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PHYTOCHEMICAL AND ANTIMICROBIAL POTENTIAL OF SEED AND BARK EXTRACTS OF *SWIETENIA MAHAGONI* (L.) JACQ.

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ABSTRACT: *Swietenia mahagoni* (L.) Jacq. belongs to the Meliaceae family and it is native to the West Indies. It was introduced to India in 1975 and now grown in any parts of India. Biological activities of the plant are due to the abundance of phenolic compounds including different terpenoids and limonoids. The plant possesses various secondary metabolites which are responsible for its anti-bacterial, anti-fungal, anti-malarial, anti-diabetic anti-oxidant, anti-ulcer, anti-viral, anti-diarrhoeal, anti-pyretic and anti-inflammatory properties. This antimicrobial activity encouraged to work on identification of phytochemical and antimicrobial investigation of this herbal plant. The seeds and barks of *Swietenia mahagoni* is subjected to phytochemical screening for secondary metabolites and disk diffusion method for its antimicrobial activity against standard MTCC strains, two bacteria namely *Staphylococcus aureus* (gram+) (MTCC no. 6908), *Escherichia coli* (gram-) (MTCC no. 77) and one fungus *Aspergillus niger* (MTCC No.1344) were used for the study. The qualitative phytochemical tests reveal the presences of phytocompounds including alkaloids, terpenoids, tannins, and glycosides as major active constituents. The seed and bark extracts exhibit positively significant antimicrobial activity against the standard strains. The fungal activity was good in seed extracts, and bacterial activity was significant in bark extracts.

INTRODUCTION: India has rich resources of herbal medicine to cure various ailments. Infectious diseases are the world leading cause of premature deaths and number one cause of deaths in tropical countries ¹. Worldwide almost 57 million people die because of these Infectious diseases ². Plants constitute major drugs to cure various human ailments.

According to the World Health Organization (WHO), traditional medicines using plant extracts continue to provide health coverage for over 80% of the world's population ^{3, 4}. It is reported that 41% of medicine in the USA and 50% in Europe contain constituents from natural products which prove that the trend of using natural products is increased ¹.

Meliaceae plants are attracting considerable interest, because of their significant biological activities. Secondary metabolites like alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, glycosides that hold various pharmacological properties are due to the presence of bioactive plant compounds ⁵.

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Biological activities of the plant are due to the abundance of phenolic compounds including different terpenoids and limonoids. The chemical entities of this plant have been proved for their anti-bacterial, anti-fungal, anti-malarial, anti-diabetic, anti-oxidant⁶, anti-ulcer, anti-viral, anti-diarrhoeal, anti-pyretic and anti-inflammatory properties⁷. *Swietenia mahagoni* (L.) Jacq. belongs to the family of Meliaceae⁸, it is also called as West Indian Mahogany. It is extensively used as medicine for several diseases and widely grown plant of Indonesia⁹. *S. Mahogany* (L.) Jacq. is a large, deciduous and economically important timber tree, it is mainly cultivated in the tropical zone, such as India, Malaysia and Southern China^{10, 11}. *Mahogany* can reach 75 feet in height¹², leaves are evergreen or semi-evergreen, flowers are unisexual, and the tree is monoecious¹³. The leaves, fruits, bark, seeds and roots of *S. mahagoni* have been used traditionally for the treatment of hypertension, diabetes, malaria, amoebiasis, coughs, tuberculosis, antiseptic, astringent¹⁴, diarrhea and tonic^{15, 4, 16}. *S. mahagoni* seeds are also reported to have medicinal value for the treatment of hypertension, diabetes, malaria, cancer, coughs, and intestinal parasitism; the stem bark decoctions are applied as antiseptic in cuts and wounds¹¹. Furthermore, the seed extracts *S. mahagoni* have been accounted to possess antimicrobial activity¹⁷ and used for leishmaniasis and abortion medicine by an Amazonian Bolivian ethnic group and as a folk medicine in Indonesia¹⁸. Seeds are used to cure diabetes, and also it possesses anti-inflammatory, anti-mutagenicity and anti-tumor activities¹⁹.

The seed oil is being used as an alternative body ointment therapy for a range of skin cuts, itches, and wounds to ameliorate the healing process in African countries. A decoction of bark is used to increase appetite²⁰. The bark serves as an antipyretic, bitter tonic and astringent²¹. The bark decoction is extensively used as a febrifuge, which can be associated with its use as an anti-malarial drug²². It is also evidenced that the aqueous extract of its seed and bark are used in local people of East Medinipur, (West Bengal), Balasore (Orissa) traditionally for curing psoriasis, diabetes and diarrhea²³. This tree yields perhaps the most famous timber in the world and is largely used for furniture and decorative articles²⁴.

It has now become very rare due to overharvesting of the plant *Swietenia mahagoni* (L.) Jacq. and it is substituted by *S. macrophylla* king^{24, 25}. *S. macrophylla* king differs in its leaves, leaflets and fruits²⁶. Considering the above evidence, the study was planned to identify the Phytochemicals of the *Swietenia mahagoni* (L.) Jacq. seed and bark extract responsible for their antimicrobial property.

MATERIALS AND METHODS:

In-vitro Studies Qualitative Phytochemical Screening:

Plant Materials: The plant specimen *Swietenia mahagoni* (L.) Jacq. barks were collected locally in Mettupalayam forest area, Tamil Nadu, India. The collected specimen was authenticated by Government of India Ministry of Environment, Forest and Climate Change, Botanical Survey of India, Southern Regional Centre, T.N.A.U campus, Coimbatore, India (Accession no. BSI/SRC/5/23/2017/Tech-565) and the sample specimen was kept in the herbarium library. The seed of the plant was purchased from the Forest Genetic Zone, Coimbatore, Government of Tamil Nadu in April 2018.

Preparation of Plant Materials: The *S. mahagoni* plant bark was stripped from trunk and seeds were removed from its seed coat. The collected bark and seed were chipped and dried under shade for four weeks at room temperature to remove the excess moisture. The dried samples were powdered in an herbal grinding mill.

Preparation of Plant Extracts: The extracts were prepared from nine solvents according to its polarity from low to high. The solvents used for the extraction are petroleum ether, benzene, n-hexane, chloroform, ethyl acetate, acetone, ethanol, methanol and aqueous. The seed and bark samples measuring 10 grams were added to 100 ml of solvent and kept in a shaker for 24 h. The samples are filtered using Whatman no. 1 filter paper and the filtrates were used for further analysis.

Phytochemical Qualitative Analysis: The plant extracts were assessed for the existence of the phytochemical analysis by using the following standard methods. The extracts of *S. mahagoni* seed and bark were subjected to preliminary qualitative screening of various Phytoconstituents

such as alkaloids, flavonoids, phenols, phlobatannins, Saponin, tannins, terpenoids and glycosides which determine the major secondary metabolites in these as shown in **Table 1**.

Test for Alkaloids:

Wagner's Reagent: It is the general reagent for deduction of alkaloids. 1.27 g of iodine and 2 g of potassium iodide was dissolved in 5ml of distilled water, and the volume was made 100 ml with distilled water. 2 ml of prepared extract and few drops of Wagner's reagent forms reddish brown precipitate indicate the presence of alkaloids²⁷.

Mayer's Reagent: 2 ml of prepared extract and few drops of Mayer's reagent forms white or pale yellow color indicate the presence of alkaloids²⁸.

Test for Flavonoids: 2 ml extract was mixed with zinc dust and concentrated HCl was added dropwise. Formation of reddish pink to brown color indicates the presence of flavonoids²⁹.

Test for Phenols: 2 ml of extract was mixed with 2ml of 2% solution of FeCl₃. Formation of blue-green color precipitate indicates the presence of phenols²⁹.

Test for Phlobatannins: (Precipitate Test) 2 ml of prepared extract was mixed with 2ml of hydrochloric acid (1%) and heated. Formation of red precipitate indicates the presence of phlobatannins³⁰.

Test for Saponin: (Foam Test) 5 ml of extract was diluted with 5 ml of distilled water, and a drop of sodium hypo chloride was added to the solution and shaken well. Formation of froth indicates the presence of saponin³¹.

Test for Tannins: (Ferric chloride test) Distilled water and extract was taken in 1:4 ratios and 10% solution of ferric chloride is added dropwise, and few drops of dilute H₂SO₄ was added. The solution turns to a yellow or green color indicates the presence of tannin.

Test for Terpenoids: (Salkowski's test) Extract was mixed with 2 ml of chloroform, and 2 ml of concentrated H₂SO₄ was added carefully and shaken gently. A deep red color indicates the presence of terpenoids^{17,32}.

Test for Glycosides: (Keller-Kiliani Test) 2ml extract was mixed with 2 ml of glacial acetic acid containing 2 drops of 2% of FeCl₃. The mixture was poured into another test tube containing 2 ml of concentrated sulphuric acid. A brown ring at the interphase indicates the presence of glycosides^{33,34}.

In-vitro Qualitative Antimicrobial Evaluation:

Evaluation of Antimicrobial Activity: The bark and seed extract were subjected to antimicrobial assays by disc diffusion test (Kirby-Baurer) technique.

Microorganisms: In the present study, two bacteria namely *Staphylococcus aureus* (gram+) (MTCC no. 6908), *Escherichia coli* (gram-) (MTCC no. 77) and one fungus *Aspergillus niger* (MTCC no. 1344) were used for the study. The test microbes were collected from Microbial type culture collection & gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India.

Preparation of Inoculum: The inoculums for the experiment were prepared in fresh Nutrient broth for bacteria and sabouraud's broth for fungi, from preserved slant culture. The inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards. The turbidity of the culture may be adjusted by the addition of sterile saline or broth (if excessive or by further incubation) to get required turbidity Leonard Jarrett *et al.*³⁵

Plant Extract Preparation: The seed and bark crude extracts of *Swietenia mahogany* were dissolved in dimethyl sulfoxide (DMSO) to prepare the dilute solutions. For one gram of extract (dry extract), 10ml DMSO is added to prepare the solution and stored in the refrigerator. Finally, these seed and bark samples were stored and labeled, and the labeled specimens were further taken for antimicrobial activities bioassay²².

Determination of Antibacterial Activity: The disk diffusion (Kirby-Baurer) technique, which is of the recommended standards of the National Committee for Clinical Laboratory Standards (NCCLS), was used for antimicrobial test¹⁰. Determination of antibacterial activity was tested against crude extracts of nine solvents (from low to high polarity) namely Pet-ether, Benzene, n-

Hexane, Chloroform, Ethyl Acetate, Acetone, Ethanol, Methanol, Aqueous extracts of seed and bark of *Swietenia mahogany* by the disc diffusion method. Nutrient agar medium was prepared and the standard MTCC bacterial strains³⁶ were spread by streaking the sterile swab all over the surface of the medium 3 times by rotating the plate through an angle of 60° after each application. Finally, pass the swab around the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed. The standard sterile disc - SDO67 measuring 8 mm diameter (discs are soaked overnight in crude solvent extracts) were used to load the plant sample.

Each sterile plate were divided into three parts, in each part samples disc such as for bark and seed were named by its crude solvent extracts *i.e.*, Pet ether, n-Hexane, Benzene, Chloroform, Ethyl acetate, Acetone, Ethanol, Methanol, Aqueous were loaded with 100 µg/disc and placed on the agar plates inoculated with respective microorganisms. Further, the plates were placed at room temperature for 1 h to allow diffusion of extract into the agar³⁷. Then the plates were incubated for 24 h at 37 °C temperature. Ciprofloxacin 10 µg/disc was used as positive control. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone around the sample disc after 24 h³⁸; the zone values were recorded in mm **Table 2**.

Determination of Antifungal Activity:

Sabouraud's Dextrose Broth was prepared and the standard MTCC fungal strain *Aspergillus niger* was spread over the plate. Leave the inoculums to dry at room temperature with the lid closed. The standard sterile disc - SDO67 (discs are soaked overnight in crude solvent extracts) were used to load the plant sample. Each sterile plate was divided into three parts and named according to its solvent extracts were loaded with 100 µg/disc and placed on the dextrose plates inoculated with *Aspergillus niger*. Further, the plates were placed at room temperature for 1 h to allow diffusion of extract into the Dextrose Broth. Then the plates were incubated for 24-72 h at 28 °C temperature. Fluconazole 10 µg/disc was used as positive control. The antifungal activity was evaluated by measuring diameter of the inhibition zone around the sample disc after 72 h, and the zone values were recorded in mm.

RESULTS AND DISCUSSION:

Phytochemical Screening: The qualitative analyses of chemical constituents of nine crude extracts of *S. mahagoni* bark and seed have been analyzed in this study. These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes. The observations and inferences made in the phytochemical tests are presented in **Table 1**. The biological activities of the plant are due to the presence of various plant secondary metabolites which contribute significant properties like anti-bacterial, anti-fungal, anti-malarial, anti-diabetic anti-oxidant, anti-ulcer, anti-viral, anti-diarrhoeal, anti-pyretic and anti-inflammatory activities.

The plant of Meliaceae family are known to produce active secondary metabolites (limonoids; modified triterpenes) which have been reported to possess a wide range of activities including antimalarial, cytotoxicity against cell lines³⁹; terpenoids are also known to possess antibacterial, antifungal activities¹. Biological activities of the plant are due to the presence of phenolic compounds which includes different terpenoids and limonoids⁷. The significant pharmacological activities like anti-bacterial, anti-malarial, anti-viral, anti-cancer and anti-inflammatory activities are due to the presence of terpenoids, phlobatannins are responsible for the wound healing, *i.e.*, anti-inflammatory property in cuts and wounds⁴⁰. Antimicrobial activities against pathogens are due to the presence of alkaloids, saponin, tannins, and flavonoids in medicithe nal plants²⁸. Glycosides also have vast therapeutic efficacy as they are found in almost every medicinal plant. The studies have confirmed that saponin possesses the unique property of precipitating and coagulating red blood cells³¹.

The test result reveals that significantly positive results for the presence of alkaloids, tannins, terpenoids, and glycosides for all the seed and bark extracts. From the result, it shows considerably high terpenoids content found in both the seed and bark extracts. The ethyl acetate, acetone, ethanol, methanol and aqueous extracts of the bark shows potentially +ve results for the presence of phytocompounds, among other extracts acetone bark extract, shows a high level of phyto-

compounds for the majority of test methods. So it is concluded that, phytochemical screening *Swietenia mahagoni* (L.) Jacq. seed and bark extract reveal

the presence of maximum classes of phytoconstituents in bark extract when compared to seed.

TABLE 1: RESULTS OF PHYTOCHEMICAL ANALYSIS OF *S. MAHAGONI* SEED AND BARK EXTRACTS

<i>Swietenia mahagoni</i> (L.) Jacq. plant part	Name of the Solvent	Phytoconstituents							
		Alkaloids	Flavonoids	Phenol	Phlobatannins	Saponin	Tannins	Terpenoids	Glycoside
Seed	Pet-ether	-	-	-	-	+	+	+++	+++
	Benzene	++	-	-	-	+	+	+++	+
	n-Hexane	+	-	-	-	-	+	++	+
	Chloroform	-	-	-	-	+	-	++	+
	Ethyl acetate	+	-	-	+	+	+	+++	++
	Acetone	+	-	-	-	-	++	++	++
	Ethanol	++	-	-	-	+	++	+	+++
	Methanol	+	-	-	-	+	+	+++	++
	Aqueous	+	+	-	-	-	+	+	++
	Bark	Pet-ether	++	-	-	-	-	++	+
Benzene		+	-	+	-	+	+	+	+
n-Hexane		+	-	-	-	-	+	+	+
Chloroform		-	-	-	-	-	-	+	+
Ethyl acetate		++	++	++	++	-	++	+++	+++
Acetone		+++	+++	+++	+++	+++	++	+++	+
Ethanol		+	+	+++	++	+	+++	+++	+
Methanol		+	+	++	-	++	+++	+++	++
Aqueous		++	+	++	+++	+++	++	++	-

+++ = High; ++ = Moderate; + = Low; - = Absence

Evaluation of Antimicrobial Activity: The antimicrobial activity of crude extracts of *Swietenia mahagoni* (L.) Jacq. seed and bark are shown in **Table 2, Fig. I, II, III** were evaluated *in-vitro* using disk diffusion method.

Evaluation of Antimicrobial Activity of Seed Extract: The *S. mahagoni* seed revealed that pet ether extract shows strong antibacterial activity for *Staphylococcus aureus* with the maximum zone of 20 mm. The n-hexane seed extract shows a maximum of 17 mm zone against *Escherichia coli* when compared other solvent extracts.

In the fungal test, it is found that n-hexane and chloroform extract shows the good result of 11 mm zone against *Aspergillus niger*. So, it could be concluded that among all the seed extracts n-hexane shows comparatively better results both for bacterial and fungal activity.

Evaluation of Antimicrobial Activity Bark Extract: The results of *S. mahagoni* bark shows positive activity for almost all the solvent extracts against *Staphylococcus aureus* and *Escherichia coli* bacteria and shows minimum zone for the fungal test against *Aspergillus niger* in few bark extracts.

TABLE 2: ANTI-MICROBIAL ACTIVITY OF *S. MAHAGONI* SEED AND BARK BY DISK DIFFUSION METHOD

Solvents	Zone of inhibition in (mm)					
	<i>S. mahagoni</i> seed			<i>S. mahagoni</i> bark		
	Gram +ve	Gram -ve	Fungi	Gram +ve	Gram -ve	Fungi
	<i>S. aureus</i>	<i>E. coli</i>	<i>A. niger</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>A. niger</i>
Ciprofloxacin	30	38	-	30	38	-
Fluconazole	-	-	11	-	-	11
n-Hexane	19	17	11	10	13	09
Benzene	10	14	10	15	15	10
Chloroform	14	15	11	12	15	09
Pet ether	20	16	10	11	14	11
Acetone	12	13	09	19	24	0
Ethyl acetate	11	16	09	17	22	0
Ethanol	10	16	10	18	23	0
Methanol	14	14	09	18	23	0
Aqueous	10	10	09	12	14	0

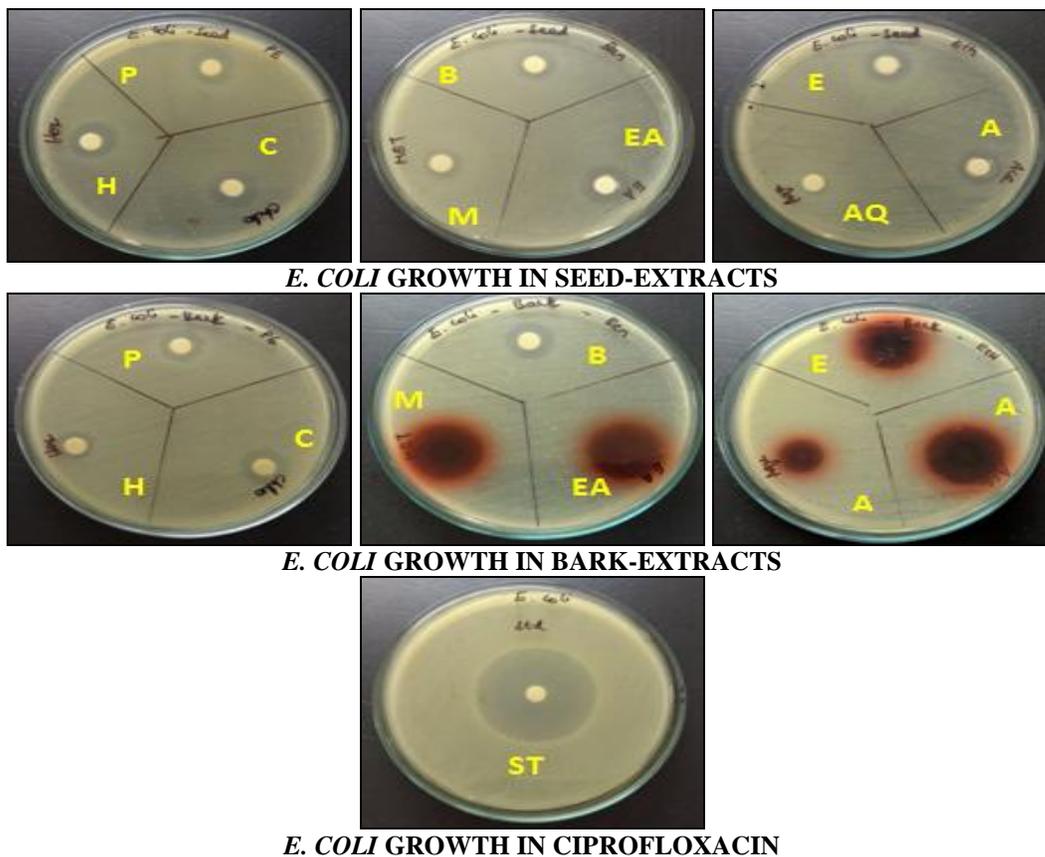


FIG. 1: ANTIBACTERIAL ACTIVITY OF *S. MAHAGONI* SEED AND BARK AGAINST *ESCHERICHIA COLI*
 ST: Standard; P: Pet ether; H: n-Hexane; C: Chloroform; B: Benzene; EA: Ethyl Acetate; A: Acetone; E: Ethanol; AQ: Aqueous

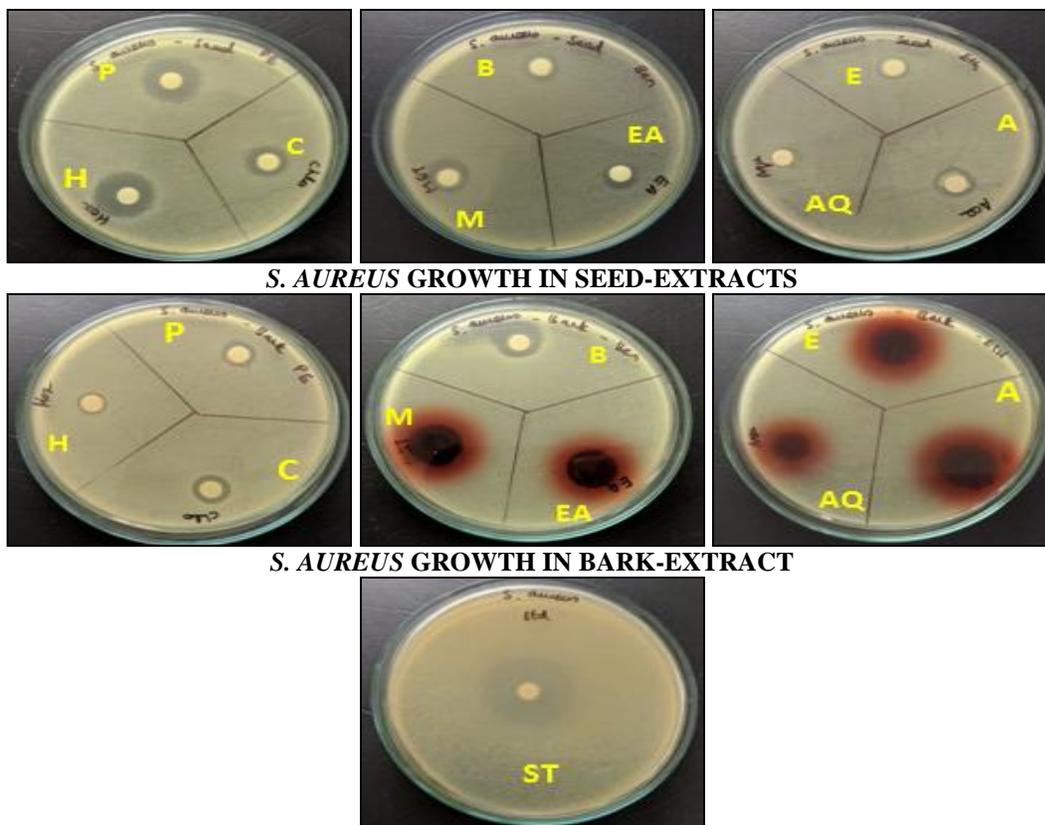


FIG. 2: ANTIBACTERIAL ACTIVITY OF *S. MAHAGONI* SEED AND BARK AGAINST *STAPHYLOCOCCUS AUREUS*
 ST: Standard; P: Pet ether; H: n-Hexane; C: Chloroform; B: Benzene; EA: Ethyl Acetate; A: Acetone; E: Ethanol; AQ: Aqueous

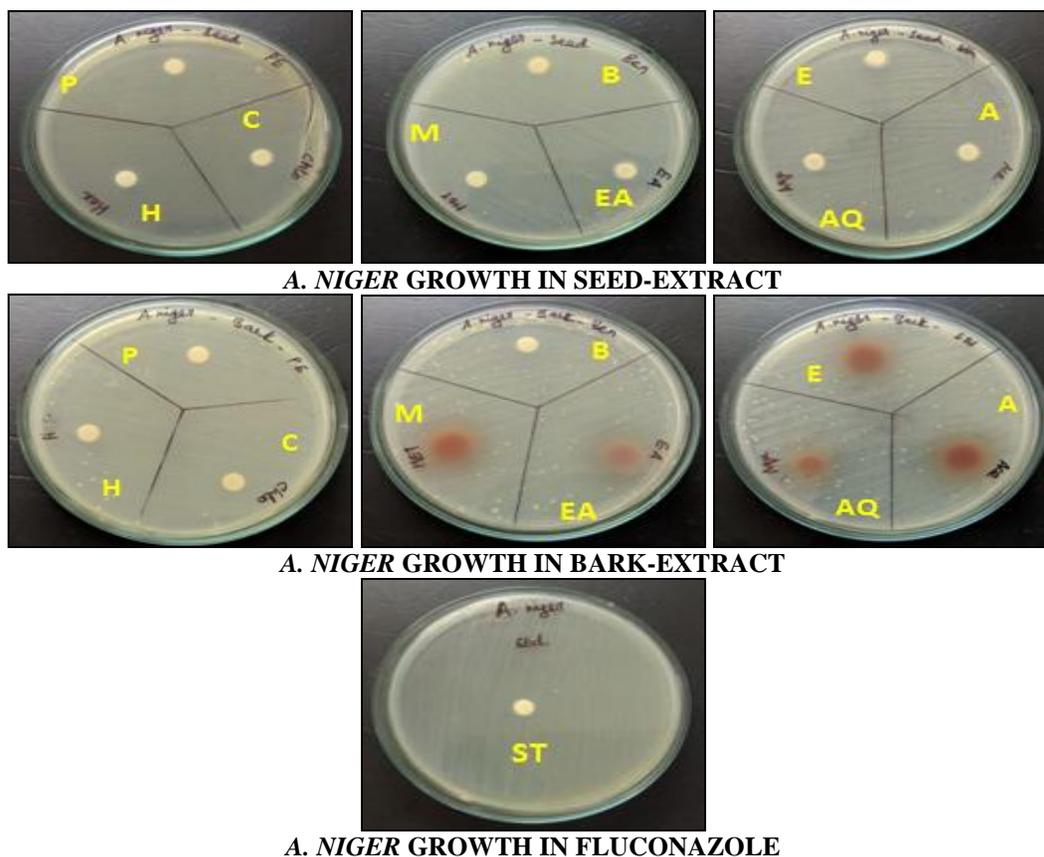


FIG. 3: ANTIFUNGAL ACTIVITY OF *S. MAHAGONI* SEED AND BARK AGAINST *ASPERGILLUS NIGER*

ST: Standard; P: Pet ether; H: n-Hexane; C: Chloroform; B: Benzene; EA: Ethyl Acetate; A: Acetone; E: Ethanol; AQ: Aqueous

CONCLUSION: Hence, the study could be concluded that, in the qualitative phytochemical screening of different solvent extracts of *Swietenia mahagoni* (L.) Jacq. reveal the presence of various compounds. The acetone bark extract shows more efficient results where most of the said phytochemical were present. The *S. mahagoni* seed and bark were analyzed for its antibacterial activity by disk diffusion test, and results were observed to be potential for selected strains. The bacterial and fungal activity of seed shows notable zone against bacteria and fungi in n-hexane extract. The crude acetone extract of *S. mahagoni* bark reveals positive results for both the test bacteria (*Staphylococcus aureus* and *Escherichia coli*). Overall, the results of *S. mahagoni* bark extracts shows potentially positive results when compared to the seed extracts. Thus the results of the study agreement with the results of previous work of the plant^{10, 18, 12}.

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CONFLICT OF INTEREST: Nil

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