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Phytochemical analysis and antimicrobial activity of *Madhuca longifolia* and *Clerodendrum infortunatum*: Medicinal plants for application as textile finishes

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Abstract
In the plant kingdom *Madhuca longifolia* (Mahua) and *Clerodendrum infortunatum* (Bhant) bears widely proven spectrum of medicinal properties. Mahua oil is used in medicine as embroilment, cure the skin diseases, rheumatism, headache, laxative, piles and haemorrhoids. Under the present study, phytochemical analysis of different extract of leaves of *Clerodendrum infortunatum* and oil cakes of *Madhuca longifolia* were prepared with the five different solvents viz; distilled water, methanol, acetone, dichloromethane and hexane, which revealed the presence of various bioactive compounds. Quantitative analysis showed that among both the plant sources, *Madhuca longifolia* (Mahua) yielded higher total phenols (70.43±0.183mg/g in methanol) in comparison with *Clerodendrum infortunatum* (29.17±0.119mg/g in methanol). Methanol gave good results for extraction of phenols. Quantification of flavonoids and tannins showed that *Clerodendrum infortunatum* (Bhant) leaves yielded higher flavonoid content (12.17±0.132 mg/g in acetone) and total tannins (13.87±0.086 mg/g in acetone) among both the plant extracts. Among the extraction solvents, acetone gave good results for extraction of flavonoids and tannins compared to distilled water, methanol, dichloromethane and hexane. The antimicrobial activity of both the plant extracts showed maximum zone of inhibition at 5% against both test organisms viz. *Staphylococcus pasteuri* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative). Therefore the extracts of these two plants can be used for antimicrobial finish on textiles by various methods of finish application.

Keywords: *Madhuca longifolia*, *Clerodendrum infortunatum*, phytochemical screening, flavonoids, phenols and tannins

Introduction
Certain treatments are applied to improve the look and qualities of textile goods. These treatments are called finishes. A finish is a treatment given to a fabric, to change its appearance, handling /touch or performance. Its purpose is to make the fabric more suitable for its end use. The finishes may be basic or functional. Basic finishes also called as aesthetic finishes are applied to almost all the fabrics, with an aim to improve their appearance, feel and body. Functional finishes are applied to improve the performance of a fabric for some specific purpose, for example- fireproof, waterproof, bulletproof, crease-resistant finish and antimicrobial. Increasing global competition in textiles has created many challenges for textile researchers. The rapid growth in technical textiles and their end-uses has generated many opportunities for the application of innovative finishes. Novel finishes of high added value for apparel fabrics are also greatly appreciated.

Antimicrobial textiles with improved functionality find a variety of applications such as health and hygiene products, specially the garments worn close to the skin. In the last few decades, with the increase in new antimicrobial fibre technologies and the growing awareness about cleaner surroundings and healthy lifestyle, a range of textile products based on synthetic antimicrobial agents such as triclosan, metal and their salts, organometallics, phenols and quaternary ammonium compounds, have been developed and quite a few are also available commercially (Purwar & Joshi, 2004) [4]. Although the synthetic antimicrobial agents are effective as well as durable on textiles, but still they are a cause of concern due to the related side effects such as water pollution and various others. Hence, there is a great demand for

antimicrobial textiles based on eco-friendly agents which help to reduce the ill effects associated due to microbial growth on textile material effectively. The use of natural plant products for antimicrobial finishing of textile materials has been widely reported. There is a vast source of medicinal plants with active antimicrobial ingredients such as tulsi, pomegranate, eucalyptus etc. Although, there are many natural products rich in antimicrobial agents, the study on their use in textiles is very limited and not well documented. These natural products are associated with various benefits like lower incidence of adverse reactions as compared to synthetic pharmaceuticals and reduced cost, can be exploited as an attractive eco-friendly alternative to synthetic antimicrobial agents for textile applications. Textile materials may be responsible for disease transmission and the spread of new strains of diseases from the main sources to elsewhere. However, textile materials as necessary materials for clothing and daily life are possible means for prevention of infectious diseases and pathogens if they have antimicrobial properties. By treating the textiles with an antimicrobial finish cross contamination during use can diminish considerably.

These finishes can be applied by various methods onto the textiles such as dip dry, spraying, microencapsulation, nano encapsulation and exhaust method. Microencapsulation is now widely used in textile finishing also. Many special and functional properties can be imparted to the fabrics by microencapsulating the core material. This core material can be any substance having a special function to perform for the fabric. Encapsulation has allowed moisturisers, therapeutic oils and insecticides to be incorporated into fabrics. Microencapsulation of anti-microbial agents is also gaining popularity in sportswear and medical textiles. Sharma and Goel (2018) [16] developed microcapsules by using two essential oils viz; eucalyptus and cedarwood oil as a natural insecticide and applied onto the textile substrate. The other review papers in this area of antimicrobial textiles, on the other hand, cover a range of antimicrobial agents mostly synthetic and only a few natural products.

Hippocrates (in the late fifth century B.C.) reported 300-400 medicinal plants (Schultes, 1978) [15]. Plants also have an almost limitless ability to synthesize aromatic substances, such as phenols and secondary metabolites. In many cases, these substances serve as plant defense mechanisms against microorganisms.

Clerodendrum infortunatum, commomly known as *"Bhant"*. It is very common undershrub in India. It belongs to the family Verbenaceae, and bears widely proven spectrum of medicinal properties. This plant is quite rich in its characteristic medicial terpenoid-clerodin, along with other important metabolites. It is a frequently used medicinal plant ever since ages. Leaves are used as an alternative for "Chiretta". Leaves and roots are applied on the exterior specially for skin diseases and alopecia and are recommended in headache too. Leaves of this plant are also used in homeopathy such as the fresh leaves are employed for colic due to worms, diarrhoea connected with nausea, chronic fever along with loss of appetite and also in enlargement of liver and spleen with indigestion and constipation. The alcoholic extract of the complete plant showed anti-protozoal activity against *Entamoeba histolytica.* It also exhibited hypoglycaemic activity in albino rats. The fresh juice of leaves from this plant has successfully been used against malaria particularly among children. Roots of this plant are very efficient against rheumatism, muscular sprains and cramps. This plant is extensively available throughout the year and also in a sufficient amount (Jha and Prasad, 2016) [7], but apart from such extensive uses of *Clerodendrum infortunatum* has gained very little attention of phytochemists. *Madhuca longifolia* is the botanical name of *Madhuca* tree which belongs to family- Sapotaceae. Medium to large sized deciduous tree, spreading branches and a large rounded crown. Mahua trees are growing widely under dry tropical climatic conditions. *Madhuca longifolia* distributed in Andhra Pradesh, Gujarat, Madhya Pradesh, Odisha, Chhatisgarh, Jharkhand, Bihar and Uttar Pradesh. It is an important tree for poor people, to a great extent valued for its flowers and seeds known as tora (Ekka and Ekka, 2014) [4]. The leaves of mahua tree contain saponin and an alkaloid glucoside. Sapogenin and other basic acid have been found in the seeds. The flowers of this plant are well-known for their nutrient content and high reducing sugar (Mishra and Padhan, 2013) [9]. The seeds oil has emollient properties and has been used in skin diseases, rheumatism and head ache. It is also used as a laxative in piles and haemorrhoids and as an emetic. The leaves and bark of *Madhuca longifolia* have astringent properties because of its tannin content. The cake is used as manure either alone or in combination with other cakes and fertilisers. Previous studies were focussed on various parts of the *Madhuca longifolia* plant but very limited researches were carried out on the oil cakes of this plant. Oil cakes were either used as manure or fertilisers or used for fish killing. So in the present study oil cakes of *Madhuca longifolia* and leaves of *Clerodendrum infortunatum* were used for qualitative and quantitative analysis of phytochemicals and antimicrobial testing so that on the basis of these tests these extracts can be recommended for application of finishes on textile substrate.

Materials and Methods

Collection of plant source

Oil cakes of *Madhuca longifolia* and leaves of *Clerodendrum infortunatum* were purchased from local market of Pantnagar, G.B.P.U.A & T (Uttarakhand) and from the roadsides of G.B.P.U.A & T, Pantnagar respectively in the month of August and September, 2016. Botanical identification of the plant was confirmed taxonomically in the department of Biological Science, College of Basic Sciences and Humanities, G.B.P.U.A & T, Pantnagar

Plate 1: *Madhuca longifolia* (oil cakes of Mahua)

Plate 2: *Clerodendrum infortunatum* (leaves of Bhant)

Preparation of plant source

The freshly collected leaves of *Clerodendrum infortunatum* were washed thoroughly with distilled water thrice to make them free from impurities and air-dried under shade at the room temperature for 7-10 days. Dried leaves of *Clerodendrum infortunatum* and oil cakes of *Madhuca longifolia* were grounded into powdered form with the help of domestic electric grinder then sieved using a muslin cloth. Powder of plant leaves was stored separately in the airtight container for further use.

Plate 3: Powder of *Madhuca longifolia* oil cakes

Plate 4: Powder of *Clerodendrum infortunatum* leaves

Preparation of extract

In the present study five solvents in the order of increasing polarity were selected namely distilled water, methanol, acetone, hexane and dichloromethane. Soxhlet method of extraction was carried out during the study as this is considered as the most useful apparatus for solid-liquid extraction. This method is most common, standard and conventional for extracting the active compounds of plants.

Conventional soxhlet extraction method given by Azmir *et al.* (2013) [2] was used for the plant extraction. Forty gram powder of leaves was placed separately in a thimble holder. The distillation flask was filled with 400 ml of solvent and temperature was maintained at the boiling point of particular solvent (Distilled Water- 100°C, Methanol- 64.7°C, Acetone- 56°C, Hexane- 68°C and Dichloromethane- 39.6°C). In the solvent flask, solute was separated from the solvent using distillation. Solute was left in the flask and fresh solvent was passed back into the thimble. This operation was repeated until the solvent passing through thimble became colourless.

Phytochemical screening of plant extracts

Qualitative and quantitative screening helps in identification of the secondary metabolites present in the leaf extract and quantification of these bioactive compounds respectively. Therefore the researcher carried out the phytochemical screening of plant extract of *Madhuca longifolia* and *Clerodendrum infortunatum* which were used in the present study.

Secondary Metabolites	Name of the test	Reference
Qualitative tests		
Alkaloids	Dragendorff's test	Sasikala and Sundaraganapathy (2017)
	Hager's test	Sasikala and Sundaraganapathy (2017)
	Mayer's test	Sasikala and Sundaraganapathy (2017)
	Wagner's test	Raaman (2006) [13]
	Tannic Acid Test	Sasikala and Sundaraganapathy (2017)
Flavonoids	Ammonia test	Vimalkumar *et al.* (2014) [22]
	Lead acetate test	Vimalkumar *et al.* (2014) [22]
	Alkaline reagent test	Singh *et al.* (2013)
	Ferric chloride test	Vimalkumar *et al.* (2014) [22]
Tannins	Ferric chloride Test	Zohra *et al.* (2012) [23]
	Match Stick test	Kaur (2018) [8]
Phenolic compounds	Ferric chloride test	Vimalkumar *et al.* (2014) [22]
	Lead acetate test	Vimalkumar *et al.* (2014) [22]
	Dilute iodine solution test	Vimalkumar *et al.* (2014) [22]
Saponins	Foam Test	Zohra *et al.* (2012) [23]
	Lead acetate Test	Devmurari (2010) [3]
Terpenoids	Salkowski test	Elezabeth and Subramanian (2013) [5]
Quantitative tests	Total Phenolic Content	Ainsworth and Gillespie (2007) [1]
	Total Flavonoid Content	Quettier *et al.*, 2000
	Total Tannins Content	Sun *et al.*, 1998 [20]

Test microorganisms

Two cellulose degrading test microorganisms including one Gram Positive- *Staphylococcus pasteuri* and Gram Negative- *Pseudomonas aeruginosa* were used for the study. These strains were procured from department of Microbiology, College of Basic Sciences and Humanities, G.B.P.U.A & T, Pantnagar, Uttarakhand. The selected bacterial strains were maintained at 4^0 C in nutrient broth in Microbiology lab of the university (G.B.P.U.A&T).

Preparation of nutrient agar medium

Nutrient agar (28 grams) was dissolved in 1000 ml distilled water. The components were gently heated to dissolve completely. The nutrient agar medium was sterilized in an autoclave at 15 psi (121^0C) for 15 minutes and cooled at room temperature in a laminar.

Preparation of test inoculums

Active culture for bacteria was prepared by 0.325 gm nutrient broth in 25 ml distilled water. The nutrient broth was then sterilized in an autoclave at 15 psi (121^0C) for 15 minutes and then cooled at room temperature in a laminar. After cooling a loopful of bacteria from the sub cultured petriplate was inoculated in the beaker and then incubated for 24 hours at 37°C in an incubator cum shaker so that continuous shaking was maintained in the beaker for proper growth of bacteria. After 24 hours the inoculated culture became turbid. This indicated the complete growth of bacteria.

Antimicrobial activity of plant extracts

The antimicrobial activity of plant extracts was assessed as per agar well diffusion method. Nutrient media and nutrient broth was prepared separately in distilled water and autoclaved at 120°C for five minutes at a pressure of 15 lb. The nutrient agar was poured in the sterilized petriplates and placed in laminar for solidification. Later, 150 ml nutrient media was inoculated with 1 ml of bacterial working culture and poured on the upper surface of solidified media and allowed to solidify. Three wells equidistant to each other were created using a cork borer. About 40 µl of plant extract was loaded to each well and the plates were incubated for 24 hours at 37 °C. After the incubation period, the diameter of the growth inhibition zones was recorded in centimetres. Methanol was used as control.

In the present study various concentrations ranging between 2 percent to 5 percent of diluted plant extracts by the agar well diffusion method were used in the analysis. The lowest concentration at which zone of inhibition observed was recorded as the minimum concentration of the extracts and was selected for further experimentation.

Results and Discussion

1. Percentage Yield of Plant Extracts of *Madhuca longifolia* and *Clerodendrum infortunatum*

Extraction yield is the measure of solvent efficiency to extract specific components from the original plant material. Therefore the percentage yield was calculated to quantify the amount of extract with respect to specific solvent. The extraction from plant source was carried out using five solvents such as distilled water, methanol, acetone, hexane and dichloromethane. The yield (quantity) of the extracts obtained per 200 ml of solvent used and 20 gram powder of selected plants was shown in Figure 1. Among the leaves and oil cakes extracts of *Clerodendrum infortunatum* (Bhant) and *Madhuca longifolia* (Mahua), yield of both the plants was found to be higher in methanol and acetone (12.01%, 23.75% and 8.45%, 18.77% respectively) followed by dichloromethane, distilled water and hexane (8.22%, 18.04%, 5.240%, 15.54% and 5.18%, 10.81% respectively).

While comparing the yield of both the plants, percentage yield of *Madhuca longifolia* was found to be higher in all the solvents.

In general irrespective of the plant sources, percent yield of both the plant extracts was found to be higher in methanol and acetone solvent compared to distilled water, dichloromethane and hexane.

The results were in accordance with Vaghasiya *et al.* (2007) [21] the percentage yield of extracts prepared from selected plants were found to be more in methanol than in acetone. The results are also supported by Gupta *et al.* (2016) [6] that the percentage yield of hot methanolic extract of S. cumini (L.) was found to be highest (19.5%) followed by P. oleracea (18%) and P. guajava (L.) (17.6%).

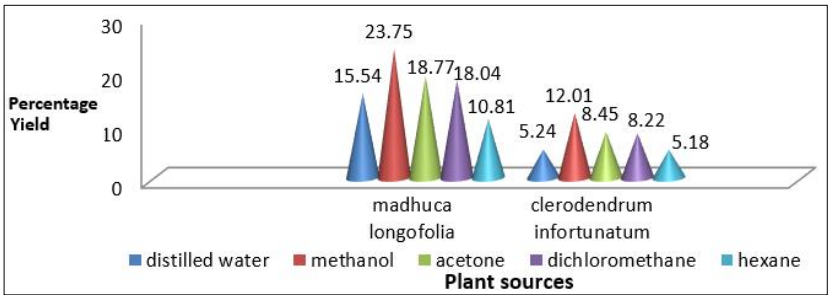


Fig 1: Percentage yield of *Madhuca longifolia* and *Clerodendrum infortunatum*

Qualitative analysis of *Madhuca longifolia* and *Clerodendrum infortunatum*

The extracts of *Madhuca longifolia* and *Clerodendrum infortunatum* were tested to identify various phytochemicals. The results of which are as follows-

***Madhuca longifolia* (Mahua)**

To test the presence of bioactive compounds in the extract of oil cake of *Madhuca longifolia*, various tests against secondary metabolites were carried out. The results of which are as follows-

The results of qualitative screening of *Madhuca longifolia* are presented in Table 1. Results revealed that alkaloids were present in higher concentration in methanolic extract as depicted by Dragendroff's test. Further, lower concentration was found in distilled water and acetone. Alkaloids were not found in any of the extract prepared in dichloromethane and hexane. Hager's test revealed the moderate presence of alkaloids only in the methanolic extract whereas none of the extract showed positive results for alkaloids as depicted in Hager's test. Mayer's test also confirmed the moderate concentration of alkaloids in methanolic extract of *Madhuca longifolia*. Whereas, lower concentration of alkaloids was found in distilled water and acetone. Further wagner's test revealed the higher concentration of alkaloids in methanol followed by distilled water revealed lower presence of alkaloids in the extracts of *Syzygium aromaticum*. However tannic acid test revealed moderate presence of alkaloids only in methanolic extract. Extracts in distilled water, acetone, dichloromethane and hexane showed negative results for alkaloids.

Ammonia and ferric chloride test confirmed lower concentration of flavonoids in methanolic extract and none other test confirmed for positive results for *Madhuca longifolia*.

Match stick test revealed higher concentration of tannins in methanolic extract. Moderate concentrations of tannins were found in distilled water and acetone. Whereas, lower concentration was found in dichloromethane and hexane. Further ferric chloride test conferred higher concentration of tannins in methanol and acetone. On the other hand moderate concentration was found in distilled water. Yet lower concentration of tannins was found in dichloromethane and hexane.

Ferric chloride test revealed lower concentration of phenolic compounds in all the extracts except dichloromethane. Further lead acetate test revealed moderate concentration of

Table 1: Qualitative screening of Mahua (*Madhuca longifolia*) extracts

S. No	Phytochemical Tests	Solvents				
		Distilled Water	Methanol	Acetone	Dichloromethane	Hexane
1.	**Alkaloids**					
a)	Dragendroff's Test	+	+++	+	-	-
b)	Hager's Test	-	++	-	-	-
c)	Mayer's Test	+	++	+	-	-
d)	Wagner's Test	+	+++	-	-	-
e)	Tannic Acid Test	-	++	-	-	-
2.	**Flavonoids**					
a)	Ammonia Test	-	+	-	-	-
b)	Lead acetate Test	-	-	-	-	-
c)	Alkaline reagent Test	-	-	-	-	-
d)	Ferric chloride Test	-	+	-	-	-
3.	**Tannins**					
a)	Match stick Test	++	+++	++	+	+
b)	Ferric chloride Test	++	+++	+++	+	+
4.	**Phenolic compounds**					
a)	Ferric chloride Test	+	+	+	-	+
b)	Lead acetate Test	+	++	+	-	+
c)	Dilute Iodine Solution Test	+	++	+	-	+
5.	**Saponins**					
a)	Foam Test	++	+++	+	+	+
b)	Lead Acetate Test	+++	++	+	+	+
6.	**Terpenoids**					
a)	Salkowski Test	+	++	+++	+	+

+++ = High concentration, ++ = Moderate concentration, + = Low concentration, - =Absent

phenolic compounds in methanol. Meanwhile, extracts prepared in distilled water, acetone and hexane showed lower concentration of phenolic compounds. On the other hand dilute iodine solution test revealed moderate concentration of phenolic compounds in methanol whereas lower concentration was found in distilled water, acetone and hexane.

Foam test revealed higher concentration of saponins in methanolic plant extract of *Madhuca longifolia* followed by moderate concentration was found in distilled water. However, extract prepared in acetone, dichloromethane and hexane showed lower concentration of saponins. Further lead acetate test revealed higher concentration of saponins in distilled water whereas moderate concentration of saponins was found in methanolic extract. Yet lower concentration of saponins was found in acetone, dichloromethane and hexane. Meanwhile higher concentration of terpenoids was only present in the plant extract prepared in acetone. On the other hand extraxts prepared in methanol confirmed the moderate concentration of terpenoids in methanolic extract. Yet lower concentration was found in distilled water, dichloromethane and hexane.

The secondary metabolites present in methanolic extract of *Madhuca longifolia* was also confirmed by Sinha *et al.* (2017) [19] that secondary constituents such as alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, tannins and so on were present in extract of *Madhuca longifolia.*

***Clerodendrum infortunatum* (Bhant)**

To test the presence of phytochemicals in the extract of *Clerodendrum infortunatum* (Bhant) various tests for alkaloids, flavonoids, tannins, phenolic compounds, saponins and terpenoids were conducted. The results of tests performed are presented in the Table 2 and the details of the tests are as follows-

It is evident from Table 2 that Dragendroff's test revealed higher concentration of alkaloids in *Clerodendrum infortunatum* (Bhant) extracts of methanol. Further moderate presence of alkaloids was found in distilled water, acetone, dichloromethane and hexane. Hager's test showed the moderate presence of alkaloids in the extracts of methanol, dichloromethane and hexane. Mayer's test confirmed the moderate presence of alkaloids in the methanolic extract while lower concentration was found in distilled water and acetone. Further wagner's test revealed the higher concentration of alkaloids in methanol and acetone followed by dichloromethane revealed lower presence of alkaloids in the extracts of *Clerodendrum infortunatum*. However tannic acid test divulged moderate presence of alkaloids in distilled water, methanol, acetone and dichloromethane. On the other hand only hexane revealed lower concentration of alkaloids.

Ammonia and alkaline reagent test revealed the moderate concentration of flavonoids in methanolic extract of *Clerodendrum infortunatum*. Yet lower concentration was found in distilled water and acetone.

Table 2: Qualitative screening of Bhant (*Clerodendrum infortunatum*) extracts

S. No	Phytochemical Tests	Solvents				
		Distilled Water	Methanol	Acetone	Dichloromethane	Hexane
1.	**Alkaloids**					
a)	Dragendroff's Test	++	+++	++	++	++
b)	Hager's Test	+	++	+	++	++
c)	Mayer's Test	+	++	+	-	-
d)	Wagner's Test	-	+++	+++	+	-
e)	Tannic Acid Test	++	++	++	++	+
2.	**Flavonoids**					

a)	Ammonia Test	+	++	+	-	-
b)	Lead acetate Test	++	+++	++	++	++
c)	Alkaline reagent Test	+	++	+	-	-
d)	Ferric chloride Test	-	+++	+++	+	-
3.	**Tannins**					
a)	Match stick Test	+	++	+	++	++
b)	Ferric chloride Test	++	++	++	+	++
4.	**Phenolic compounds**					
a)	Ferric chloride Test	++	+++	++	++	++
b)	Lead acetate Test	+	++	+	++	++
c)	Dilute Iodine Solution Test	+	++	+	-	-
5.	**Saponins**					
a)	Foam Test	+	+	+	-	-
b)	Lead Acetate Test	+	+	+	+	+
6.	**Terpenoids**					
a)	Salkowski Test	-	++	+	-	+

+++ = High concentration, ++ = Moderate concentration, + = Low concentration, - =Absent

Dichloromethane and hexane does not showed positive results for presence of flavonoids. Lead acetate test revealed higher concentration of flavonoids only in methanol. However, all the other solvents revealed the moderate presence of flavonoids for Lead acetate test. Meanwhile, ferric chloride test revealed higher presence of flavonoids in methanol and acetone in the extract of *Clerodendrum infortunatum* yet lower concentration of flavonoids was found only in dichloromethane.

Match stick test revealed moderate concentration of tannins in methanol, dichloromethane and hexane. However, extracts prepared in distilled water and acetone showed lower presence of tannins. Further ferric chloride test confirmed moderate concentration of tannins in all the extracts prepared in distilled water, methanol, acetone and hexane yet lower concentration of tannins was only found in dichloromethane.

Ferric chloride test revealed higher concentration of phenolic compounds only in methanol. However, extracts prepared in distilled water, acetone, dichloromethane and hexane showed moderate presence of phenolic compounds for ferric chloride test. On the other hand lead acetate test confirmed the moderate presence of phenolic compounds in methanol, dichloromethane and hexane. Yet lower concentration of phenolic compounds was found in distilled water and acetone. Further dilute iodine solution test revealed moderate concentration only in methanolic extract while lower concentration of phenolic compounds was found in distilled water and acetone.

Foam test revealed lower concentration of saponins in distilled water, methanol and acetone plant extract of *Clerodendrum infortunatum*. However, lead acetate test showed lower concentration of saponins in all the solvents. Meanwhile moderate concentration of terpenoids was only present in the plant extract prepared in methanol. However lower concentration was found in acetone and hexane.

The results were in accordance with Sheel *et al.* (2014) that methanolic extract of *Clerodendron infortunatum* possess sterols, terpenoids, Alkaloids, carbohydrates, glycoside, and tannins**.** Prasad *et al.* (2012) [10] also confirmed the presence of phenolic compounds, glycosides, anthraquinones, terpenoids, flavinoids, tannins, lignin and saponins in the selected Clerodendrum sp.

Quantitative screening of plant extracts

To quantify the amount of phenols, flavonoids and tannins some tests were carried out namely Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and Total Tannin Content (TTC). The results of which are as follows-

Total phenolic content (TPC)

Total Phenolic Content in *Madhuca longifolia* and *Clerodendrum infortunatum* was calculated by using the standard curve of gallic acid shown in figure 3.

Figure 2, highlights the total phenolic content of the extracts (mg/g) of dry leaf. It is observed that *Madhuca longifolia* gave higher TPC in methanol (70.43±0.183mg/g) followed by acetone (46.88±0.121mg/g), distilled water (25.02± 0.224mg/g), dichloromethane (23.65±0.085 mg/g) and hexane (10.54±0.274mg/g). On the other hand *Clerodendrum infortunatum* (Bhant) leaves recorded maximum TPC in methanol (29.17±0.119mg/g) and acetone (28.31±0.022mg/g) extracts followed by dichloromethane (20.22±0.098mg/g), distilled water (11.17±0.085mg/g) and hexane (9.89±0.112mg/g).

In general irrespective of extraction solvents *Madhuca longifolia* (Mahua) yielded higher total phenols in comparison with *Clerodendrum infortunatum* shown in figure 2. Meanwhile, among the extraction solvents, methanol gave good results for extraction of phytochemicals compared to distilled water, acetone, dichloromethane and hexane.

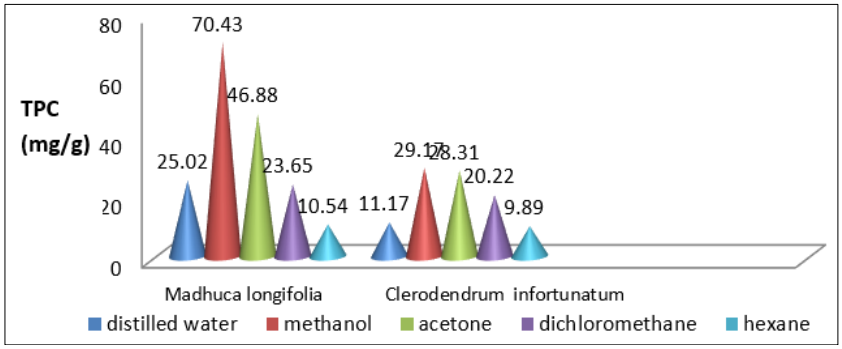


Fig 2: Total Phenolic Content of *Madhuca longifolia* and *Clerodendrum infortunatum*

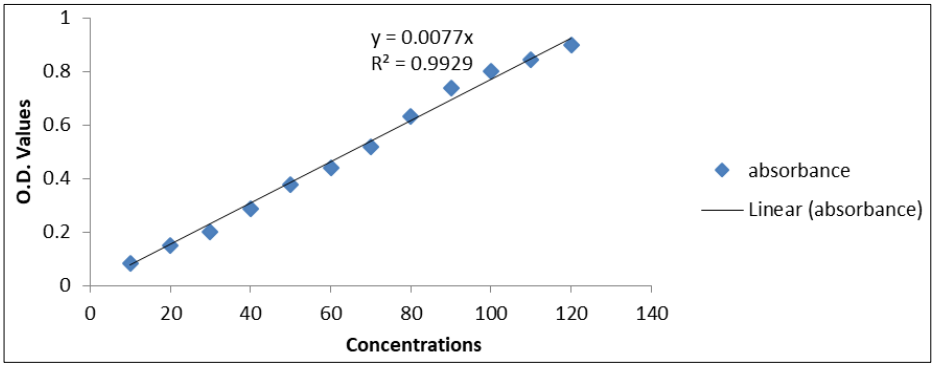


Fig 3: Calibration Curve for Total Phenols

Total flavonoid content (TFC)

To calculate the Total Flavonoid Content, quantitative analysis of both the plant extracts were carried out. The amount of total flavonoid was calculated by using the calibration curve of quarcetin shown in figure 5.

The total flavonoid content of the extracts (mg/g) of dry leaf was observed to be that *Madhuca longifolia* gave higher TFC in methanol (10.06±0.118 mg/g) followed by acetone (5.64±0.118 mg/g), dichloromethane (4.23±0.113 mg/g), distilled water (3.92±0.174 mg/g) and hexane (1.24±0.115 mg/g). On the other hand *Clerodendrum infortunatum* (Bhant) leaves reported maximum TFC in acetone (12.17±0.132 mg/g) and methanol (9.171±0.132 mg/g) extracts followed by dichloromethane (5.16±0.087 mg/g), distilled water (4.12±0.112 mg/g) and hexane (2.19±0.087 mg/g).

In general irrespective of extraction solvents *Clerodendrum infortunatum* (Bhant) leaves yielded higher total flavonoid among both the plant extracts which is clearly shown in figure 4. Meanwhile, among the extraction solvents, acetone gave good results for extraction of flavonoids compared to methanol, distilled water, dichloromethane and hexane.

Total Tannins Content (TTC)

Total tannins content of selected plant extracts prepared in various solvents such as distilled water, methanol, acetone, dichloromethane and hexane were calculated. The standard curve of catechol shown in figure 7 was used for calculating total tannins content in plant extracts. The results obtained are as follows-

The total tannins content of the extracts (mg/g) of dry leaf was observed to be that *Madhuca longifolia* gave higher Total Tannin Content in methanol (10.37 ± 0.142 mg/g) followed by acetone (7.3 ± 0.086 mg/g), dichloromethane (4.63 ± 0.089 mg/g), distilled water (4.26 ± 0.065 mg/g) and hexane (2.14±0.117 mg/g). However, *Clerodendrum infortunatum.*

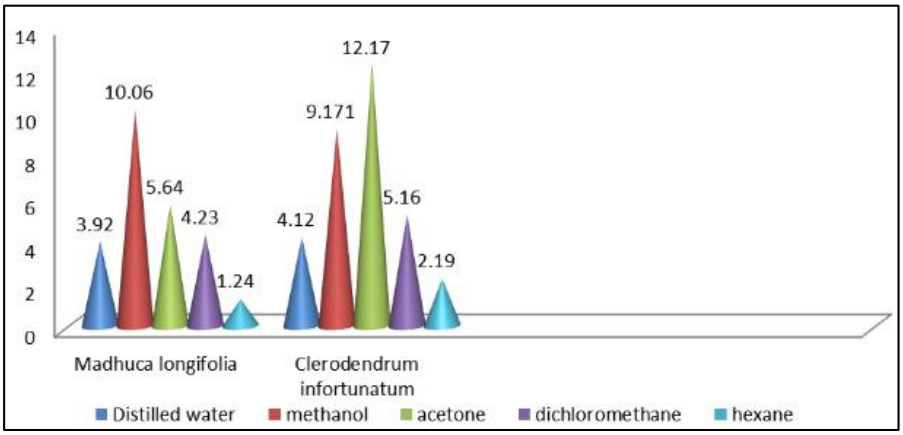


Fig 4: Total flavonoid content of *Madhuca longifolia* and *Clerodendrum infortunatum*

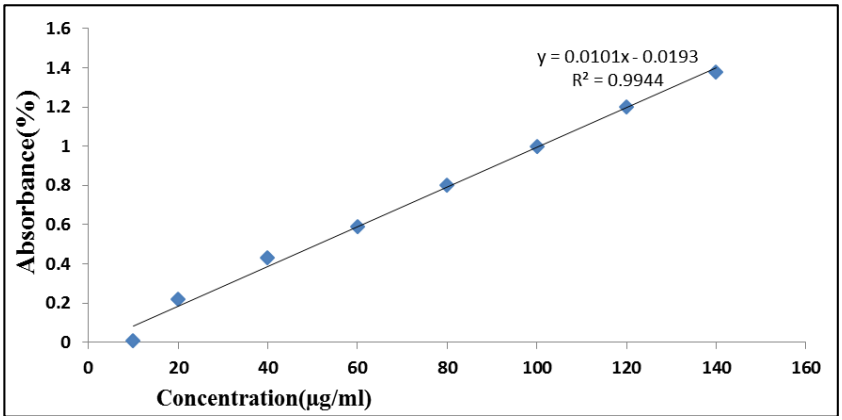


Fig 5: Calibration curve for total flavonoids

(Bhant) leaves reported maximum total tannin content in acetone (13.87±0.086 mg/g) and methanol (11.68±0.098 mg/g) extracts followed by dichloromethane (4.12±0.063 mg/g), distilled water (1.93±0.115 mg/g) and hexane (2.69±0.087 mg/g).

In general irrespective of extraction solvents *Clerodendrum infortunatum* (Bhant) leaves yielded higher total tannins among both the plant extracts which is shown in figure 6. Meanwhile, among the extraction solvents, acetone gave good results for extraction of tannins compared to distilled water, methanol, dichloromethane and hexane.

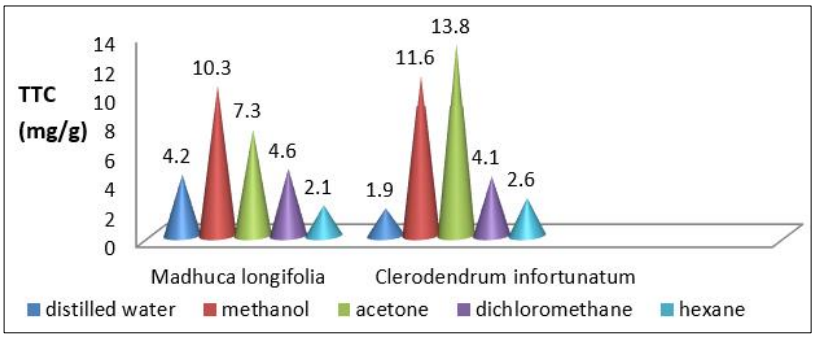


Fig 6: Total Tannins Content of *Madhuca longifolia* and *Clerodendrum infortunatum*

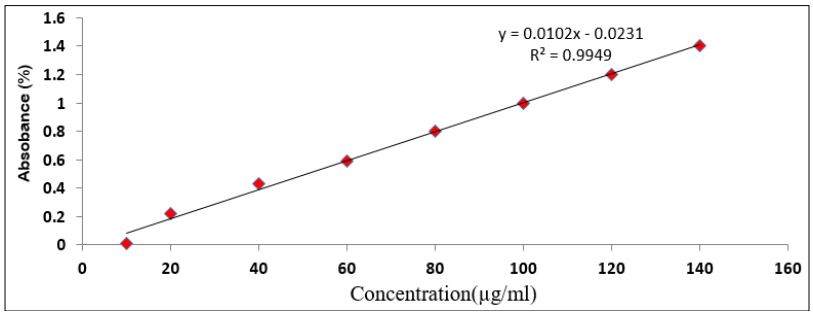


Fig 7: Calibration Curve for Total Tannins

Antibacterial activity of plant extracts

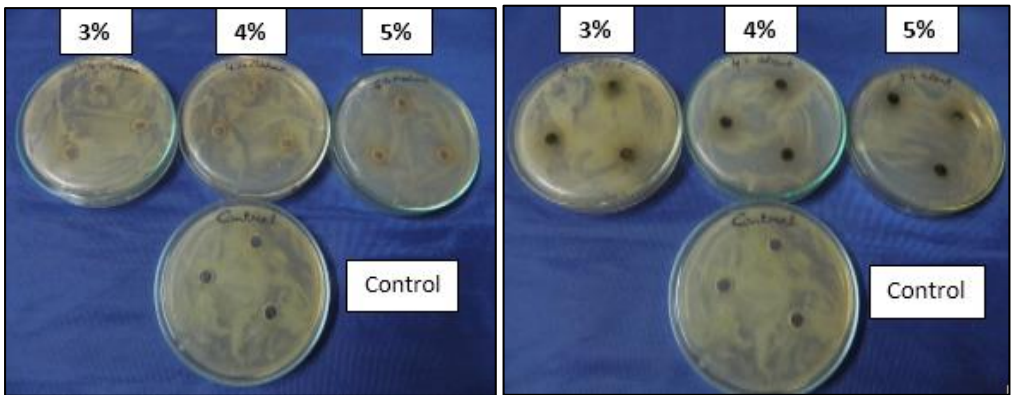
The antimicrobial activity of plant extracts against two bacterial strains (one gram positive and one gram negative) is recorded in the Table 3.

Madhuca longifolia exhibited maximum zone of inhibition at 5 percent of extract (2.2 ± 0.05cm) against *Staphylococcus pasteuri* followed by 4% (1.9 ± 0.1cm) and 3% (1.7 ± 0.11cm). In case of gram negative bacteria i.e. *Pseudomonas aeruginosa*, *Madhuca longifolia* exhibited maximum zone of inhibition at 5 percent of extract (0.7 ±0.02cm) followed by 4% (0.6 ±0.05cm) and 3% (0.6 ±0.05cm).

Clerodendrum infortunatum (Bhant) exhibited maximum zone of inhibition at 5 percent of extract (1.2 ± 0.05cm) against *Staphylococcus pasteuri* followed by 4% (1.1 ± 0.02cm) and 3% (0.7 ± 0.02cm). Whereas, in case of gram negative bacteria i.e. *Pseudomonas aeruginosa*, *Clerodendrum infortunatum* (Bhant) exhibited maximum zone of inhibition at 5 and 4% percent of extract (0.6 ±0.02cm) followed by 3% (0.6 ±0.05cm).

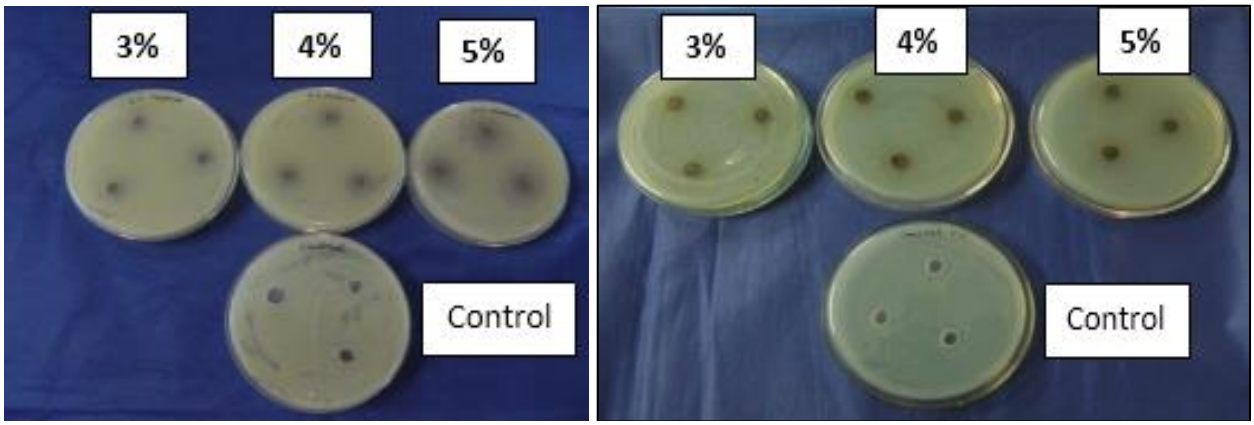
Table 3: Antibacterial activity of plant extracts

S. No.	Extracts	Zone of Inhibition (cm)					
		Gram positive bacteria			Gram negative bacteria		
		Staphylococcus pasteuri			*Pseudomonas aeruginosa*		
		3%	4%	5%	3%	4%	5%
1.	*Madhuca longifolia*	1.7 ± 0.11	1.9 ± 0.1	2.2 ± 0.05	0.6 ±0.05	0.6 ±0.05	0.7 ±0.02
2.	*Clerodendrum infortunatum*	0.7 ± 0.02	1.1 ± 0.02	1.2 ± 0.05	0.6 ±0.05	0.6 ±0.02	0.6 ±0.02



Madhuca longifolia ***Clerodendrum infortunatum* (Bhant)**

Plate 5: Antimicrobial activity of different plant extracts against *Staphylococcus pasteuri* in three concentrations



Madhuca longifolia ***Clerodendrum infortunatum* (Bhant)**

Plate 6: Antimicrobial activity of different plant extracts against *Pseudomonas aeruginosa* in three concentrations

Conclusion

Analysis of the percentage yield of both the plants viz; *Madhuca longifolia* and *Clerodendrum infortunatum* showed that, percentage yield of *Madhuca longifolia* was found to be higher in all the solvents. Irrespective of the plant sources, percent yield of both the plant extracts was found to be higher in methanol and acetone solvent compared to distilled water, dichloromethane and hexane. Various qualitative tests confirmed the presence of secondary metabolites in the extracts of oil cake of *Madhuca longifolia* and leaves of *Clerodendrum infortunatum.* Further quantitative analysis confirmed that among both the plant sources, *Madhuca longifolia* (*Mahua*) yielded higher total phenols (70.43±0.183mg/g in methanol) in comparison with *Clerodendrum infortunatum* (29.17±0.119mg/g in methanol). Among the extraction solvents, methanol gave good results for extraction of phenols. Quantification of flavonoids and tannins showed that *Clerodendrum infortunatum* (Bhant) leaves yielded higher total flavonoid (12.17±0.132 mg/g in acetone) and total tannins (13.87±0.086 mg/g) among both the plant extracts. Among the extraction solvents, acetone gave good results for extraction of flavonoids and tannins compared to distilled water, methanol, dichloromethane and hexane. The antimicrobial activity of both the plant extracts showed maximum zone of inhibition at 5% against both test organisms viz. *Staphylococcus pasteuri* and *Pseudomonas aeruginosa.* Therefore the plant extracts of *Madhuca longifolia* (oil cakes) and *Clerodendrum infortunatum* (dried leaves) can be used separately or a ratio based consortia can be prepared to apply antimicrobial finish on textiles against two microorganisms i.e. *Staphylococcus pasteuri* and *Pseudomonas aeruginosa* which are cellulose degrading. The finish can be applied with various techniques such as dip dry, spray, microencapsulation, nano encapsulation and many more.

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