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Evaluation for Antimicrobial and Antioxidant Activity of Methanolic, Chloroform and Aqueous Extracts of *Alangium salvifolium*

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Abstract: This research work has been conducted to explore antimicrobial efficacy and ethno botanical analysis of plant *Alangium salvifolium* and to find an active constitute against different microorganism. The plant *Alangium salvifolium* also known as Alangium it generally fond in tropical regions like India, Australia, and some parts of Asia. It is used in traditional medicine due to its rich chemical composition. Alangium salvifolium contains alkaloids, glycosides, flavonoids, steroids, triterpenoids, tannins, and other compounds. Different parts of the plant, including the leaves, roots, bark, and seedsare used for their medicinal properties. Plant consist of anti-inflamatory, antipyretic,and anticancer activities. The plant is also used to treat many disease like rheumatism, leprosy, gastric ulcers, wound healing, epilepsy, scabies, and more. In the present work we find the different extracts of plant alangium salvifolium has significant anti microbial and anti oxidant activity against some microorganism.

Key words - Alangium salvifolium, methanolic extracts, antimicrobial activity, antioxidant.

1. INTRODUCTION

Plants have been a cornerstone of medicine for millennia, and importance of it is continues to grow. The World Health Organization (WHO) estimates that 80% of the global population relies on traditional medicine, much of it plant-based, for primary healthcare(Ouedraogo et al. 2012). Over 21,000 plant species are recognized for their medicinal uses, highlighting the vast potential of the plant kingdom(Patel et al. 2012). India, with its rich history of Siddha, Unani, and Ayurveda, exemplifies this tradition. A critical area of research is the use of phytochemicals to combat antimicrobial resistance. The rise of drug-resistant pathogens poses a significant threat to global health, and plant-derived antimicrobials offer a promising source of new antibiotics(Kasote et al. 2015). Researchers are actively screening various plant species, especially those used in traditional medicine, to identify novel antibacterial compounds. Traditional medicine systems, with their long history of practical application, are a valuable resource in this search. Many traditional remedies have already shown effectiveness against antibiotic-resistant bacteria, highlighting the importance of further research in this area. The exploration of plant-based medicines offers a potential solution to the growing challenge of drug resistance and reinforces the enduring value of natural remedies(Hongal et al. 2024).

The effectivity of antimicrobial agent is based on its specific inhibitory activity and mechanism on particular microorganism. There is no single antibacterial agent which can work against all microorganisms due to their diverse physiological characteristics. Microorganisms can be categorized based on their oxygen requirement (aerobes or anaerobes), cell wall composition (Gram-positive or Gram-negative), growth stage (spores or vegetative cells), optimal growth temperature, and acid/osmosis resistance, etc. These characteristics influence the effectiveness of different antimicrobial agents.In addition to microbial characteristics, the antimicrobial agent's characteristics also play a role in its efficacy. Someantimicrobial agents inhibit essential metabolic or reproductive pathways of microorganisms, while some are target the cell wall or membrane synthesis pathway.

The plant *Alangium salvifolium*, is generally fond in tropical regions like India, Australia, and some parts of Asia(Prakash et al. 2013). It is used in traditional medicine due to its rich chemical composition. Alangium salvifolium contains alkaloids, glycosides, flavonoids, steroids, triterpenoids, tannins, and other compounds(Prakash et al. 2013). Different parts of the plant, including the leaves, roots, bark, and seedsare used for their medicinal properties(Shravya, Vinod, and Sunil 2017). Plant consist antioxidant, anti-inflamatory, antipyretic, antimicrobial, and anticancer activities. The plant is also used to treat many disease like rheumatism, leprosy, gastric ulcers, wound healing, epilepsy, scabies, and more(Singh, Singh, and Vidyasagar 2014). Alangium species have a rich history in traditional medicine across Asia, particularly in China, India, the Philippines, and Thailand. Different parts of various Alangium species have been employed to treat a wide range of ailments(Nasrullah et al. 2015).

Alangium salvifolium is a small tree which have 3-10 meters height. It has spiny branches, pale brown bark, and numerous holes on its trunk. The leaves are alternate, simple, and lack stipules. They are narrowly oblong ovate-lanceolate in shape and have a glabrous surface. The petiole is up to 1.5 cm long and hairy. The flowers are bisexuals, regular and have 5-10 metros parts. They are white in color and arranged in axillary fascicles. The fruits are small, nearly globular and purplish-red when ripe. They are crowned by a persistent calyx-limb. The flowering season is from February to June(Ratra and Gupta 2015). It is native to tropical Australia, India, Madagascar, Western Africa Southern and western Pacific Ocean islands Eastern Asia (China, Malaysia, Indonesia, India, and

Philippines) and New Caledonia. In India, it is found various location including Hyderabad forests, Sitamata wildlife sanctuary, Rajasthan, Chittoor district of Andhra Pradesh like Tirupati, Talakona, Chandragiri and Aragonda(Ratra and Gupta 2015).

2. MATERIALS AND METHODS

2.1 Collection of plant:

Plant leaves of *Alangium Salvifolium*were collected from Botanical garden of O.R.B.M.S.college campus, Tankara, Morbi, Gujarat, India. Plant leaves collected during the month of December. The collected plant specimens were authentically identified. Collected materials were brought to the laboratory, wasShed with tap water followed by distilled waterand dried leaves under sun light for 4-5 days. The dried leaves were homogenized to a fine powder about 350gm weighted and stored in an airtight bottle.

2.2 Preparation of Methanolic extraction of *Allangium salvifolium*

About 150 gm of dried leaf powder placed in thimble with covered the filter paper. Soxhlet extractor is placed onto flask containing 99% Methanol.Soxhlet equipped with a condenser. The solvent is heated with heating mantle. The solvent vapor travels up a distillation arm and floods into the chamber housing the thimble of solid. Dry powder in chamber slowly fills warm solvent. Desired compound dissolved in the warm solvent. When the soxhlet chamber is almost full, the chamber is emptied by the siphon and solvent running back down to the distillation flask. This process continuous until the leaf powder reduced its color green to slight colorless.

2.3 Preparation of Chloroform extraction of *Alangium salvifolium*

About 150 gm of dried leaf powder placed in thimble with covered the filter paper. Soxhlet extractor is placed onto flask containing 99% Chloroform. Soxhlet equipped with a condenser. The solvent is heated with heating mantle. The solvent vapor travels up a distillation arm and floods into the chamber housing thimble contain dry powder. Dry powder in chamber slowly fills with solvent.

2.4 Preparation of Aqueous extrect of *Alangium salvifolium*

An aquous extract involes taking dry leaves powder about 15 gram in flask and add 250ml distilled water. Cover the neck of flask with copper foil and place the flask in rotatory shaker machine for about 1 hour. And then filter the mixture though filter paper to obtain the final extract.

2.5 Phytochemical analysis of Methanolic, Chloroformand Aqueous extractions

The dried pulverized plant material (15g) was extracted with double distilled water. The aqueous extracts were filtered using Whatman No.1 filter paper and the qualitative phytochemical analysis for the presence of tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides and steroids was carried out immediately without storage according to standard procedures(Muthoni Guchu et al. 2020).

2.5.1Tannin test

Take 2ml different extract in test tube which is tested. Then, three drops of ferric reagent were added to the sample except blank tubes. Observe grey or black color formation which is indicates presence of tannin(Muthoni Guchu et al. 2020).

2.5.2 Phlobatannin test

Two millilitres (2 mL) of the solution of the extract were added into dilute HCl and observed forred colored precipitate that was indicate presence of the phlobatannin(Muthoni Guchu et al. 2020).

2.5.3 Saponin test

Foam test: 2 ml solution of extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes.Observe the of stable foam devepment suggests the presence of saponins(Muthoni Guchu et al. 2020).

2.5.4 Flavonoids test

Alkaline reagent test: Two to three drops of sodium hydroxide were added to 2 mL of each extract. Initially, a deep yellow color appeares but it gradually become colorless by adding few drops of dilute HCL, indicating that flavonoids were present(Muthoni Guchu et al. 2020).

2.5.5 Terpenoid test

Salkowski test was used to detect terpenoids. Extract (2ml) was mixed with chloroform (1 ml), and concentrated sulphuric acid (2 ml) was carefully added to form a layer. Observe reddish brown coloration of the inter face is formswhich indicates positive results for the presence of terpenoids(Muthoni Guchu et al. 2020).

2.5.6 Steroid test

The crude plant extracts (2 ml) was taken in a test tube and dissolved with chloroform (2 mL), then added equal volume of concentrated sulfuric acid to the test tube by sides. If upper layer in the test tube turns into red color ring indicates steroid is present(Muthoni Guchu et al. 2020).

2.5.7 Phenol test

Take 0.5 gm. sample of each extract boil in 5ml methanol with boiling water bath. After boiling let cool it down and filter it with Whatman filer paper. Add 5 drop of 5% Ferric Chloride ($FeCl_3$) and mixed it. If green precipitate form that indicates the phenol is present(Muthoni Guchu et al. 2020).

2.5.8 Cardiac Glycoside Test

0.5 gm each extract dissolve in 2 ml Glacial Acetic Acid containing 2 drop of 10% Ferric Chloride($FeCl_3$)solution. Add 1 ml of Conc. Sulfuric Acid (H_2SO_4) on side o test tube. Observe the violet band forms at boundary which indicates positive test(Muthoni Guchu et al. 2020).

2.5.9 Alkaloid test

Take 2 ml of extract and add 2ml of dilute HCl in test tube. Add few drops of Mayer's reagent in test tube Observe formation of creamy precipitate in test tube, which indicates positive test(Muthoni Guchu et al. 2020)

2.5.10 Antibacterial activity of Methanolic, Chlorofom and Aqueous extract

Anti-bacterial activity was performed according to method described by Zahan brice boris with slight modification(Zahan et al. 2012)Each inoculum of all 4 bacterial strains was spread onto Petri dishes containing Nutrient agar plates. Wells, each 6 mm in diameter, were formed by employing a sterile Pasteur pipette tip. The wells were filled with 60 µl of each prepared extract. As a positive control, a well was filled with streptomycin, a reference antibiotic. Following a 1-hour pre-diffusion period at room temperature, the Petri dishes containing the extracts were incubated at 37˚C for 24 hours. Following the incubation period, inhibition zone diameters were measured. To minimize bias and obtain mean values, the experiment was conducted in triplicate. The results of the antibiogram tests were interpreted using the standard criteria outlined in Table 2.

2.5.11 Antioxidant activity of *Alangium salvifolium*

Determination of 1, 1-dipheny-l-2-picrylhydrazyl (DPPH) radical scavenging activity:

DPPH free radical scavenging activity was determined following the established protocol of Bracaet al. (2002). Briefly, 0.004% w/v of DPPH radical solution was prepared in methanol and then 900 µl of this solution was mixed with 100 µl of extract or standard ascorbic acid solution for Methanol Extract and BHT - Butylated hydroxytoluene for Cloroform Extract (12.5 to 200 µg/ml) and kept in a dark place for thirty minutes. Then, absorbance was measured at 517 nm. Perform the precticl for Mthanol Extract and Chloroform Extract both.The following formula was used to quantify the scavenging capacity against DPPH radicals, presented as % inhibition.

Inhibition (%) = (A0 - As) / A0 × 100

Where A0 = Absorbance of control group, As = Absorbance of sample(Nasrullah et al. 2015).

After collect the raw data ; Data would Statistical analysis of the data was performed using GraphPad Prism software.

2.5.12 Ferric reducing antioxidant power (FRAP) assay

The Fe3+ reducing power was determined by the method of Oyaizu (1986) with slight modifications. Shortly, 1 ml of extract or standard ascorbic acid solution was taken in a test tube and mixed with 2 ml of phosphate buffer solution (0.2 M, pH 6.6). Then 2 ml of potassium ferricyanide (1%) was added and incubated at 50°C for 20 min. After this process, 2 ml of trichloroacetic acid (10%) was added. After that add 1 ml $FeCl_3$ (0.1%) solution. Dilute test sample uniformly with Distilled water as require. The absorbance was measured at 680 nm and the reducing power of the extracts was compared with the standard ascorbic acid.Perform this practical for Methanolic Extract, Chloroformic Extract and Water Extract. The raw data is statistically analyzed by Graph pad Prism(Nasrullah et al. 2015).

3. RESULTS AND DISCUSSIONS

3.1 Phytochemical analysis

The phytochemical analysis of different extract of plant identified several bioactive constituents; includingTannin ,Flavanoid , Trepenoid, Steroid , Phenol , Cardiac Glycoside and Alkaloidpresent in leaves of Alangium salvifolium.

Table No.1-Phytochemical analysis of Plant Leaves

N0.	Test name	Methanol Extract	Chloroform Extract	Water Extract
1	Tannin Test	+	+	+
2	Phlobatannin Test	-	-	-
3	Saponin Test	-	-	-
4	Flavanoid Test	+	-	+
5	Trepenoid Test	+	+	+
6	Steroid Test	+	+	+
7	Phenol Test	+	+	+
8	Cardiac Glycoside Test	+	+	+
9	Alkaloid Test	+	+	+

The phytochemical analysis of different extract of plant identified several bioactive constituents, including Tannin, Flavanoid ,Trepenoid, Steroid , Phenol , Cardiac Glycoside and Alkaloid known for their medicinal and physiological activities. Phenolic compounds, a major group of plant metabolites, possess various biological properties such as ant apoptosis, antiaging, and antioxidant effects. Tannins can interfere with protein synthesis, while flavonoids display antimicrobial properties and strong anticancer activities(Yadav and Agarwala 2011).Alkaloids are recognized for their cytotoxicity and medicinal uses, with reports highlighting their analgesic and antispasmodic effects alkaloid which is capable of reducing headaches associated with hypertension(Ajayi, Ajibade, and Oderinde 2011)Steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones(Ajayi, Ajibade, and Oderinde 2011). Cardiac Glycosides may lower blood pressure. Overall, the study suggests that these phytochemical compounds represent valuable bioactive constituents, reinforcing the importance of these plants as reservoirs of medicinal compounds.

3.2Antimicrobial Activity

The result of the study was indicated that three extract prepared from the leaves of Alangium salvifolium methanol, chloroform and Aqueous showed inhibitory activity against bacteria. Gram positive bacterial strains (Bacillus, Staphylococcus aureus) and Gram negative bacterial strains (Escherichia coli and Salmonella) showed different zone of inhibition (Table 2, Graph 1). Methanol extract of 15 mg / ml concentration showed highest inhibition zone against Staphylococcus aureus; and lowest zone of inhibition obtain Bacillus against Aqueous extract (3.7 mg/ml). Bacillus show highest inhibition against methanol extract (15 mg / ml) and lowest inhibition against aqueous extract (3.7 mg / ml).Staphylococcus aureus shows highest inhibition against methanol extract (15mg/ml); and lowest inhibition against Aqueous extract (3.7 mg/ml). Escherichia coli shows highest inhibition against methnol (15 mg/ml)

and Aqueous extract (5 mg / ml); and lowest inhibition against all three extract (3.7 mg/ml).Salmonella shows highest inhibition against methanol extract (15 mg/ml); and lowest inhibition against methanol extract (3.7 mg/ml). The highest zone of inhibition produced by the methanol extract demonstrates that methanol was a better extracting medium for the antimicrobial activity. Staphylococcus aureus was the bacteria most inhibited by the extracts. Thus, several antibacterial agents inhibit the biochemical activities of microorganisms by distorting the structural arrangement of membrane phospholipids. Growth inhibition of antimicrobial agents does not only result from the loss of intracellular materials but instead may be dependent on the inhibition of fatty acid biosynthesis or other antimicrobial action like inhibition in DNA or RNA synthesis or in other way. Methanol extract had the best antimicrobial activity on all the tested bacterial strain(Dahiru, Malgwi, and Sambo 2013)

Table No. 2- Anti microbial activity of Plant extracts

Antimicrobial activity of different extract					
Extract	Concentration (mg /ml)	Zone Of Inhibition (cm)			
		Bacillus	Staphylococcus aureus	E. coli	Salmonella
Standard	3.7	0.78	0.75	1.15	0.85
	7.5	0.98	1.1	1.31	1
	15	1.1	1.15	1.39	1.1
Chloroform	3.7	0.36	0.66	0.61	0.41
	7.5	0.4	0.76	0.65	0.61
	15	0.45	0.78	0.85	0.78
Methanol	3.7	0.33	0.71	0.61	0.33
	7.5	0.38	0.87	0.81	0.56
	15	0.63	0.96	0.89	0.83
Aqueous	3.7	0.3	0.55	0.61	0.36
	7.5	0.43	0.70	0.83	0.53
	15	0.48	0.95	0.89	0.7

3.3 Antioxidant Activity of *Alangium salvifolium*

DPPH Radical scavenging activities of methanolicExtract :Methanolic Extract of Alangium salvifolium perform in vitro DPPH radical scavenging activities in a dosedependent trend table 3. Methanolic plant extract exhibited significantly higher DPPH radical scavenging activities than the standard (L- ascorbic acid). At all the studied concentration the methanolic extract of 0.06 mg/ ml shows highest scavenging activity.Lowest scavenging activity of DPPH assay was exhibited at 0.25mg/ml(Nasrullah et al. 2015).

Table No.3- DPPH assay of Methanolic Extract

DPPH assay of Methanolic Extract			
Concentration	Absorbance of Extract	Absorbance of Standard	Scavenging Activity
1	0.356	0.046	673.91
0.5	0.196	0.043	355.81
0.25	0.15	0.04	275
0.12	0.24	0.033	627.27
0.06	0.626	0.03	1986.66

DPPH Radical scavenging activities of chloroform Extract: Chloroform Extract of Alangium salvifolium perform in vitro DPPH radical scavenging activities in a dose dependent trend table 4. The raw data is statistically analyzed by Graph pad Prism. Chloroform plant extract exhibited significantly higher DPPH radical scavenging activities than the standard (BHT- Butylatedhydroxytoluene). At all the studied concentration the Chloroform extract of 1 mg/ ml shows highest scavenging activity.Lowest scavenging activity of DPPH assay was exhibited at 0.06 mg/ml.

Table No.4-DPPH assay of Chloroform Extract

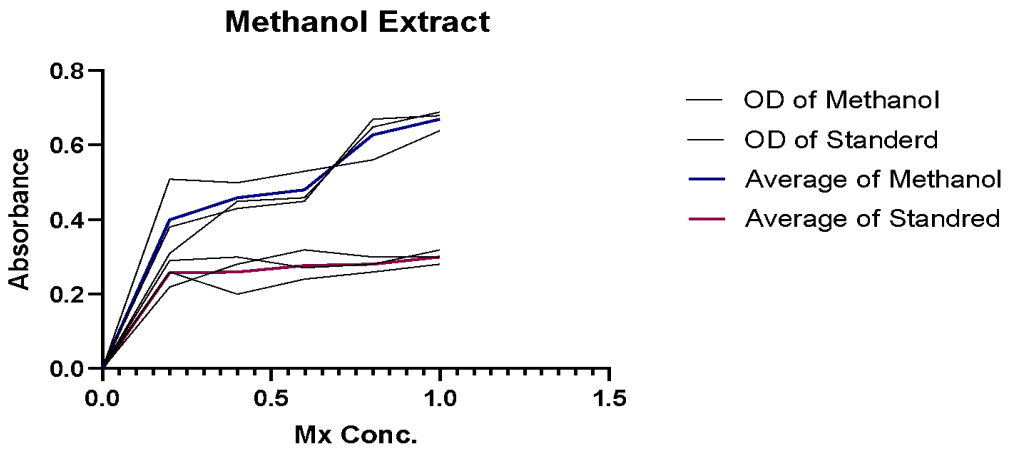
DPPH assay of Chloroform Extract			
Concentration	Absorbance of Extract	Absorbance of Standard	Scavenging Activity
1	0.61	0.06	1010.66
0.5	0.53	0.12	429.66
0.25	0.45	0.25	155
0.12	0.64	0.43	105.83
0.06	0.65	0.68	27.58

This study investigated the DPPH scavenging activity of methanolic extracts of Alangium salvifolium .The results demonstrated a concentration-dependent relationship, meaning that higher concentrations of the extracts exhibited greater antioxidant activity(Muthoni Guchu et al. 2020). This observation aligns with previous research on other plant extracts, such as *Ageratum conyzoides* and *Caesalpiniapulcherrima*, where a similar concentration-dependent effect was observed(Muthoni Guchu et al. 2020b).The study's findings are consistent with metabolomic analyses of various plant extract, which also revealed significant antioxidant potential in different plant parts (leaves, flowers, and pods).

Ferric reducing antioxidant power (FRAP) assay:

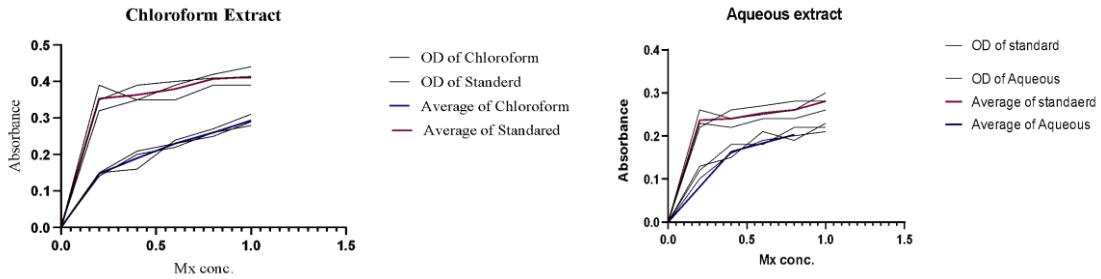
Generally, the methanolic, Chloroform and Aqueous extract of Alangium salvifolium studied medicinal plants exhibited remarkable concentration dependent increases absorbance values at a wavelength of 700 nm. At all the tested concentrations the Methanol extract of Alangiunm salvifolium have higher absorbance than Standard Ascorbic Acid .Where Chloroform and Water Extract of Alangium salvifolium have lower absorbance than Standard absorbance. Notably, at all the tested extract the Methanolic Extract produced significantly higher absorbance than Chloroform and Water Extract.

Figure:-1 FRAP assay of methanolic Extract of leaves



Synthetic antioxidants like propyl gallate (PG), butylatedhydroxyanisole (BHA), and butylatedhydroxytoluene (BHT), while commonly used to combat oxidative stress, have been linked to adverse health effects, including liver damage and malignancies. Furthermore, their effectiveness in animal models and humans is limited. Consequently, there's a growing trend towards replacing these synthetic compounds with natural antioxidants derived from plants, which are generally considered safer, more readily available, and more affordable.study aimed to investigate the phytochemical composition and in vitro antioxidant activity of extracts from *Alangium Salvifolium*.

Figure:-2 FRAP assay of Chloroform extract of plant leaves



4. Conclusion

The ferric reducing antioxidant power (FRAP) assay was employed, a method that quantifies the ability of a substance to reduce ferric ions (Fe3+) to ferrous ions (Fe2+). The formation of Fe2+ is measured by monitoring the absorbance at 720 nm. Increased absorbance at this wavelength indicates a greater reducing power(Muthoni Guchu et al. 2020).The study's findings revealed a concentration-dependent increase in absorbance values for the methanolic extracts of the studied plants, demonstrating considerable ferric reducing antioxidant power. These results are consistent with previous in vitro studies, such as the work by Aliyu et al., who demonstrated the antioxidant capacity of leaf extracts from *Bauhinia rufescens Lam.* Similarly, Adesanoye and Farombi observed dose-dependent ferric reducing activity in methanolic leaf extracts of *Vernoniaamygdalina*, which aligns with the present study's findings(Adesanoye and Farombi 2014). *A. salvifolium*wang is extensively used as traditional medicine in india.The Alangium salvifolium is the mostly used to cure and prevent a lot of disease such as epilepsy, scabies, gonorrhea, jaundice, hepatitis, diabetes, syphilis and asthma. Numerous studies have been conducted on different parts of Alangium salvifolium, but this plant has not yet developed as a drug by pharmaceutical industries. This present study confirmed that the methanolic leaf extract ofplant Alangium salvifolium can be most effective against human pathogenic bacteriaand it contains significant amount of phytochemical compoundlikeTannin , Flavanoid , Trepenoid, Steroid , Phenol , Cardiac Glycoside and Alkaloidand methanolicLeaf extract of A. salvifolium consist antioxidant activity which can be also usefull in health sector.

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