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In Vitro Evaluation of Antibacterial Properties of *Moringa oleifera, Dalbergia sissoo* and *Alstonia scholaris*

Namrata Prasad[1*], Dipika Nandi[1], Surabhi Arora[1], Amit Pandey[2]
1.Graphic Era University, 566/6 Bell Road, Clement Town, Dehradun- 248001, Uttarakhand, India
2.MRD LifeSciences[TM] Pvt. Ltd., B-3/46 & 47, 2nd Floor, Vibhuti Khand, Behind- Indus Tower, Gomti Nagar, Lucknow- 226010 (U.P.), India
* E-mail of the corresponding author: np280591@gmail.com

Abstract
In this paper leaves of *Moringa oleifera, Dalbergia sissoo* and *Alstonia scholaris* in different solvent were subjected to antibacterial analysis against selected bacterial pathogens and phytochemical analysis was done. To investigate the antibacterial activities of Drumstick tree (*Moringa oleifera*), Sheesham (*Dalbergia sissoo*) and Dita bark (*Alstonia scholaris*) were tested against bacterial pathogens (*Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*). The dry crude sample extracts were tested for its antibacterial activities using 'agar well diffusion technique'. The solvents used were methanol, acetone, ethyl acetate and chloroform, compare to all, methanolic extracts showed best results with *Staphylococcus aureus* and *Pseudomonas* in case of *Dalbergia sissoo*; methanolic extracts also showed best results with *Escherichia coli* and *Pseudomonas* in case of *Alstonia 1cholar* while acetone and ethyl acetate showed best results with *Staphylococcus aureus* in case of *Moringa oleifera.* These samples were further taken to determine the MIC value. The MIC value was determined using broth dilution method. Acetone and ethyl acetate extracts of *Moringa oleifera* were subjected to get MIC against *Staphylococcus aureus* and it was found to be 0.003mg/ml and 0.096mg/ml respectively. MIC values for methanolic extract of *Dalbergia sissoo* were 0.386 mg/ml for *Staphylococcus aureus* and 0.005 mg/ml for *Pseudomonas aeruginosa.* In case of methanolic extract of *Alstonia scholaris* MIC values were 41.67 mg/ml both for *Pseudomonas aeruginosa* and *Escherichia coli.* Important sources of phytochemicals of immense medicinal and pharmaceutical potential were present.
Keywords: Antibacterial activity, Methanol, acetone and ethyl acetate plant extract, MIC (Minimum Inhibitory Concentration) and phytochemical analysis.

1. Introduction

1.1. Antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic).

1.2. Antibiotics: An antibiotic (against life) is a compound or substance that kills or slows down the growth of bacteria. Antibiotics include a chemically heterogeneous group of small organic molecules of microbial origin that, at low concentrations, are deleterious to the growth or metabolic activities.

1.3. Antibacterial: An antibacterial is a compound or substance that kills or slows down the growth of bacteria. The term is often used synonymously with the term *antibiotic(s)*; today, however, with increased knowledge of the causative agents of various infectious diseases, *antibiotic(s)*has come to denote a broader range of antimicrobial compounds, including antifungal and other compounds.

1.4. Following samples were used:

1.4.1. Moringa oleifera (synonym: *Moringa pterygosperma*) is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae. They are known to be anti- helminthic, antibiotic, detoxifiers, immune builders and have been used to treat malaria (Abalaka *et al.,* 2012) [10].

1.4.2. Dalbergia sissoo (or Indian Rosewood) is a deciduous rosewood tree. Toxicology: Ethanolic extract of the fruits of *Dalbergia sissoo* exhibited molluscicide effect against eggs of the freshwater snail *Biomphalaria pfeifferi*.

1.4.3. Alstonia scholaris (Apocynaceae is used solely for medicinal purposes, ranging from Malaria and epilepsy to skin conditions and asthma. In Ayurveda it is used as a bitter and as an astringent herb for treating skin disorders, malarial fever, urticaria, chronic dysentery, diarrhea, in snake bite and for upper purification process of Panchakarma. The Milky juice of the tree is applied to ulcers. The bark contains the alkaloids ditamine, echitenine and echitamine and used to serve as an alternative to quinine. A decoction of the leaves was used for beriberi.

1.5 Pathogens used:

1.5.1. Escherichia coli: Gram negative, Bacilli. (MTCC 739)

1.5.2. Pseudomonas aeruginosa: Gram negative, Bacilli. (MTCC 2453)

1.5.3. Staphylococcus aureus: Gram positive, Cocci. (MTCC 2940)

The present study was carried out to evaluate antibacterial activity of *Moringa oleifera, Dalbergia sissoo &*

Alstonia scholaris against bacterial pathogens (*Escherichia coli, Pseudomonas aeruginosa & Staphylococcus aureus*) and phytochemical analysis which are responsible for antibacterial activity.

2. Methodology

2.1. Preparation of plant extract: were prepared using different solvents methanol, acetone, ethyl acetate and chloroform with sample solvent ratio of 1:10. Samples were kept in dark for 48 hours to dissolve secondary metabolites and air dried and dissolved in same amount of 100mM Tris- HCl or Di methyl sulfoxide (DMSO).

2.2. Antibiogram analysis: was performed to evaluate the antimicrobial properties of plant extract with the help of Agar well diffusion method. It is done to check the sensitivity of antibiotics against various pathogens. If the antibiotic will be effective it will show Zone of Inhibition against pathogens, whereas, if culture is resistant then it will show full growth.

2.3. Antibacterial activity: Nutrient Agar plates were prepared and 10µl of each bacterial pathogen (*Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*) was spread over the plates. Wells were made and 50µl of each sample was loaded in each well and plates were incubated at 37ºC for overnight and zone of inhibition was observed.

2.4. MIC (Minimum Inhibitory Concentration): is the lowest concentration of an antimicrobial that will inhibit the visible growth of microorganisms. An MIC is used to measure the activity of an antimicrobial agent against an organism.

Nutrient broth was prepared and 3ml was poured in pair of six test tubes. In the first test tube 0.5ml of sample was dissolved. Further for remaining test tubes serial dilution method was followed by putting 0.5ml from first test tube to the second and so forth till the sixth test tube. The set of six test tubes were inoculated with 10µl of the pathogen. Other set of six were kept as control. Overnight incubation at 37ºc was done. Optical density was taken at 620nm.

2.5. Phytochemical analysis: are the main constituents of any plant sample, which are responsible for secondary metabolites also. The other works of these phytochemical are flavouring, colors etc. (Thenmoxhi *et al*., 2010) [18]. These are tested using various tests.

The dried leaf powder of the plant was boiled in water, ten times the quantity of the extract. The extract was then filtered and was used for testing of different compounds.

2.5.1. Saponins: 2ml of the filtrate is dissolved in 2ml distilled water and left for thirty minutes. Bubbles or froth indicate the presence of saponins.

2.5.2. Tannins: To 1ml of filtrate 3-4 drops of 10% Ferric chloride was added. Blue color indicates Gallic tannins and green color indicate catecholic tannins.

2.5.3. Phenols: Equal volumes (0.5ml) of extract and ferric chloride solutions were mixed and the presence of phenols was indicated by a deep bluish green color.

2.5.4. Glycosides: 2.5ml of dilute sulphuric acid was added to 0.5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 0.5ml of Fehling solution added. Glycosides were indicated by a brick red precipitate.

2.5.5. Volatile oils: 2ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. White precipitate formation indicates presence of volatile oils.

2.5.6. Flavanoids: To 1ml of extract 2ml of 10% NAOH was added. Intense yellow coloration indicates flavanoids which turn colorless with the addition of Dil. HCl.

2.5.7. Alkaloids: To 1ml of sample 1ml of Ammonium chloride and 1ml chloroform was added. Dil. HCl was added and acid layer was taken in another test tube. Drops of Iodine in Potassium Iodide were added. Red or brown color indicates alkaloids.

2.5.8. Anthraquinones: About 0.5 ml of the extracts was boiled with 10% Hcl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of Chloroform was added to the filtrate. Few drops of 10% Ammonium chloride were added to the mixture and heated. Formation of rose-pink colour indicates the presence of authraquinones.

2.5.9. Reducing Sugars: 1ml of extracts was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for few minutes. An orange red precipitate indicates the presence of reducing sugar.

2.5.10. Phlobatanins: The extract (0.5ml) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins.

2.5.11. Terpenoids: Salkowski test: 0.2ml of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated Sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the Terpenoids.

2.5.12. Steroids: 0.5ml of extract was dissolved in 5ml of Chloroform and equal volume of Con. Sulphuric acid is added by the side of the test tube. Upper layer turns red and acid layer shows yellow color

with gree flouroscene indiating the presence of steroids.

3. Results

Fig. 1.: Samples used (*Moringa oleifera, Dalbergia sissoo* and *Alstonia scholaris*)

(a) (b)

Fig. 2.: Pathogen's growth observed after spreading (Pathogens used: *Escherichia coli* (a), *Pseudomonas aeruginosa* and *Staphylococcus aureus* (b))

3.1 Antibiogram analysis

Table no. 1: Antibiogram analysis of *Moringa oleifera* showed:

	ZONE OF INHIBITION (in mm)			
SOLVENTS	Methanol	Acetone	Chloroform	Ethyl acetate
PATHOGENS				
S. aureus	11	15	13	15
Pseudomonas	11	00	00	00
E. coli	12	00	00	00

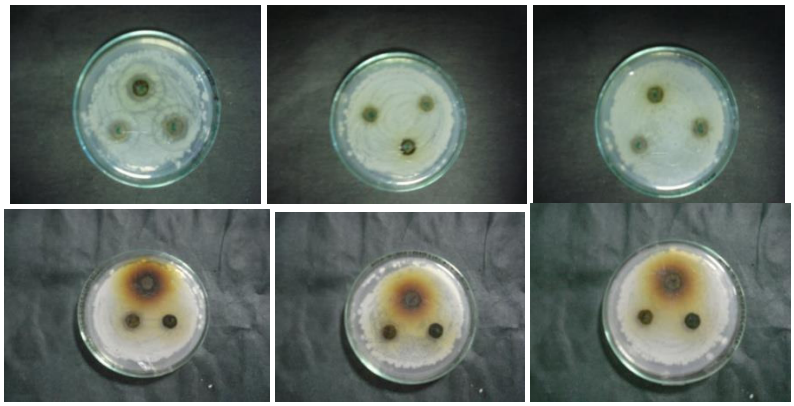

Fig. 3: ZOI for *Moringa oleifera* (top three plates of methanolic extract with each pathogen *S. aureus, Pseudomonas* and *E. coli* respectively, bottom three plates are of ethyl acetate extract with pathogens in the same order).

Table no. 2: Antibiogram analysis of *Dalbergia sissoo* showed:

	ZONE OF INHIBITION (in mm)			
SOLVENTS	Methanol	Acetone	Chloroform	Ethyl acetate
PATOGENS				
S. aureus	**14**	00	00	12
Pseudomonas	**14**	00	00	11
E. coli	**12**	00	00	00

Fig. 4: ZOI for *Dalbergia sissoo* (plates of methanolic extract with each pathogen *S. aureus, Pseudomonas* and *E. coli* respectively).

Table no. 3: Antibiogram analysis of Alstonia scholaris showed

	ZONE OF INHIBITION (in mm)			
SOLVENTS	Methanol	Acetone	Chloroform	Ethyl acetate
PATOGENS				
S. aureus	**12**	00	00	10
Pseudomonas	**19**	00	12	15
E. coli	**19**	00	14	12

Fig. 5: ZOI for *Alstonia scholaris* (plates of methanolic extract with each pathogen *S. aureus, Pseudomonas* and *E. coli* respectively).

3.2. Initial concentrations:

Table no. 4: Initial concentrations (Stock) for MIC tests:

	Moringa oleifera	*Dalbergia sissoo*	*Alstonia scholaris*
Solvent	Initial concentration		
Methanol	1000 mg/ml	500 mg/ml & 250 mg/ml	1000 mg/ml & 250 mg/ml
Acetone	125 mg/ml	500 mg/ml	1000 mg/ml
Chloroform	500 mg/ml	500 mg/ml	500 mg/ml
Ethyl acetate	125 mg/ml	500 mg/ml	500 mg/ml

3.3. MIC Results:

Table no. 5: MIC results for *Moringa oleifera*

Moringa oleifera			
Test tube no.	Concentration (mg)	O.D for Acetonic extract against *S. aureus*	O.D for Ethyl acetate extract against *S. aureus*
1.	20.83	0.00	0.00
2.	3.47	0.36	0.64
3.	0.58	0.21	0.50
4.	**0.096**	0.30	0.20
5.	0.016	0.17	0.43
6.	**0.003**	**0.05**	0.35

Table no. 6: MIC results for *Dalbergia sissoo*

Dalbergia sissoo				
Test tube no.	Concentration (mg)	O.D for Methanolic extract against *S. aureus*	Concentration (mg)	O.D for Methanolic extract against *Pseudomonas*
1.	83.33	0.00	41.67	1.50
2.	13.89	0.45	6.94	0.00
3.	2.315	0.65	1.16	0.43
4.	**0.386**	**0.32**	0.19	0.49
5.	0.064	0.56	0.032	0.48
5.	0.01	0.77	**0.005**	**0.22**

Table no. 7: MIC results for *Alstonia scholaris*

Alstonia scholaris				
Test tube no.	Concentration (mg)	O.D for Methanolic extract against *Pseudomonas*	Concentration (mg)	O.D for Methanolic extract against *E. coli*
1.	**41.67**	**0.15**	**41.67**	**0.14**
2.	6.94	0.23	6.94	0.27
3.	1.16	0.31	1.16	0.41
4.	0.19	0.31	0.19	0.50
5.	0.032	0.38	0.032	0.46
5.	0.005	0.39	0.005	0.43

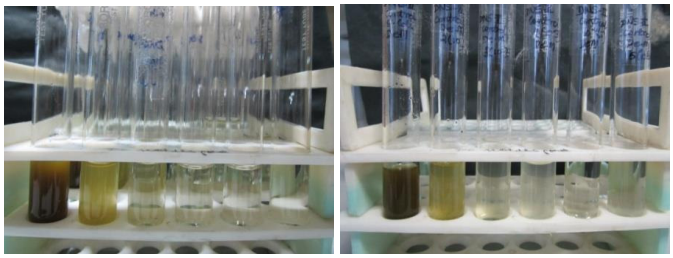

Fig. 6: MIC tubes for *Alstonia scholaris* methanolic leaf extract against *Pseudomonas.*
Fig. 7: MIC tubes for *Alstonia scholaris* methanolic leaf extract (control tubes).

3.4 Phytochemical analysis:

Table no. 8: Phytochemical analysis for *Moringa oleifera, Dalbergia sissoo* and *Alstonia scholaris.*

	Moringa oleifera	*Dalbergia sissoo*	*Alstonia scholaris*
Phytochemicals	Result	Result	Result
Saponins	+	-	+
Tannins	+	+	+
Phenol	+	+	+
Glycosides	-	-	-
Volatile oil	-	+	-
Flavanoids	-	+	-
Alkaloids	-	+	-
Anthraquinones	-	-	-
Reducing sugars	+	-	+
Phlobatanins	-	-	-
Terpenoids	+	+	-
Steroids	-	-	-

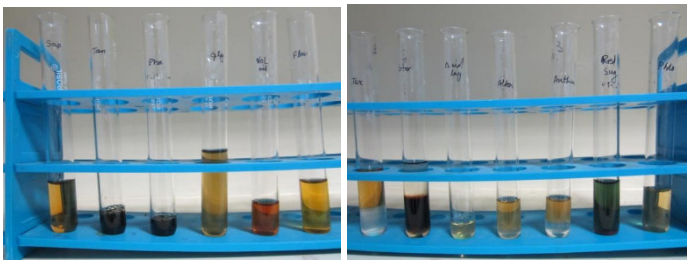

Fig 8: Phytochemical test of *Alstonia scholaris.*

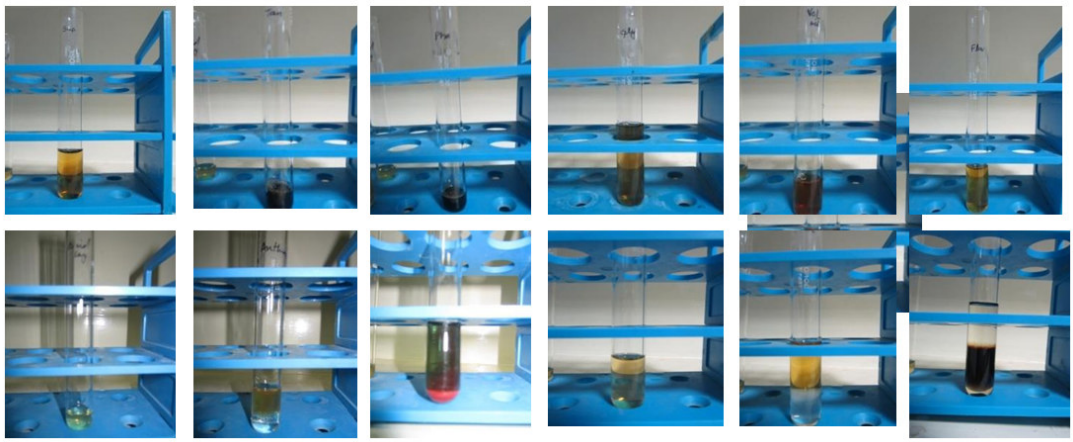

Fig 9: Phytochemical test of *Alstonia scholaris.*
Top left to right: Saponins(+), Tannins (+), Phenol (+), Glycosides (-), Volatile oil (-), Flavanoids (-)
Bottom left to right: Alkaloids (-), Anthraquinones (-), Reducing sugars (+), Phlobatanins (-), Terpenoids (-), Steroids (-).

4. Conclusion

In this paper we have shown that plant samples demonstrated antibacterial activity against different bacterial pathogens. Herbal medicines are valuable for primary health care system. These plants show antibacterial activity, but more pharmacological investigations are necessary. Present time the emergence of multi drug resistant in human and animal pathogenic microbes as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antibacterial drug of plant origin. The antibacterial activity of leaf extract of *Moringa oleifera, Dalbergia sissoo* and *Alstonia scholaris* and their potency was quantified by the ZOI measurement. All plants parts are having antibacterial property compared to which leaves show the maximum activity.

The solvents used were Methanol, Acetone, Chloroform and Ethyl acetate and after antibiogram analysis it was found that acetone and ethyl acetate show maximum activity in case of *Moringa oleifera* against *S. aureus* with ZOI 15mm for both solvents. While methanolic extracts of leaf showed maximum activity in case of *Dalbergia sissoo* and *Alstonia scholaris*. For *Dalbergia sissoo* it was against *S. aureus* and *Pseudomonas* with ZOI 14mm for both pathogens and for *Alstonia scholaris* it was against *Pseudomonas* and E. coli with ZOI 19mm for both pathogens.

MIC is the least concentration of antibiotics which will inhibit the growth of microorganisms. The MIC value was determined using broth dilution method. Acetone and ethyl acetate extracts of *Moringa oleifera* were subjected to get MIC against *Staphylococcus aureus* and it was found to be
0.003mg/ml and 0.096mg/ml respectively. MIC values for methanolic extract of *Dalbergia sissoo* were 0.386 mg/ml for *Staphylococcus aureus* and 0.005 mg/ml for *Pseudomonas aeruginosa*. In case of methanolic extract of *Alstonia scholaris* MIC values were 41.67 mg/ml both for *Pseudomonas aeruginosa* and *Escherichia coli*.

The phytochemical analysis of *Moringa oleifera* showed the presence of Saponins, Tannins, Phenols, Anthraquinones and Terpenoids. For *Dalbergia sissoo* phytochemicals found were Tannins, Phenols, Volatile oils, Flavanoids, Alkaloids and Terpenoids. *Alstonia scholaris* showed Saponins, Tannins, Phenols and Reducing sugars as phytochemicals. The mechanism of action of phytochemicals may be via lysing the cell, increasing permiability of the cell wall and membrane, inhibition of protein and DNA synthesis and or by inhibiting the transport of nutrients across the cell wall or membrane.

Earlier literature indicated that medicinal plants are the back bone of the traditional medicine and the antimicrobial activity of the pant extract is due to different chemical agent in the extract, which was classified as active antimicrobial compounds these compounds attracts beneficial and repel harmful
organisms, so as photoprotectants and respond to environment changes. Glycosides serve as defense mechanism against predation by many micro organism, insects and herbivores.

5. Future Prospects

Traditional medicines are now the mainstay of drug discovery for the treatment of emerging and old diseases.
The present research works includes isolation and purification of therapeutics, microbial from the active extracts and carry out further pharmacological evaluation by several methods like NMR, GC-MS, and HPLC to screen and isolate bioactive agents.

It can further be served as drug with fewer side effects and less cost.
However, there is a need to ensure that, what is known is used for improvement of the health of people there is a need to establish the necessary expertise for development of traditional medicines and deliberate effort should be made to encourage local industrial production of herbal medicines so that cultivation may become possible and hence contribute to poverty reduction.

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