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Phytochemical Screening and Antimicrobial Activity of Plant Extracts for Textile Applications

Shameembanu A. Byadgi[1*], Sadhana D. Kulloli[1] and C. K. Venugopal[2]

[1]Department of Textile and Apparel Designing, College of Community Science, University of Agricultural Sciences, Dharwad, India.
[2]Department of Horticulture, College of Agricutlure, University of Agricultural Sciences, Dharwad, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author SAB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SDK and CKV managed the analyses of the study. Author CKV managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To carry out the qualitative and quantitative phytochemical screening and assess the antimicrobial activity of banyan (*Ficus benghalensis*), castor (*Ricinus communis*) and clerodendron (*Clerodendron inerme*) leaf extracts.
Place and Duration of Study: Department of Textile and Apparel Designing, College of Community Science, University of Agricultural Sciences, Dharwad, Karnataka, between July 2014 and June 2017.
Methodology: Extraction of phytochemicals was carried out by different solvents *viz*., 70% ethanol, 70% methanol and distilled water. The phytochemical screening was carried out for the presence of various bio-active constituents according to standard procedures. Total phenolic content (TPC) was determined by Folin-Ciocalteu reagent assay method. Antimicrobial activity of crude extracts of plants was assessed by agar well diffusion method.

**Corresponding author: E-mail: shama29@rediffmail.com;*

Results: The qualitative screening revealed the presence of constituents such as flavonoids, alkaloids, tannins, phenols and saponins in all the leaf extracts. However, terpenoids were absent in banyan leaf extracts. Irrespective of solvents, castor extracts yielded higher total phenols followed by banyan and clerodendron extracts. Further, the antibacterial activity of the crude ethanol extract of castor against bacterial species *viz*., *Staphylococcus aureus*, *Escherichia coli* and fungal strain, *Aspergillus niger* showed highest antibacterial and antifungal activity compared to banyan and clerodendron extracts.

Conclusion: It can be concluded that the results has provided the basis for use of banyan, castor and clerodendron extracts as potential agents for applying antimicrobial finish to textiles. Hence, there is a need to explore the applicability of these plant resources which are rich in phytochemicals/phenolics and may have beneficial effects on health.

Keywords: Antimicrobial activity; Aspergillus niger; banyan; castor; clerodendron; Escherichia coli; phytochemicals; Staphylococcus aureus.

1. INTRODUCTION

Textile materials are vulnerable to microorganisms' attack which would cause many cross infections and allergic reactions to the wearer. Microorganisms can deteriorate the performance properties of fabrics and produce discomfort to the wearer. Control of microbial growth may be achieved using physical or chemical agents that either kill microorganisms or inhibit their further growth. Therefore, in order to protect the wearer from such infection, the textile fabrics can be finished with antimicrobial agents. Basically, with a view of protecting the hygiene in the textile substrate, the antimicrobial finish is a recent innovation in finishes used for health care applications. With this threat gaining its stature day by day, there is a wide variety of commercial antimicrobial agents available in the market but it gives toxic effect to the environment and the wearer. Hence, there is a necessity and expectation for a wide range of textile products finished with eco-friendly antimicrobial properties [1].

Medicinal plants possess numerous properties *viz*., antimicrobial, aroma and wound healing; attributed to the presence of various complex chemical substances with different composition. Phytochemicals are divided as primary and secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's *etc*. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides [2]. Plant sources such as banyan, castor, clerodendron represent major medicinal species and are extensively used in Indian traditional medicinal system for curing various diseases/ ailments. Hence, the aforesaid plants were selected for the present study.

Banyan (*Ficus benghalensis*) belongs to the family Moraceae, is native to Asia including India where it grows from low altitudes to 2000 ft (610 m), especially in dry regions. The roots are given for persistent vomiting and infusion of bark is considered as a tonic & astringent and is also used in diarrhoea, dysentery and diabetes; the milky juice (latex) of stem bark is used for the treatment of rheumatism and other inflammatory diseases. Studies revealed that the plant possess anti-inflammatory, anti-diarrhoeal, antidiabetic, antioxidant, antibacterial and antifungal activities [3].

Castor (*Ricinus communis*) belonging to family Euphorbiaceae is a tall glabrous and glaucous annual sometimes shrubby or almost small tree, 2-4 m high; found throughout India, mostly growing wild on waste land and also cultivated for its oil seeds. The Sanskrit name *'Erandah'* describes the property of the drug to dispel diseases. The plant is used in the treatment of paralysis, epilepsy, distention of the uterus and is considered as a reputed remedy for all kinds of rheumatic infections. Phytochemical studies revealed the presence of anti-inflammatory, antidiabetic, anticancer, purgative and antimicrobial activities [4].

Clerodendron (*Clerodendron inerme*) is a genus of flowering plants belonging to the family Lamiaceae, widely distributed in tropical and subtropical regions of the world, includes small trees, shrubs and herbs. Common names include glory bower and bag flower. Ethno-medical importance of various species of *Clerodendron* genus has been reported in various indigenous systems of medicines and as folk medicines for treating various health and skin disorders. The plant is reported to possess anti-inflammatory, antioxidant, antimalarial, antibacterial, antifungal and antiviral activities [5].

Phytochemical surveys are being seen as the first step towards the discovery and structural elucidation of useful natural organic constituents for the development of antimicrobial agents in the fields such as pharmaceuticals, food processing, textile wet processing and many more. Since, textile materials provide a medium for growth of micro organisms, the mode of action of plants producing antimicrobial effects on selected textile materials can be better investigated if the active ingredients are identified and characterized [6]. Recognising the importance of plant materials as antimicrobial agents, research has been initiated in the area of producing bioactive textiles for the protection of wearer from common microbes causing cross infections. Thus, the present study was designed with the following objectives.

- To carry out the qualitative and quantitative phytochemical screening of plant extracts
- To assess the antimicrobial activity of plant extracts

2. MATERIALS AND METHODS

2.1 Plant Sources

Based on the availability and review of literature, matured leaves of banyan (*Ficus benghalensis*), castor (*Ricinus communis*) and clerodendron (*Clerodendron inerme*) were selected for the present study. The plant sources were identified by the Department of Horticulture, College of Agriculture, University of Agricultural Sciences, Dharwad.

2.2 Herbal Extraction

The leaves of selected plant sources were cleaned with distilled water and shade dried at room temperature to remove the traces of moisture. Leaves were crushed to fine powder using mechanical grinder. Two grams of dry leaf powder of each sample were weighed and mixed with 25 ml (w/v) of each solvent (70% ethanol, 70% methanol and distilled water) separately. The extracts were incubated for 24 hours at room temperature, later centrifuged at 5000 rpm (REMI C-24 Plus refrigerated centrifuge) and the supernatants were separated. Residue was re-extracted with 25 ml of the respective solvent and the process was repeated [7]. The supernatants obtained were pooled and the extracts obtained were measured and filtered using Whatman filter paper No. 40 (125 mm).

2.3 Phytochemical Screening

2.3.1 Qualitative screening

Plants produce different class of secondary metabolites such as alkaloids, tannins, flavonoids, phenols, saponins, glycosides, terpenoids and so on that are responsible for therapeutic and defence properties. Qualitative screening helps in identification of these metabolites present in the plant extracts. Thus, qualitative screening of plant extracts was carried out according to the standard procedures as mentioned in Table 1.

Table 1. Standard qualitative tests for screening the presence of phytochemicals

Phytochemicals	Tests	Reagents	Positive results
Alkaloids	Dragendorff test [8]	Dragendorff's reagent	Prominent yellow ppt
	Wagner test [8]	Wagner's reagent	Reddish brown ppt
Flavonoids	Ammonia test [9]	1% NH_3	Yellow colour
	Sodium hydroxide test [10]	20% NaOH, HCl	Yellow colour; on addition of HCl turns to colourless
Tannins	Ferric chloride test [8]	5% $FeCl_3$	Blue-black or blue-green colouration
Phenolic compounds	Gelatin test [9]	1% gelatine solution containing 10% NaCl	White ppt
	Lead acetate test [8]	10% lead acetate	Bulky white ppt
Saponins	Foam test [10]	20 ml distilled water (mixed vigorously for 15 minutes)	Presence of froth
Terpenoids	Salkowski test [10]	0.5 ml chloroform, 1 ml conc. H_2SO_4	Reddish brown colouration at the interface

2.3.2 Quantitative screening

Total phenolic content in the extracts was determined by Folin-Ciocalteu assay method [11] with little modification using gallic acid as the reference standard. All the solvent extracts were diluted to appropriate volumes and were mixed with 2 ml of 10 per cent sodium bicarbonate solution, incubated at room temperature for 3 min, later 100 µl of Folin-Ciocalteu reagent was added to the mixture. The resulting solution was incubated for 90 min at room temperature under dark, the absorbance was measured at 765 nm using the UV-Vis Spectrophotometer (BioMate 3S UV-Visible Spectrophotometer). The TPC was expressed as gallic acid equivalent (GAE) in milligrams per gram of dry leaf.

2.4 Antimicrobial Activity of Plant Extracts

2.4.1 Selection of micro organisms

Staphylococcus aureus and *Escherichia coli* are the common cloth damaging bacteria. *S. aureus* contaminate under garments and can cause infections like furunels and boils diseases of skin. *E. coli* are non-pathogenic in normal conditions but if present in excess, will become causative agent of various diseases like urinary tract infection, diarrhea and vomiting [12].

Therefore, in the present study, bacterial species *viz.,* Gram positive *Staphylococcus aureus* (ATCC 6538) and Gram negative *Escherichia coli* (ATCC 8739) were selected and procured from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh. The fungal strain was isolated from infected peanuts by direct transfer technique that involves simple transfer of a mould from its natural habitat to a pure culture situation in the laboratory. Later, the strain was identified by the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad.

2.4.2 Screening antimicrobial activity of plant extracts

The antimicrobial activity of the plant extracts was assessed according to the Agar well diffusion method [13]. Nutrient media was poured in the sterilized petri plates and allowed to solidify (lower layer) under aseptic conditions. Later, 150 ml nutrient media was inoculated with 1 ml of 24 hours old bacterial working culture and poured on the solidified media (upper layer) and allowed to solidify. Four wells equidistant to each other were created using a cork borer (5 mm diameter). About 5 µl of plant extract was loaded to each well and the plates were incubated for 24 hours at 37℃. Seventy per cent ethanol was used as control. Zone of inhibition around the well was recorded in millimeters. Similar procedure was followed for assessing antifungal activity against *A. niger* using potato dextrose agar and potato dextrose broth. The plates were incubated at 28℃ for 48 hours and the observations were recorded [14].

2.5 Statistical Analysis

Statistical tools *i.e*., mean and standard deviation was applied to analyse the experimental data. Further, Analysis of Variance (ANOVA) for factorial experiments was used to examine the statistical significance between the variables.

3. RESULTS AND DISCUSSION

3.1 Yield of the Plant Extracts

The yield of the extracts obtained per 50 ml of solvent is presented in Fig. 1. It is observed that banyan leaves produced higher yield in methanol (36 ml/ 50 ml) followed by ethanol (35 ml/ 50 ml) and distilled water (33 ml/ 50 ml) respectively. However, clerodendron leaves produced higher yield in ethanol (42 ml/ 50 ml) compared to methanol (38 ml/ 50 ml) and distilled water (35 ml/ 50 ml). Similarly, the yield of castor leaf extract was high in ethanol (40 ml/ 50 ml) followed by methanol (39 ml/ 50 ml) and distilled water (34 ml/ 50 ml). In general, irrespective of the plant sources, yield of all the leaf extracts was higher in ethanol and methanol solvent compared to distilled water.

Almey and co-workers [15] suggested that both ethanol and methanol offered the best results to extract phenolic compounds because of the presence of polar groups. The authors found that as polarity of the solvent increased, higher extraction yields of total soluble solids and total extractable phytochemicals were obtained. Moreover, it was observed that extracting solvent significantly affected the yield of extracts indicating that different extracting solvents influenced different yields of extracts. The results are in line with the study conducted by Vastrad and co-workers [16] who revealed that the yield of plant extracts was higher in ethanol and methanol solvents.

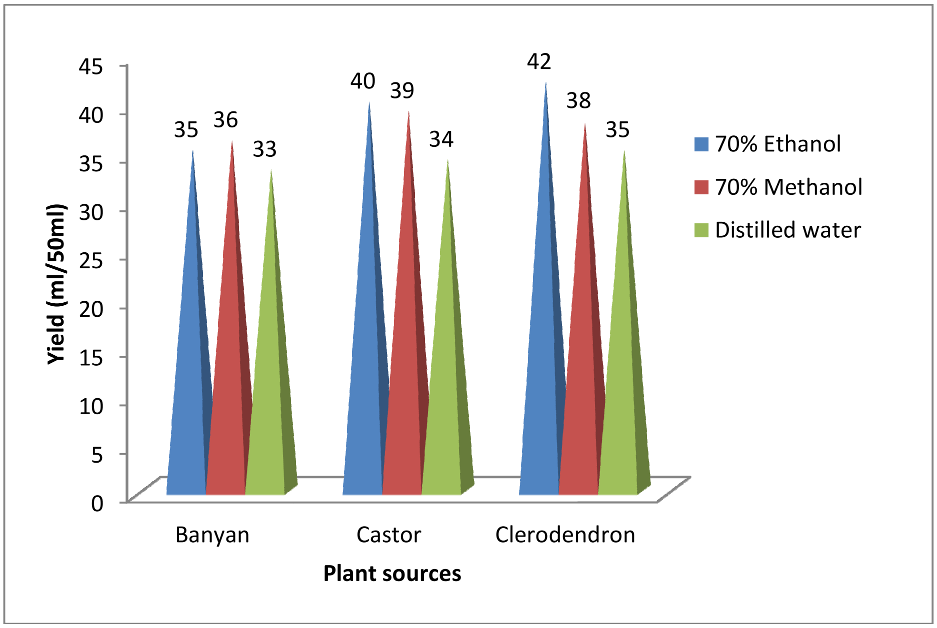


Fig. 1. Yield of the plant extracts

3.2 Phytochemical Screening of Plant Extracts

3.2.1 Qualitative screening

Table 2 revealed significant presence of alkaloids in all the leaf extracts of banyan and castor while moderate presence of alkaloids was observed in all the extracts of clerodendron confirmed by Dragendorff's and Wagner's tests. Presence of alkaloids contributes to medicinal value as well as physiological activity to the plant. They possess astringent, anti-diabetic, diuretic, anti-asthmatic, anti- cancer and anti-bacterial activities. Thus, the plant sources possessing alkaloids can be used for imparting antimicrobial finishing to textiles [2].

Significant presence of flavonoids was observed in methanol and distilled water extracts of banyan proved by ammonia test where as sodium hydroxide test conferred the presence of flavonoids only in distilled water extract. However, moderate presence of flavonoids was observed in all the leaf extracts of castor and clerodendron proved by both ammonia and sodium hydroxide tests. Flavonoids have been referred to as nature's biological response modifiers; because of their inherent ability to modify the body's reaction to allergies & virus and possess anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities [2]. They are considered as potent antioxidants with reported antimutageneic and anticarcinogenic effects.

Further, ferric chloride and lead acetate tests conferred significant presence of phenols and tannins in all the leaf extracts of banyan, castor and clerodendron. On the other hand, gelatin test gave positive results for phenols and tannins in all the extracts of banyan leaves and methanol extract of clerodendron leaves. Phenols possess a number of biological activities such as antioxidant, antiseptic, disinfectant fungicide and pesticides [17]. The antiviral, antibacterial and anti-tumour activities of tannins are very important in imparting antimicrobial property to the textiles. Tannins are also used in the dyestuff industry as caustics for cationic dyes (tannin dyes), and also in the production of iron gallate inks [2].

Saponins were present in moderate amounts in all the extracts of castor; methanol and distilled water extracts of banyan and ethanol extract of clerodendron leaves as proved by foam test. Saponins confer antifungal, antioxidant, anti-inflammatory properties and can be used as mordants in natural dyeing and printing of textiles [2].

Table 2. Qualitative screening of plant extracts

Sl. no.	Phytochemical tests	Banyan			Castor			Clerodendron		
		70% ethanol	70% methanol	Distilled water	70% ethanol	70% methanol	Distilled water	70% ethanol	70% methanol	Distilled water
I.	**Alkaloids**									
a.	Dragendorff's test	++	++	++	+++	+++	++	+	+	-
b.	Wagner's test	+++	+++	+++	+++	++	+++	++	++	++
II.	**Flavonoids**									
a.	Ammonia test	++	+++	+++	+	+	++	++	+++	++
b.	Sodium hydroxide test	-	-	+++	+	++	+++	+	+	++
III.	**Phenols and tannins**									
a.	Ferric chloride test	+++	+++	+++	+++	+++	++	+++	+++	+
b.	Gelatin test	+	+	+	-	-	-	-	+	-
c.	Lead acetate test	+++	+++	+++	+++	+++	+++	+++	+++	+++
IV.	**Saponins**									
a.	Foam test	-	+++	++	+	++	+++	++	-	-
V.	**Terpenoids**									
a.	Salkowski test	-	-	-	++	+	+++	-	-	+++

+++ = *Strongly present;* ++= *Moderately present;* + = *Poorly present;* - = *Absent*

On the other hand, Salkowski test depicted significant presence of terpenoids in all the extracts of castor leaves and distilled water extract of clerodendron leaves but absent in banyan leaf extracts. Terpenoids play a defensive role in plants being toxic, metabolic inhibitors and exerts antimicrobial activity.

However, the meticulous activity diffused by each bio-active compound needs to be characterized further in order to develop novel products for improving the quality characteristics of textile materials.

3.2.2 Quantitative screening

Table 3 highlights on the total phenolic content of the plant extract (mg/ g of dry leaf). It is observed from the Table that banyan leaf extracts exhibited higher TPC in methanol (29.302 ± 2.10 mg/ g) followed by ethanol (27.214 ± 2.312 mg/ g) and distilled water (18.472 ± 1.527 mg/ g) extracts. Conversely, clerodendron leaves depicted maximum TPC in both ethanol (26.708 ± 3.362 mg/ g) and methanol (26.248 ± 2.048 mg/ g) extracts as compared to distilled water (19.194 ± 2.023 mg/ g) extract. Further, castor leaves also exhibited maximum TPC in ethanol (33.522 ± 2.519 mg/ g) and methanol (33.305 ± 2.521 mg/ g) extracts followed by distilled water (26.78 ± 2.306 mg/ g) extract. Ethanol is said to be the most suitable solvent in the extraction of phenolic compounds due to its ability to inhibit the reaction of polyphenol oxidase that causes the oxidation of phenolics and its ease of evaporation compared to water [15]. Though, ethanol and methanol gave similar results with respect to total phenolic content, ethanol was selected for further experiment because ethanol is more polar than methanol and also due to the cytotoxic nature of methanol, it is not preferred in textile wet processing.

3.3 Antimicrobial Activity of Plant Extracts

The antimicrobial activity of herbal extracts against bacterial and fungal species is recorded in Table 4. It is observed that, castor extract exhibited maximum zone of inhibition against *S. aureus* (14 ± 1.83 mm) followed by clerodendron (12 ± 0.96 mm) and banyan (11 ± 0.96 mm) extracts. Further, it was observed that, castor (12 ± 1.63 mm) and banyan (12 ± 2.22 mm) extracts showed greater zone of inhibition against *E. coli* compared to clerodendron (10 ± 0.82 mm) extract. Similarly, castor extract exhibited good antifungal activity against *A. Niger* (13 ± 1.71 mm) than clerodendron and banyan extracts (Fig. 2). However, 70 per cent ethanol (control) exhibited least antibacterial and antifungal activity.

Table 3. Total phenolic content (TPC) of plant extracts

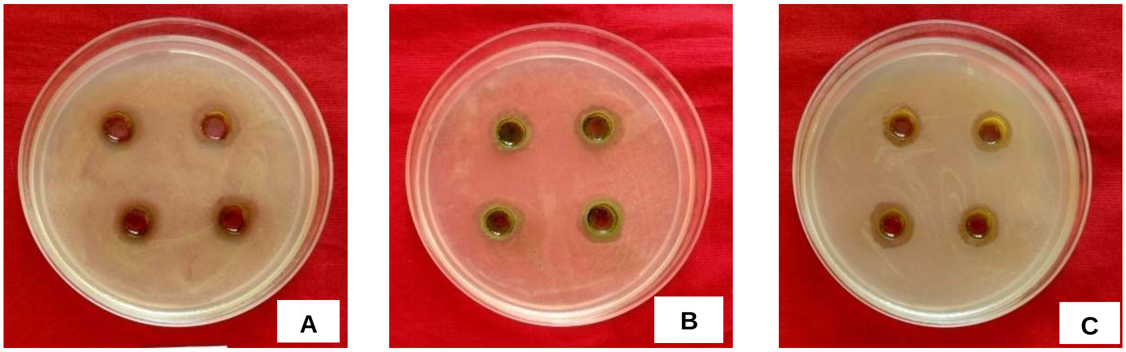
Sl. no.	Plant sources	Total phenolic content (mg/g dried leaf)		
		70% ethanol	**70% methanol**	**Distilled water**
1.	Banyan	27.214 ± 2.312**	29.302 ± 2.10**	18.472 ± 1.527**
2.	Castor	33.522 ± 2.519**	33.305 ± 2.521**	26.78 ± 2.306**
3.	Clerodendron	26.708 ± 3.362**	26.248 ± 2.048**	19.194 ± 2.023**
C.D. (0.01)	Sources	2.33		
	Solvents	2.33		
	Sources x Solvents	4.04		
C.V. (%)		**6.74**		

Mean ± Standard deviation
***Highly significant @ 1 per cent level of significance*

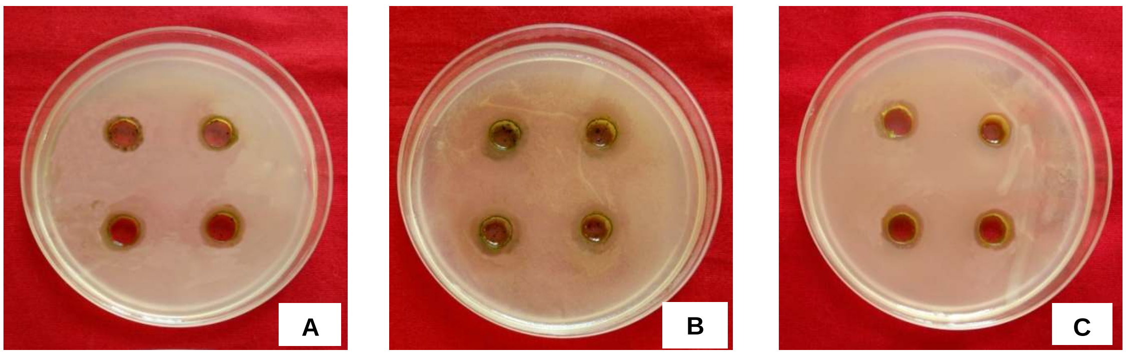
Table 4. Antimicrobial activity of plant extracts

Sl. no.	Extracts	Zone of inhibition (mm)		
		Antibacterial activity		**Antifungal activity**
		S. aureus	***E. coli***	***A. niger***
1.	70% ethanol (control)	09 ± 0.82	08 ± 0.82	09 ± 0.82
2.	Banyan	11 ± 0.96	12 ± 2.22	10 ± 1.26
3.	Castor	14 ± 1.83	12 ± 1.63	13 ± 1.71
4.	Clerodendron	12 ± 0.96	10 ± 0.82	12 ± 1.26

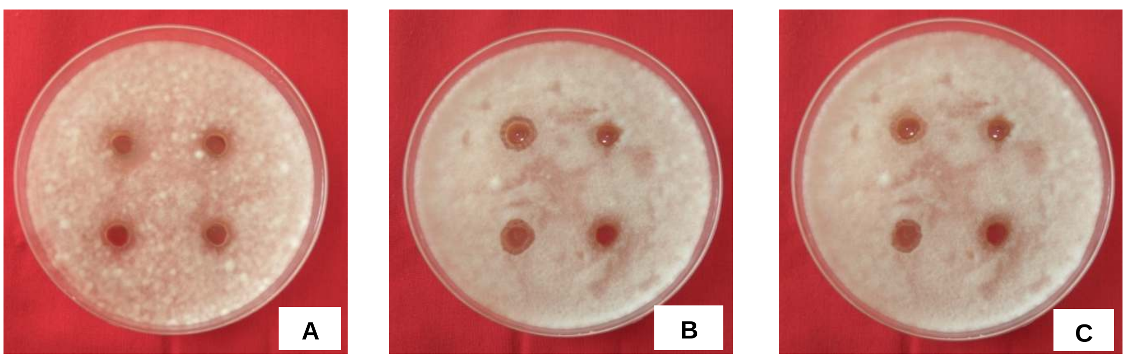
Mean ± S.D.; S. aureus – Staphylococcus aureus; E. coli – Escherichia coli; A. niger – Aspergillus niger



Antibacterial activity against *Staphylococcus aureus*: Castor (A), Banyan (B) and Clerodendron (C)



A**ntibacterial activity against *Escherichia coli*: Castor (A), Banyan (B) and Clerodendron (C)**



Antifungal activity against *Aspergillus niger:* Castor (A), Banyan (B) and Clerodendron (C)

Fig. 2. Antimicrobial activity of plant extracts

The variation in the antimicrobial activity of the plant extracts can be attributed to inoculum size, type of media used, type of solvent used for extraction, extraction procedure, incubation time and temperature, part of the plant used and its time of collection, method of extraction procedure, incubation time and temperature, method of antimicrobial assay and strain activity [18]. The resistance of plant extract to microbial attack also depends on its leaching (diffuse) mechanism *i.e.,* higher the diffusing action of plant extract better will be the antimicrobial activity. Meanwhile, among the bacterial species, higher inhibition zone was observed against Gram positive (*Staphylococcus aureus*) bacteria than Gram negative (*Escherichia coli*) bacteria. Similar observations have been made by many researchers who reported that Gram positive bacteria are more susceptible to plant's extracts as compared to Gram negative bacteria [19,20].

4. CONCLUSION

India has rich flora used in traditional medical treatments; the medicinal properties of these plants could be based on the therapeutic and antioxidant effects of different phytochemicals present in them. The results revealed that alkaloids, flavonoids, saponins, phenols and tannins were present in all the leaf extracts. However, terpenoids were absent in banyan leaf extracts. Further, irrespective of solvents, castor extracts yielded higher total phenols followed by banyan and clerodendron extracts. The crude extract of castor showed higher zone of inhibition against bacterial and fungal species compared to clerodendron and banyan extracts. Among various functionality, the antimicrobial property of fabric is being considered to be important with garments, which are in direct contact with human body. Also, research interest is focused on exploring the efficacy of new types of antibacterial agents that are permanently fixed on the fiber, controlling the multiplication of microorganisms even after many launderings. Hence, it can be concluded that there is a need to explore the applicability of these plant resources for textile applications which are rich in phytochemicals/ phenolics and may have beneficial effects on health.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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