are given in Table 3. Most of the phytochemical were found in extracts, according to the findings.

Table 3. Qualitative Phytochemical analysis of *Alangium salvifolium* Extract

S. No.	Experiment	Result	
		Ethyl acetate	Ethanol
Carbohydrates Test			
1.	Molisch's Test	-	+
2.	Fehling's Test	-	+
3.	Benedict's Test	-	+
4.	Bareford's Test	-	+
Test for Alkaloids			
1.	Mayer's Test	-	+
2.	Hager's Test	-	+
3.	Wagner's Test	-	+
4.	Dragendroff's Test	-	+
Test for Terpenoids			
1.	Salkowski Test	+	+
2.	Libermann-Burchard's Test	+	+
Test for Flavonoids			
1.	Lead Acetate Test	+	+
2.	Alkaline Reagent Test	+	+
3.	Shinoda Test	+	+
Test for Phenolic Compounds and Tannins			
1.	$FeCl_3$ Test	+	+
2.	Lead Acetate Test	+	+
3.	Gelatine Test	+	+
4.	Dilute Iodine Solution Test	+	+
Test for Saponins			
1.	Froth Test	+	-
Test for Protein and Amino acids			
1.	Ninhydrin Test	+	+
2.	Biuret's Test	+	+
3.	Million's Test	+	+
Test for Glycosides			
1.	Legal's Test	-	+
2.	Keller Killiani Test	-	+
3.	Borntrager Test	-	+

4.4 In-vitro Antioxidant Activity

In the present investigation, extracts' antioxidant activity in vitro was evaluated by DPPH radical scavenging activity, and SOS activity, of *Alangium salvifolium* seeds. The results are summarized in Tables 4.

4.4.1 DPPH Assay

The IC50 value for the scavenging of DPPH radicals in ethanolic and ethyl acetate extracts of *Alangium salvifolium* seed was determined to be 75.41 and 33.9 g/mL, and exhibited percent inhibition 62.40% and 81.72% respectively. The standard inhibitory concentration (IC50) for ascorbic acid was found to be 20.73 g/mL, with an inhibition efficiency (IE) of 86%.

Table 4. DPPH radical scavenging activity of Ascorbic acid

Concentration (µg/mL)	Absorbance	% Inhibition
20	0.465	51.71339564
40	0.403	58.15160955"
60	0.354	63.23987539
80	0.278	71.13187954
100	0.134	86.08515057
Control	0.963	
IC50		20.73

Table 5. DPPH radical scavenging activity of Ethyl acetate extract

Concentration (µg/mL)	Absorbance	% Inhibition
20	0.718	25.44132918
40	0.637	33.85254413
60	0.552	42.67912773
80	0.473	50.88265836
100	0.362	62.40913811
Control	0.963	
IC50		75.41

Table 6. DPPH radical scavenging activity of Ethanolic extract

Concentration (µg/mL)	Absorbance	% Inhibition
20	0.523	45.69055036
40	0.469	51.298027
60	0.394	59.08618899
80	0.248	74.24714434
100	0.176	81.72377985
Control	0.963	
IC50		33.9

4.4.2 SOS Radical Scavenging Activity

There was SOS activity in both the ethyl acetate and ethanolic extracts of *Alangium salvifolium* seed, with a percent inhibition of 58.59 and 75.66, respectively, and an IC50 value of 79.95 and 48.45 g/mL.

Table 7. SOS radical scavenging activity of Ascorbic acid extract

Concentration (µg/mL)	Absorbance	% Inhibition
20	0.411	52.59515571
40	0.363	58.13148789"

60	0.287	66.89734717
80	0.219	74.74048443
100	0.123	85.81314879
Control	0.867	
IC50		17.54

Table 8. SOS radical scavenging activity of Ethyl acetate extract

Concentration (µg/mL)	Absorbance	% Inhibition
20	0.673	22.37600923
40	0.548	36.79354095
60	0.496	42.79123414
80	0.413	52.3644752
100	0.359	58.5928489
Control	0.867	
IC50		76.86

Table 9. SOS radical scavenging activity of Ethanolic extract

Concentration (µg/mL)	Absorbance	% Inhibition
20	0.487	43.82929642
40	0.404	53.40253749”
60	0.345	60.20761246
80	0.283	67.35870819
100	0.211	75.66320646
Control	0.867	
IC50		34.2

5. DISCUSSION

The results of the study show that plants contain natural antioxidants that have a positive effect on maintaining health, treating diseases, and mitigating the harmful effects of toxic substances Plants have always been a rich source of phytochemical as well as various biological activities. The medicinal properties of plants depend on the phytochemical such as phenols, terpenoids, or alkaloids. Extraction of *Alangium salvifolium* seeds was carried out using different solvents such as ethanol, ethyl acetate, and petroleum ether.

Qualitative analysis is very important to identify the phytochemical constituents of medicinal plants. Plants are valuable as medicines because they contain specific bioactive components. The current investigation, the preliminary phytochemical investigation was carried out in the ethanolic and ethyl acetate extracts of *Alangium Salvifolium* seeds Qualitative phytochemical investigation of *Alangium Salvifolium* seeds reveals the presence of proactive components like carbohydrates, flavonoids, alkaloids, amino acids, polyphenols, tannins, glycosides, and steroids showed in table 3. A Qualitative study of phytochemical of *Alangium Salvifolium* seeds was assayed by various methods[12,13,14]

ROS causes oxidative deleterious effects on cellular components. Antioxidants play an important role in protecting our body from diseases by reducing ROS levels in the body[15,16]. Recent analysis suggests that the antioxidant potential of plants has therapeutic significance in diseases caused by free radicals, such as diabetes, cancer, gastrointestinal diseases, cardiovascular diseases, aging and so on. It is well known that free radicals play a role in various diseases. Antioxidants protect humans from various diseases by scavenging free radicals. They act by removing reactive oxygen species from the environment or defending antioxidant defence mechanisms[17,18].

Antioxidant activity can be measured in vitro by various assays, including DPPH radical scavenging activity and activity SOS. Stronger radical scavenging activity is associated with lower reaction mixture absorption, while stronger reducing power is associated with higher reaction mixture absorption.

Where as a stronger reducing power is associated with higher absorption of the reaction mixture. In this study, the antioxidant properties of the seed extract of *Alangium salvifolium* were investigated in vitro. Both ethyl acetate and ethanolic extract of *Alangium salvifolium* seeds showed significant scavenging activity for DPPH radicals, with the percentage of inhibition being 62.40 and 81.72, respectively, and the IC50 value calculated to be 75.41 and 33.9 µg/ml, respectively(table 5,6). Ascorbic acid was used as the control chemical, which had an IC50 value of 20.73 g/mL and an inhibition percentage of 86% (table 4). Similarly, ethyl acetate and ethanolic extract of *Alangium salvifolium* seeds SOS showed activity, exhibiting percentage inhibition of 58.59% and 75.66%, and IC50 value of 76.86 and 34.2µg/mL, respectively (table 9, 8). Ascorbic acid was used as the control chemical, which had an IC50 value of 17.54 g/mL and an inhibition percentage of 85.81% (table 7). An effective predictor of the antioxidant activity of a compound in seeds is its reducing power. Ascorbic acid, which has antioxidant properties, was used as a reference in the study. Antioxidants are compounds with lowering ability because they may donate electrons to neutralize reactive oxygen species produced during lipid peroxidation. These results suggest that oxidative stress and its effects may be averted by the use of plant extracts that contain antioxidants.

The ethanolic extract showed strong reducing power compared to the ethyl acetate extract. As a result, both extracts showed concentration-dependent activity. This study suggests that the extracts (ethanol and acetate) from the seeds of *Alangium salvifolium* could be very important for the treatment of various diseases caused by free radicals. Therefore, it can be concluded that the extract from the seeds of *Alangium salvifolium* can reduce free radicals.

6. CONCLUSION

In the present study, the seeds of the selected

plant *Alangium salvifolium* showed significant bioactive components and antioxidant activity. The significance of the study lies in its contribution to the development of innovative plant phytochemical that could serve as a natural antioxidant source and for use in ethnomedicine. As a result, this research showed how different solvents affect the extraction of phytochemical and antioxidant properties. The high content of phytochemical and antioxidant activity of *Alangium salvifolium* seeds support their use in ethnomedicine, and their ability to maintain health, and treat a variety of diseases. *Alangium salvifolium* seeds have great potential to combat oxidative stress. Therefore, it is recommended to expand its use for health maintenance and replace synthetic antioxidants with natural ones. It reduces the use of chemotherapeutic agents and thus avoids the side effects of chemical-based medicines without interfering with the biochemical functions of healthy cells when those chemicals are encountered. The discovery of novel treatments, the standardization and validation of existing herbal remedies, and other associated issues, however, all require a completely integrated approach.

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She has contributed to experimentation, data analysis, and interpretation for this work.