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Antimicrobialactivity of Aqueous – Chloroform Extract of *Swieteniamahagoni*SEEDS AGAINST Disease Causing Bacterial Strains Found In Foods

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Abstract
Medicinal plants are natural resources that produce valuable phytochemical that are frequently used in the treatment of various diseases. A substantial part of the population in developing countries, use folk medicines for their daily healthcare. For this reason, research is carried out, to determine the toxicity of medicinal plants, the aim being to develop effective new drugsthat are non-toxic and inexpensive. The phytochemical screening of an aqueous seed extract of SwieteniaMahagonishows the presence of all metabolites except glycosides and tannin. The phenolic content in alkaloid rich fraction ofS.mahagoni seed extract was highest yield in chloroform. The antibacterial activity is carried out using disc diffusion method the maximum activity was shown against gram positive and gram negative bacteria was E.coli and S.aureus. In MIC method the plant extract shows maximum activity against B..subtilis and S,aureus. Antimicrobial mode of action using leakage of membrane and biofilm inhibition method maximum inP.aureginosa and in E.coli

Objective
Antibacterial agents areimportantforfightingagainstpathogens that causediseases. Exploration of suchsubstances from natural sources is the need of theday. With this objective Extracts were prepared from aqueousandthe chloroform of the seedsofSwieteniamahagoni were tested fortheir antibacterialactivity.

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I. Introduction

Herbal extracts are made by contacting the plant part needed with a suitable solvent at optimum conditions for a specified time, for transferring most of the plant active ingredients to the solvent. The solvent can be removed get the concentrated final product. Most commonly used solvents include methanol, ethanol, water, acetic-acid, chloroform, carbon-di-oxide and alcohol mixtures.

According to theWorld HealthOrganization(WHO),more than 80%of the world'spopulation rely on traditionalmedicinefortheir primary healthcare needs. This has captured the interest of manyresearchers to explore localmedicinal plants for valuable medicinal traits.Medicinalplantsare natural resourcesyieldingvaluablephytochemicalproducts,which are oftenused in the treatment of variousdiseases. A substantialpart of thepopulation in developingcountries, usefolk medicinesfortheir dailyhealthcare.For this reason,research is carried out, to determine the toxicity of medicinal plants. Herbalmedicinesare often used to stimulate theimmune systemin an attempt topreventdisease,as wellas to stimulate theimmune system in an attempt to preventdisease, as wellas to induce specific cures.

Theuseof phytomedicines is becoming more scientifically based, with increasing emphasis placed on provenproductsafety and efficacy. Theuseof plant-basedmedicationshasbecomeextremely popularin theUnited StatesandEurope, with the botanical industry in the USearning $1.5billion perannum and the Europeanmarketnearlythree times as much (Ernst E et al., 1998).*mahagoni*was brought to India by the British. In1795, for thefirsttime,several*mahogany*trees were introducedas seedlingsfrom Jamaicainto the Botanic Gardens in Calcutta. By 1799,the plant got established in India. The trees continued to flourish, but several treeswere destroyed in the great cyclone of1864. The trees wereabout 71 years ofage, about 12 feet ingirth at 4 ft above the ground.

Botanical Classification (Taxonomy)

Kingdom: Plantae
Order: Sapindales
Family: Meliaceae
Genus: Swietenia
Species: *Swieteniamahagoni*

Common Names

Mahaagoni, SeemainukkuTheenkaaivedhai- (Tamil), *Hiribevu, Davala, Mahaagani,Maaghani-* (Telugu), *Mehgoni-* (Bengali),*Hebbevu-* (Kannada), *Peruvian mahogany*tree, *Spanish mahogany*tree, West Indian *mahogany* (England). (Ernst E *et al.,* 1998) (Falah S *et al*.,2008)

Different Species

*Swietenia humilis, Swieteniamacrophylla,Swieteniamahagoni,Swieteniaaubrevilleana*amongthese, the first 3 species in the genusSwieteniaare said to beimportant. They occur fromMexico to Brazil, and in the Caribbean region.

In Indonesia, theseed extract has alsobeenused to treatmalaria. Thecrushed seeds mixed with Attaleaphaleratаseed oil can be used to treat theskin problem such as allergy and to healwounds. It is alsoreportedthat the seeds of theplant also use asan abortion medicine in the ethnic group of Bolivian Amazonian (Moghadamtousi*etal.*,2013). Meanwhile, the barkof the plant has been used to treat diarrhea and fevers in Mexico. It is also usedas anastringentfor wound (Moghadamtousi*et al.,* 2013).

Antioxidant Activity

Falah S*et al.,* (2008) had reported thefindings of a newcompound,s*wietemacrophyllanin* togetherwith two known compoundcatechin and epicatechinfrom thebark of Swieteniamacrophylla. The antioxidant activities ofthesecompounds were evaluated using the DPPH(1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay,and theyfoundthats*wietemacrophyllanin*had the strongest antioxidant activity with an IC_{50}value of56µg/mL compared with Trolox (standardreference).

The antimicrobial activity of the leaf extracts in methanol,dichloromethane and hexane were testedagainst fourbacteria, *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and 13 Pseudomonas aeruginosa and fungus, Candida albicans* (Tan *et al.*,2009). As a result, the methanolextract showed significantantifungalpropertiesaswellas foundto be active againstthe Gram-positive bacteria tested.

II. Materialsand Methods

Preparation plant extraction

The aqueousdecoction of theSwietenia*mahagoni*seed (10 g) wasextracted exhaustively with 100% aqueous solution by combiningmaceration(24h) with subsequent extractionat60°C. The aqueous andchloroformextracts were alsosimultaneouslyprepared andevaporated in a vacuum to a thickresidueand left for 10–12 hrs at 5–10°C.The dark green resinous solid were separated by filtration,treated with hot water, cooled,andfiltered. The extractedsamples weresubjected to phytochemical screening.

Fig- 1 Seeds of *Swieteniamahagoni*

Fig- 2Preparation of the aqueous extract

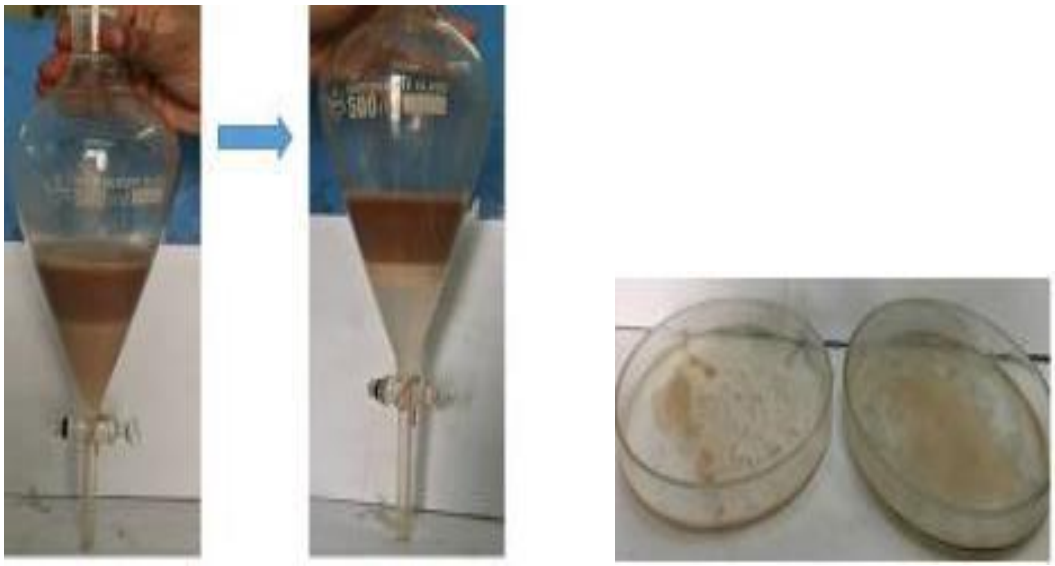
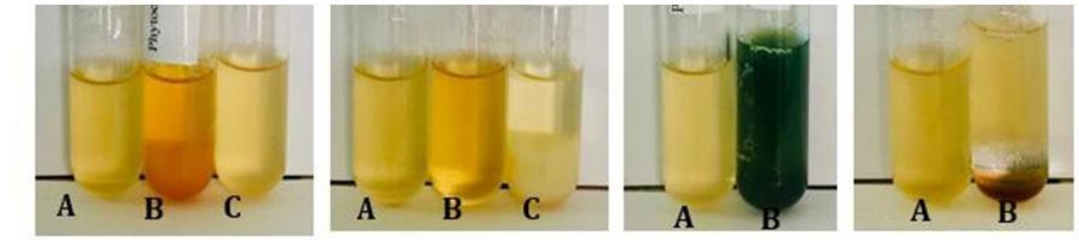


Table-1 Phytochemicalscreeningsofaqueousdecoctionfromtheseeds of *Swieteniamahagoni*

Sl. No.	PhytochemicalConstituents	Observation	Aqueousdecoctionfromtheseedsof*Swieteniamahagoni*
1	**Alkaloids** -Dragendorff's test -Mayerstest	Orange/red precipitate Creamprecipitate	+ +
2.	**Flavonoids** -AlkaliReagent -Leadacetatetest	Intenseyellow colourPrecipitateformed	+ +
3.	**Glycosides** -Keller-Killianitest	Pink colour (Ammonialayers)	-
4.	**Tannin** -FeCl3test	Blue-blackcolour	-
5.	**Saponins** -Frothingtest	Foam	+
6.	**Terpenoids** -Salkowskitest	Reddishbrowncolourringformed in theinterface	+
7.	**Polyphenols** -Ferrozine test	Reddish blue	+
8.	**Anthocyanin** **-Ammoniatest**	Pink colorin ammonia layer	+

+Positiveresult; -Negativeresult

Fig-3Screening of Phytochemicals

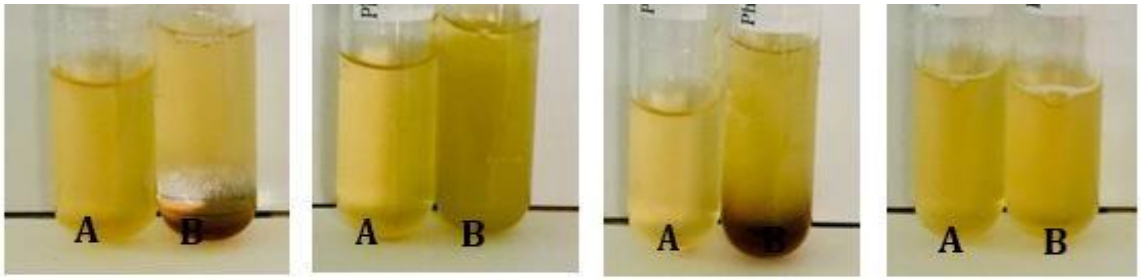


Test for Alkaloids A- Control; B- Dandruff's Test; C- Mayers Test

Test for Flavonoids A- Control; B- Alkali Reagent Test; C-Lead acetate test

Test for Anthocyanin A- Control; B- Ammonia Test

Test for Polyphenol A- Control. B- Ferrozine Test



Test for Terpenoids
A- Control
B- Salkowiski Test

Test for Glycosides
A- Control
B- Keller-kilani Test

Test for Tannin
A- Control
B- $FeCl_3$ Test

Test for Saponin
A- Control
B- Froth Test

The partial characterization of alkaloid rich fraction from the seedsof *Swieteniamahagoni*by TLC

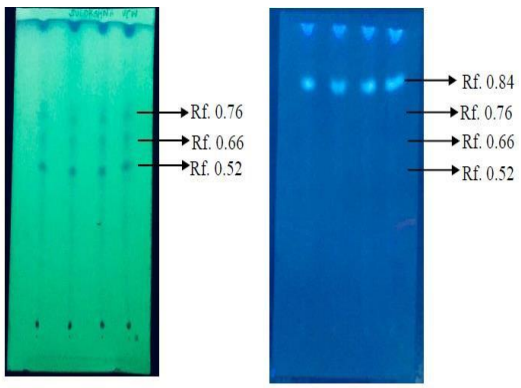
The alkaloidrichfraction from the seedsof *Swieteniamahagoni*loaded on Pre-coated TLC plates (60 F254 Merck) and developed with asolvent system of ratio of1:0.5:0.1 (Hexane, ChloroformandMethanol)wereefficient to extract the antibacterial compound it is used for further studies. Thedevelopedplate wasviewed under UV 240nm and 360nm (Table-2)

Table-2Partialcharacterizationofalkaloidrichfractionfromtheseedsof*Swieteniamahagoni*by TLC

S.No	Alkaloidrichfractionfrom theseeds of*Swieteniamahagoni*		
	UV240nm	UV360nm	Visible
1.	-	0.84	-
2.	0.76	0.76	-
3.	0.66	0.66	-
4.	0.52	0.52	-

$$R_f = \frac{\text{Distance moved by the solute (component)}}{\text{Distance moved by the solvent front}}$$

Fig-4Partialcharacterizationofalkaloid richfractionfromtheseedsof*Swieteniamahagoni*byTLC



TLC plate viewed under UV light 240 nm

TLC plate viewed under UV light 360 nm

Antibacterial activity of *Swieteniamahagoni*-Disc diffusion method

Thedisc diffusion test showed that alkaloidrichfractionfrom theseeds of*Swieteniamahagoni*wereactive on alltestedmicroorganisms,including Gram-positive,andGram-negative*bacteria.Staphylococcusaureus,Escherichiacoli,Pseudomonasaeruginosa*and*Bacillussubtilis*in the ranges of concentration ofextract 25, 50, 75 and 100µg/ml respectively(Table-3andFig- 5).Alkaloid rich fraction fromthe seedsof*Swieteniamahagoni* showed the moderateactivitiesagainstboth Gram-positive,andGram-negative bacteria*Escherichiacoli*and*Staphylococcusaureus*with inhibition zones of12.5 ± 1.3 mm and 14.6 ± 2.1 mmrespectively.Incontrast, thealkaloid rich fractionfrom theseeds of*Swieteniamahagoni*showedresistant-activity on Gram negative bacteria's*Bacillus subtilis and Pseudomonasaeruginosa*(16.3±1.8and 15. ± 1.7 mm) respectively.

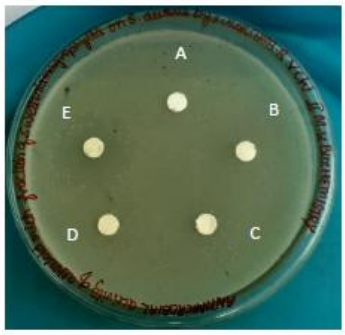
Table-3Antibacterialactivityof the plant extract

Pathogenicbacteria	**AlkaloidrichfractionexhibitedtheZoneofinhibition(mm)[a]**				
	Positivecontrol10µlAmpicillin	**Different concentrationsample-1(µl/ml)**			
		25 µl	**50** µl	**75** µl	**100**µl
Staphylococcusaureus	13mm	7.6±2.4	9.3±1.6	12.3±1.7	14.6±2.1
Escherichiacoli	12mm	6.6±2.4	8.3±2.4	10.4±1.4	12.5±1.3
Pseudomonasaeruginosa	12mm	8.1±1.0	10.4±2.7	12.3±1.7	15.3±1.7
Bacillussubtilis	11mm	7.9±2.1	11.2±1.7	13.4±0.5	16.3±1.8

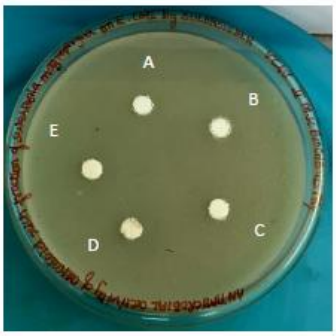
Minimum Inhibitory concentration (MIC) of the plant extract

The alkaloid rich fraction from the seeds of *Swieteniamahagoni* showed inhibitory activities against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa & Bacillus subtilis* strains but their effectiveness varied. The MIC concentrations were mostly very high and ranged from 25 to 100 µg/ml. The sample (extract) was most active against *Bacillus subtilis and Staphylococcus aureus* were moderately resistant to all tested samples. This will be of substantial advantage in fighting the menace of antibiotic refractive pathogens that are so prevalent in recent years

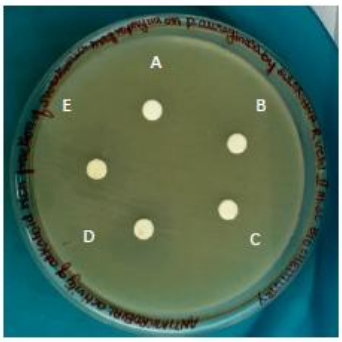
Fig-5Antibacterial activity of the plant extract



Staphylococcus aureus



Escherichia coli



Pseudomonas aeruginosa

Bacillus subtilis

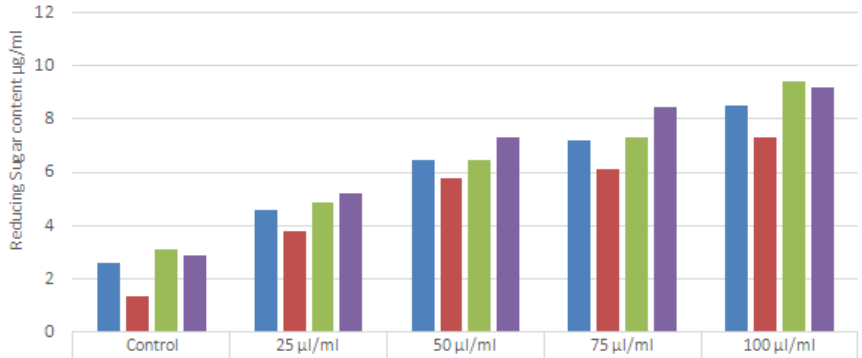
A- Control: B-25 µg/ml: C-50µg/ml; D-75µg/ml; E-100µg/ml

*The inhibitory diameter was measured by means of calipers. All the assays were duplicated, and the mean values were recorded

Antimicrobial mode of action of*Swieteniamahagoni*seed – Leakage of membrane method-Estimation of reducing sugar

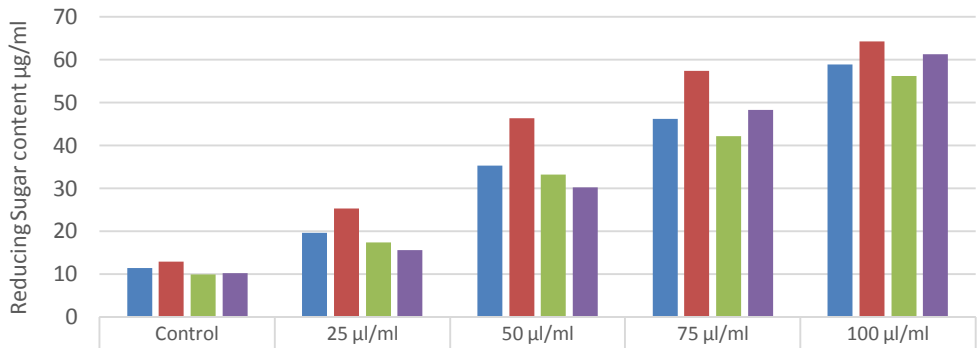
In alkaloid rich fraction from the seeds of *SwieteniaMahagoni* the amount of reducing sugar, estimated at the 18th hour ranged from 9.87 to 56.21µg/ml and 12.87 to 64.32µg/ml of bacterial dry weight of 11.46 to 58.96 µg/ml in *Staphylococcus aureus*, 12.87 to 64.32 µg/ml in *E.coli* and 9.87 to 56.21mg/ml in *Pseudomonas aeruginosa* and 10.21 to 61.24 µg/ml in *Bacillus subtilis* of alkaloid rich fraction from the seeds of *Swieteniamahagoni* treated of pathogenic bacterial cultures (Graph-1 and 2).

GRAPH-1. EFFECT OF ALKALOID RICH FRACTION FROM THE SEEDS OF *Swietenia mahagoni* ON LEAKAGE OF MEMBRANE OF PATHOGENIC BACTERIA (Reducing sugar estimation 0 Hours)



	Control	25 µl/ml	50 µl/ml	75 µl/ml	100 µl/ml
Staphylococcus aureus	2.6	4.6	6.47	7.2	8.54
Escherichia coli	1.35	3.78	5.78	6.14	7.34
Pseudomonas aeruginosa	3.1	4.89	6.47	7.31	9.41
Bacillus subtilis	2.87	5.2	7.3	8.45	9.21

GRAPH-2. EFFECT OF ALKALOID RICH FRACTION FROM THE SEEDS OF *Swietenia mahagoni* ON LEAKAGE OF MEMBRANE OF PATHOGENIC BACTERIA (Reducing sugar estimation 18 Hours)



	Control	25 µl/ml	50 µl/ml	75 µl/ml	100 µl/ml
Staphylococcus aureus	11.46	19.64	35.31	46.21	58.96
Escherichia coli	12.87	25.34	46.31	57.45	64.32
Pseudomonas aeruginosa	9.87	17.34	33.21	42.21	56.21
Bacillus subtilis	10.21	15.64	30.21	48.31	61.24

Antimicrobial mode of action of *Swieteniamahagoni*seed – Leakage of membrane method-Estimation of protein

The amount of proteininthealkaloid rich fraction from theseedsof*Swieteniamahagoni* treated both cultures was estimated, and theOD value were the referred with standard graph of BSA. The result onestimationof protein washigherthanthe control which inferred alkaloid rich fraction from the seeds of *Swieteniamahagoni* was potent against the pathogen eveninthe initial stage. The amount of protein estimated at the 18th hour ranged from7.5to 56.3µg/ml in *Staphylococcus aureus*, 10.2to 61.2µg/ml in*E.coli*, 8.7to 63.21µg/ml in*Pseudomonas aeruginosa* and 9.45to58.6µg/mlin*Bacillus subtilis*of alkaloid rich fraction from the seeds of*Swieteniamahagoni* treatedcultures. Refer table4and 5

Table-4 Estimation of protein -0 Hours - Control

Microorganism	Control	25 µl/ml	50 µl/ml	75 µl/ml	100 µl/ml
Staphylococcus aureus	1.8	3.51	4.89	5.64	6.46
Escherichia coli	2.1	4.89	6.87	7.31	8.45
Pseudomonas aeruginosa	1.78	2.87	6.45	7.12	7.89
Bacillus subtilis	2.4	3.7	5.1	6.45	7.78

Table-5 Estimation of protein -18 hours - Test

Microorganism	Control	25 µl/ml	50 µl/ml	75 µl/ml	100 µl/ml
Staphylococcus aureus	7.5	15.4	31.4	42.3	56.3
Escherichia coli	10.2	18.45	42.3	54.2	61.2
Pseudomonas aeruginosa	8.7	16.31	37.56	50.4	63.21
Bacillus subtilis	9.45	17.31	34.12	47.31	58.6

Effect of alkaloid rich fraction of *swietenia mahogany*seedon inhibition of biofilm formation in pathogenic bacteria

Effect ofthe alkaloid rich fraction of*Swieteniamahogany*seedin different concentration onthe inhibition of biofilm formation was studied by spectroscopic assay. The alkaloid rich fraction fromthe seeds of*SwieteniaMahagoni*exhibitedmoretoxicityonthebiofilm formation of*Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*. Thepotentialof100µl/ml concentration of alkaloid richfraction from theseedsof*Swieteniamahagoni*,on inhibitingbiofilm formation after 18hoursincubation was maximuminStaphylococcus aureus *(69 96%), Escherichia coli (84%) and Pseudomonas aeruginosa (67%)* thanthe corresponding control.

Thephytochemicalscreeningoftheaqueous-chloroformextractshowedthe presenceof alkaloids, phenols, saponins, terpenoids and flavonoids.Theextractwasfoundtobeanalkaloid rich solution. In the partial characterization ofthealkaloid rich fraction bychloroform extract of*S.mahagoni*seed using TLC-solvent (hexane,chloroform and methanol) and sample which showed four UV fluorescent compounds with Rf value (0.84, 0.76, 0.66, 0.52)respectively. The phenoliccontentinalkaloidrichfractionof*S.mahagoni*seed extract was found to behighest in the chloroform extract. Using the disc diffusionmethod, the maximum activity wasshownagainst both gram positive and gram-negativebacteriasuchas*S.aureus, E. coli, B.subtilis and P. aeruginosa*.Thezoneof inhibition of *S.aureus*and E.coli were found to be12.5±1.3 nmand 14.6±2.1 nm, respectively. Anappreciable result was not obtained with *B.subtilis* and *P. aeruginosa*. Highest MIC was shown against *B.subtilis* (0.223µg/ml) and *S.aureus* (0.246µg/ml) with significant antimicrobial activity than *P.aeruginosa* and *E.coli.* Estimation of reducing sugar using leakage of membrane method showed maximum activity in *E.coli* (64.32µg/ml) and, in *B.subtilis* (61.24µg/ml) than *S.aureus* and *P.aeruginosa* at 18 hour of the incubation period. The amount of protein estimated by leakage of membrane method at 18hour of incubation period ranged maximum in *P.aureginosa* (63.21 µg/ml) and in E.coli (61.2 µg/ml) than*B.subtilis*and *S.aureus.*

III. Conclusion

Thealkaloidrichfractionfrom theseedof *S.mahagoni*was found to inhibit the biofilm formation after 18 hoursincubation with a maximum in*S.aureus*(69.96 %) and in*E.coli*and showed potentanti-bacterialactivity. Themode of action isby disruption of selected proteins and phosphate containinglipidstoinducemembranedisruption,andultimatelyresultinginthe cellular decomposition and death of the bacterium. Testing of the seeds of *Swieteniamahagoni* for their antibacterial activity has proven its antibacterial potency in the tested bacterialspecies

References

[1]. Ernst E. (1998). Harmless herbs? A review of the recent literature. Am J Med 104: 170–178.
[2]. Falah, S., Suzuki, T. and Katayama, T., 2008. Chemical Constituents from Swietenia macrophylla bark and their antioxidant activity.Pakistan of Biological Sciences, 11, pp. 2007-20
[3]. Moghadamtousi, S.Z., Goh, B.H., Chan, K.C., Shabab, T. and Abdul Kadir, H., 2013. Biological Activities and Phytochemicals of SwieteniaMacropylla King. Molecules, 18(9), pp. 10465-10483.12.
[4]. Tan, S.K., Osman, H., Wong, K.C. and Boey, P.L., 2009a. New Phragmalin-type Limonoids from Swietenia macrophylla King. Food Chemistry, 115, pp. 1279-1285.

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