Characterization of Phytoconstituents in Leaf Extracts of Forest Species for Textile Applications

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Abstract

Antimicrobial fabric is increasingly used, and could eventually be required, in garments that are in direct contact with the human body, an environment that is ideal for microbial growth. Forest species such as *Cassia fistula, Pongamia pinnata, Tectona grandis,* and *Jatropha curcas* represent major groups of antimicrobial agents, which consist of active phytochemical constituents and can be used as antimicrobial agents for applying special finishes on textiles. In the present study, qualitative and quantitative screening of leaf extracts of forest species was carried out. Leaf extracts of *C. fistula, P. pinnata, T. grandis,* and *J. curcas* were prepared using solvents, viz., ethanol (70%), methanol (70%), choloroform, and deionized water. Extracts were determined for the presence of phytochemicals. Results of phytochemical screening revealed the presence of alkaloids, flavonoids, and tannins in all the leaf extracts of selected forest species. However, the saponins were absent in *P. pinnata* and *J. curcas* leaf extracts, and terpenoids were absent only in *P. pinnata* extracts. Further, the total phenolic content (TPC) was evaluated using the Folin-Ciocalteu assay method, whereas total flavonoid content (TFC) was analyzed using the colorimetric method. Methanolic extracts of *T. grandis, P. pinnata,* and *C. fistula* exhibited the highest TPC and TFC in the increasing order 143.74, 161.53, and 228.08 mg, and 126.21, 148.33, and 179.1 mg, respectively, while *J. curcas* exhibited high amounts of TPC and TFC content in ethanolic extract. We therefore conclude that extracts from forest species such as *C. fistula, P. pinnata, T. grandis,* and *J. curcas* can be used for applying eco-friendly and healthy finishes to textile substrates.

The plant kingdom has proved to be the most useful in the treatment of diseases, and plants provide an important source for pharmaceuticals. Historically, plants have provided a source of inspiration for novel drug compounds because plant-derived medicines have made large contributions to human health and well being (Sharma et al. 2012). Plant-based natural constituents are derived from various parts of plants, such as leaves, flowers, fruits, seeds, etc. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Natural products play an important role in drug-development programs in the pharmaceutical industry (Baker et al. 1995). The art and science of treating illness using plant sources has ancient roots in India. Medicinal herbs were selectively chosen and identified to cure specific ailments by traditional doctors called *vaidyas*. This evolved as a science of healing called Ayurveda. Ayurveda has lately been gaining popularity because of its limited side effects and its potential to cure diseases. The most important of these bioactive constituents of plants are terpenoids, flavonoids, alkaloids, tannins, and phenolic compounds. Medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effect and can be natural composite sources that can be used as anti-infective agents. Plants to be exploited for medicinal purposes have to undergo basic phytochemical screening as the first step toward the ultimate development of natural drugs (Saxena et al. 2010).

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The phytochemicals of various plant extracts can be applied to fabric as an antimicrobial finish. Garment manufacturers often add the antimicrobial agents to fabric in the garments they make. Those garments are in direct contact with the human body, which offers an ideal environment for microbial growth. Microbial infestation is dangerous to living beings and nonliving material. Consumers are now increasingly aware of the hygienic properties of commercial goods, and consumers expect a wide range of textile products to be finished with antimicrobial properties.

Researchers conduct many studies related to the application of antimicrobial finishes on textiles using natural plant sources. The major challenges in the application of natural products for textiles are that most of these sources are complex mixtures of several compounds and that the composition varies in different species of the same plant. Furthermore, the activity and composition also vary, depending on their geographical location, age, and method of extraction (Joshi et al. 2009). Forest species such as *Cassia fistula, Pongamia pinnata, Tectona grandis,* and *Jatropha curcas* represent major groups of antimicrobial agents, which consist of active phytochemical constituents.

Cassia fistula, also known as golden shower tree, is a flowering plant that belongs to the Caesalpiniaceae family. This species is native to India and has been widely distributed in Brazil and Sri Lanka. Its leaves and seeds have been traditionally used in these regions to treat numerous human ailments due to its cicatrizing, antipyretic, and analgesic effects (Luximon-Ramma et al. 2002). It is a mid-sized deciduous tree and is the national tree of Thailand. It is also the state flower of Kerala, India, and possesses immense importance among the Malayali population (Joshi 2000). It is popular as an ornamental plant and as an herbal medicine. This plant has been used in India to treat skin diseases such as leucoderma (Sartorelli et al. 2012). The root, bark, seeds, and leaves of this plant have medicinal properties.

Pongamia pinnata is a member of the Fabaceae family. It is a medium-sized glabrous tree native to Western Ghats, and it is found all over India on the banks of rivers and streams. Historically *P. pinnata* has been used as a folk medicinal plant, particularly in the Ayurveda and Siddha systems of Indian medicine (Vellingiri and Biesalski 2011). All parts of this plant have medicinal properties and are traditionally used medicinally. They have been used as a crude drug for the treatment of tumors, piles, skin diseases, wounds, and ulcers (Tanaka et al. 1992). *P. pinnata* has been exploited as a source of biomedicine, specifically as an antimicrobial and therapeutic agent (Vellingiri and Biesalski 2011). Fruits and seeds are used in many traditional remedies (Joshi 2000). Particularly, leaves have anthelmintic, digestive, and laxative uses, and leaves can be used for inflammations, piles, and wounds (Sangwan et al. 2010). *P. pinnata* is an effective remedy for all skin diseases, such as eczema, scabies, leprosy, and ulcers. Oil made from the seeds, known as *honge* oil, is an important asset of this tree and has been used in soap making and also as a lubricant for thousands of years.

Tectona grandis belongs to the family Verbenaceae and is popularly known as teak or sagwan. It occurs in all tropical and subtropical regions, it is a tall evergreen tree with yellowish blonde to reddish brown wood. It produces large leaves similar to the tobacco leaf. Its wood oil is used to treat eczema and ringworm, while wood-ash is applied to swollen eyelids (Gupta and Singh 2004). The leaves yield dye that is used to color clothes. The plant extracts exhibit potent antibacterial (Neamatallah et al. 2005), antiulcer (Pandey et al. 1982), anti-inflammatory (Kirtikar and Basu 1999), antiallergic (Krough 1962), central nervous system–depressant (Tamizhmani et al. 2003), and wound healing (Upadhya et al. 2000) activities.

Jatropha curcas belongs to the family Euphorbiaceae and is used in traditional folklore medicine to cure various ailments in Africa, Asia, and Latin America (Burkill 1994). *J. curcas* is commonly called physic nut, purging nut, or pig nut. *J. curcas* is native to tropical America, is grown in various parts of India as a field barrier, and is a common hedge plant in Konkan (Joshi 2000). The plum-sized fruit has been widely used in pharmaceuticals. Previous studies have reported that the plant has shown positive effects in the treatment of wounds (Sachdeva et al. 2011), diarrhea (Mujumdar et al. 2000), diabetes (Patil et al. 2011), tumors (Lin et al. 2003), immunomodulation (Abd-Alla et al. 2009), and rheumatism (Irvine 1961, Oliver-Bever 1986). The oil from seeds is used in the treatment of rashes and parasitic skin diseases and also as biodiesel for energy. Sap from the bark is used to dress wounds and ulcers and can also be used to stop bleeding. The leaves are used in the treatment of ulcers and tumors. The plant contains organic acids, viz., the cyclic triterpenes stigmasterol, curcacycline A, and curcin (Sachdeva et al. 2011).

Hence, the present study was taken up to screen the presence of phytochemicals in leaf extracts of *C. fistula, P. pinnata, T. grandis,* and *J. curcas* and assess the total phenolic content (TPC) and total flavonoid content (TFC). These compounds are not only present in plants as constitutive agents but are also formed in plant tissues in response to microbial attack (Harborne 1999). Flavonoids are secondary metabolites of plants and are generally located in the vacuoles of the epidermal cells of leaves as water-soluble glycosides (Harborne and Williams 2000). The basic structural feature of flavonoids is the 2-phenylbenzopyrane or flavan nucleus, which consists of two benzene rings linked through a heterocyclic pyran ring (Brown 1980).

Skin is the largest organ of the body. Maintaining skin health by way of curbing illness caused by infections is necessary. Most of the biochemical components offered by pharmaceutical industries are derivatives from the plant kingdom. Several antifungal compounds present in certain plant species have been used for controlling fungal pathogens (Tripathi and Dubey 2004, Serrano et al. 2005, Riaz et al. 2007, Bajwa et al. 2008). Botanical derivatives are more environmentally safe than synthetic chemicals (Hashim and Devi 2003).

Materials

Plant sources

Fresh leaves of *C. fistula, P. pinnata, T. grandis,* and *J. curcas* selected for the study were collected from an area of forest region in the western ghat (Sirsi region) of Karnataka state in India.

Chemicals

Analytical reagent (AR) grade ammonia, chloroform, ethyl alcohol, methanol, hydrochloric acid, sulfuric acid,

ferric chloride, sodium hydroxide, sodium chloride, sodium carbonate, lead acetate, and gelatin were purchased from Rankem chemicals, Bangalore. AR grade sodium nitrite and aluminum chloride were purchased from Thomas-Baker, Mumbai. Folin-Ciocalteu and Dragendorff's reagent was purchased from Merck, Germany. Gallic acid and rutin standards were purchased from Sigma-Aldrich, Germany.

Extraction

Preparation of extracts.—The leaves were cleaned using distilled water and shade dried for 2 hours to remove moisture from their surface. Two grams of fresh leaf was weighed, chopped into fine pieces, and ground in a laboratory mortar and pestle. Finely ground leaf was mixed in 25 mL of the solvent and incubated under agitation at 200 forward and backward strokes in an incubator shaker (Inkarp, Germany) for 24 hours at 25°C. The extract was centrifuged at 5,000 rpm (Remi Laboratory Equipments, Mumbai, India) at room temperature, and supernatant was separated. Residue was re-extracted with another 25 mL of the respective solvent, and the process was repeated. The supernatants were pooled, and the extract obtained was measured and filtered using Whatman filter paper no. 40 (125 mm). Extracts were stored under refrigeration at 8°C, and further analysis of phytochemicals took place within a week.

Phytochemical analysis.—Qualitative phytochemical screening of plant extracts was carried out for the identification of various classes of active chemical constituents like alkaloids, flavonoids, tannins, saponins, and terpenoids using different methods described by Raaman (2006), Rahul et al. (2010), and Ajayi et al. (2011).

Test for tannins and phenolic compounds

Ferric chloride test.—One milliliter of extract was separately stirred with 10 mL of distilled water and then filtered. A few drops of 5 percent $FeCl_3$ were added to the filtrate. Blue-black or blue-green coloration or precipitation was taken as an indication of the presence of tannins.

Gelatin test.—Two milliliters of a 1 percent solution of gelatin containing 10 percent NaCl was added to 1 mL of the extract. White precipitate indicates the presence of phenolic compounds.

Lead acetate test.—Three milliliters of a 10 percent lead acetate solution was added to 1 mL of extract. Appearance of bulky white precipitate confirms the presence of phenolic compounds.

Test for flavonoids

Ammonia test.—A few drops of a 1 percent NH_3 solution was added to 1 mL of the extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

Sodium hydroxide test.—A few drops of a 20 percent NaOH solution was added to 1 mL of extract. When HCl is added, the yellow color of the extract turns to a colorless solution that indicates the presence of flavonoids.

Test for alkaloids

Dragendorff test.—To 1 mL of extract, a few drops of Dragendorff's reagent were added. A prominent yellow precipitate indicates a positive test.

Wagner test.—A few drops of Wagner's reagent were added by the side of the test tube to 1 mL of extract. A reddish brown precipitate confirms the test as positive.

Mayer test.—One milliliter of extract was stirred with 5 mL of 1 percent HCl on a steam bath. The solution obtained was filtered, and 1 mL of the filtrate was treated with a few drops of Mayer's reagent. The turbidity of the extract filtrate on addition of Mayer's reagent was taken as evidence of the presence of alkaloids in the extract.

Test for saponins

For the foam test, about 1 mL of the sample extract was boiled in 20 mL of distilled water in a water bath and filtered; 10 mL of the filtrate was mixed with 5 mL of distilled water and mixed vigorously for 15 minutes to form a stable persistent froth. The presence of froth after 5 minutes was taken as an indication of the presence of saponins.

Test for terpenoids

To perform the Salkowski test, 1 mL of each extract was mixed with 0.5 mL of chloroform, and 1 mL of concentrated H_2SO_4 was added carefully to form a layer. The reddish brown coloration of the interface showed positive results for the presence of terpenoids.

Total phenolic content

TPC in the extracts was determined using the Folin-Ciocalteu assay method (Singleton and Rossi 1965) 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 a 10 percent Na_2CO_3 solution. The mixture was incubated at room temperature for 3 minutes, and 100 μL of Folin-Ciocalteu reagent was added to the mixture. The resulting solution was incubated for 90 minutes at room temperature in the dark, and the absorbance was measured at 765 nm using the ultraviolet–visible (UV-Vis) spectrophotometer (Varian, Middelburg, The Netherlands). The TPC was expressed as gallic acid equivalent in milligrams per gram of fresh leaf.

Total flavonoid content

The TFC was determined by the colorimetric method (Yun et al. 2009) with minor modifications. Aliquots (1 mL) of appropriately diluted extracts or standard solutions were pipetted into 15-mL polypropylene conical tubes containing 2 mL of double-distilled H_2O and mixed with 0.15 mL of 5 percent $NaNO_2$. After 5 minutes, 0.15 mL of a 10 percent $AlCl_3 \cdot 6H_2O$ solution was added, the mixture was allowed to stand for another 5 minutes, and then 1 mL of 1 M NaOH was added. The reaction solution was well mixed and kept for 15 minutes, and the absorbance was determined at 415 nm using the UV-Vis spectrophotometer. TFC was calculated using the standard rutin curve and expressed as milligrams of rutin equivalent (mg RE) per gram of sample.

Results and Discussion

The phytochemical screening of the leaves of selected forest species using 70 percent ethyl alcohol, 70 percent methanol, 100 percent chloroform, and deionized distilled water is presented in Table 1. The results reveal that ethanolic and methanolic leaf extracts of *C. fistula* exhibited

Table 1.—Qualitative phytochemical screening of forest species.[a]

Serial no. and phytochemical test	*Cassia fistula*				*Pongamia pinnata*				*Tectona grandis*				*Jatropha curcas*			
	E	M	C	A	E	M	C	A	E	M	C	A	E	M	C	A
1. Tannins and phenolic compounds																
a. Ferric chloride test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
b. Gelatin test	−	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+
c. Lead acetate test	+	+	−	+	+	+	−	+	+	+	−	−	+	+	−	−
2. Flavonoids																
a. Ammonia test	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+
b. Sodium hydroxide test	−	−	−	−	−	−	−	−	−	+	−	−	+	+	−	−
3. Alkaloids																
a. Dragendorff test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
b. Wagner test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4. Saponins																
a. Foam test	+	+	+	+	−	−	−	−	+	+	+	+	−	−	−	−
5. Terpenoids																
a. Salkowski test	+	+	−	−	+	−	−	−	−	−	+	+	+	+	+	−

[a] E = ethyl alcohol; M = methanol; C = chloroform; A = aqueous; + = present; − = absent.

positive results for alkaloids, flavonoids, tannins, saponins, and terpenoids. On the other hand, the leaf extracts in chloroform and aqueous solution showed the presence of alkaloids, flavonoids, tannins, and saponins, while terpenoids were absent in both the extracts.

It is further observed from Table 1 that ethanolic leaf extract of *P. pinnata* depicted the presence of alkaloids, flavonoids, tannins, and terpenoids, while negative results were obtained for saponins. The leaf extract in methanol exhibited positive results for all the phytochemicals except for saponins and terpenoids, which depicted negative results. On the other hand, only alkaloids and tannins were present in chloroform extract of *P. pinnata* leaves, whereas flavonoids, saponins, and terpenoids were found to be absent. The aqueous leaf extract depicted results similar to that of methanolic extract.

Alkaloids, flavonoids, tannins, and saponins were found to be present in ethanolic and methanolic extracts of *T. grandis* leaves, whereas terpenoids showed negative results in both the extracts. Contrarily, chloroform and aqueous extracts of *T. grandis* leaves showed the presence of all the phytochemicals. The phytochemical screening of *J. curcas* revealed the presence of all the phytochemicals except saponins in ethanolic and methanolic leaf extracts. Chloroform extract exhibited positive results for alkaloids, tannins, and terpenoids, while aqueous extract showed the presence of alkaloids, flavonoids, and tannins.

Comparatively, alkaloids, flavonoids, and tannins were commonly found in all the leaf extracts of selected forest species. However, negative results were obtained for saponins in *P. pinnata* and *J. curcas* leaf extracts, but terpenoids were absent only in *P. pinnata* extracts. These classes (alkaloids, saponins, tannins, anthraquinones, and flavonoids) of compounds are known to have activity against several pathogens and therefore aid the antimicrobial activities of *J. curcas* and suggest their traditional use for the treatment of various illness.

TPC of *C. fistula, P. pinnata,* and *T. grandis* (Figs. 1, 2, and 3) leaves was found to be high in methanolic extract, i.e., 143.74, 161.53, and 228.08 mg/g of fresh leaf, whereas *J. curcas* leaves (Fig. 4) exhibited maximum TPC in ethanolic (168.64 mg/g of fresh leaf) extract. However, the aqueous extracts depicted 14.52, 12.39, 23.05, and 3.34 mg of TPC/g of fresh leaf in *C. fistula, P. pinnata, T. grandis,* and *J. curcas* leaves, respectively.

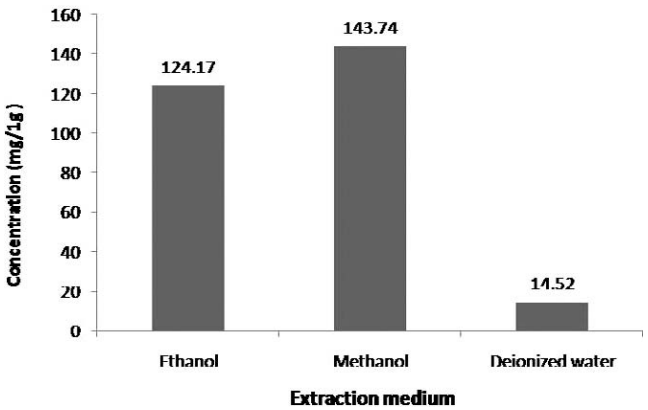

Figure 1.—Total phenolic content (milligrams per gram of fresh leaf) of cassia leaf extracts.

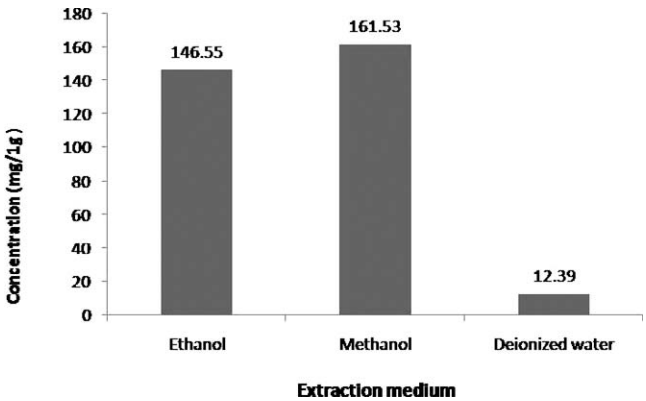

Figure 2.—Total phenolic content (milligrams per gram of fresh leaf) of pongemia leaf extracts.

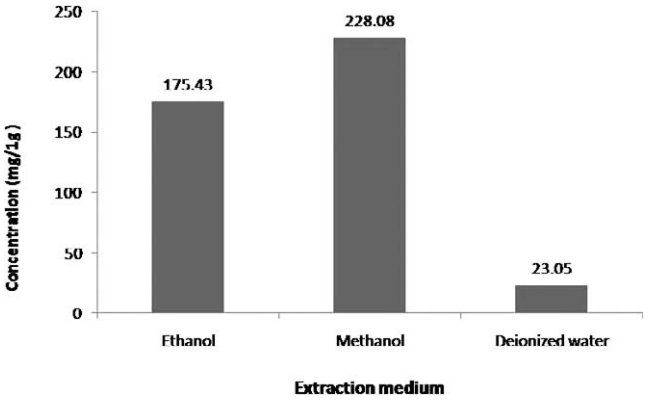

Figure 3.—Total phenolic content (milligrams per gram of fresh leaf) of teak leaf extracts.

Further, TFC of *C. fistula, P. pinnata,* and *T. grandis* (Table 2) leaves was also found to be high in methanolic extract, i.e., 126.21, 148.33, and 179.1 mg/g of fresh leaf compared with other solvent extracts, whereas *J. curcas* leaves (Table 2) exhibited maximum TFC in ethanolic (149.70 mg/g of fresh leaf) extract. However, the aqueous extracts showed less TFC compared with the other two solvents of *T. grandis, C. fistula,* and *P. pinnata* in the increasing order 16.71, 9.49, and 8.96 mg/g of fresh leaf, respectively; the aqueous extract of *J. curcas* exhibited negligible amounts of TFC content.

Textile fabrics are subjected to specific finishing treatments in order to impart desirable functional characteristics. Natural fibers are prone to microbial growth and are susceptible to moths and mildew. Naphthalene and paradichlorobenzene are commonly used at the household level as moth-proofing agents. Sprays containing fluorides and silico-fluorides are health hazards (Corbman 1983). Cotton fabrics are prone to fungal growth. Copper sulfate, boric acid, and carbolic acid are commonly used for mildew proofing. Resins based on melamine formaldehyde are used for mildew proofing (Corbman 1983).

Natural antimicrobial agents not only curb microbial growth but also render an additional healing power to the fabric. Healing power of some of the plant material has been used since ancient times. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. In many cases, these substances serve as plant defense mechanisms

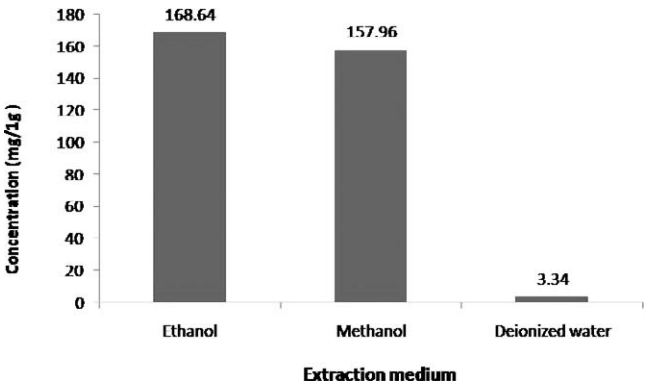

Figure 4.—Total phenolic content (milligrams per gram of fresh leaf) of jatropha leaf extracts.

Table 2.—Total flavonoid content (TFC) of extracts in forest species.[a]

Forest species	TFC (mg/g of sample)		
	E	M	A
Cassia fistula	106.4	126.21	9.49
Pongamia pinnata	121.53	148.33	8.96
Tectona grandis	153.52	179.1	16.71
Jatropha curcas	149.70	138.7	—

[a] E = ethyl alcohol; M = methanol; A = aqueous.

against predation by microorganisms, insects, and herbivores (Joshi et al. 2009). Extraction and fabric finishing techniques play a significant role in the performance of fabric. Exhaustion, direct application, and the microencapsulation techniques have proved to be efficient in imparting antimicrobial function using different plant sources (Parthiban and Thilagavathi 2012, Sumithra and Raaja 2012, Babel and Mogra 2013). American Association of Textile Chemists and Colorists (AATCC) has standardized various test procedures for testing the antimicrobial efficiency of fabrics. AATCC 147 is the standard test procedure used for qualitative assessment, AATCC 100 for quantitative analysis using different species of gram-positive and gram-negative bacteria, and AATCC 30 is used for assessing the antifungal properties of treated fabrics (AATCC 2011, 2012, 2013). However, there is a need to assess the optimal concentration at which the natural source imparts the antimicrobial function that is largely dependent on the variety and the agro climatic zone of the plant source.

Conclusions

The Indian subcontinent has a rich flora of various plants used in traditional medical treatments. The medicinal properties of these plants could be due to the phytochemicals present in them, which have antimicrobial activity. The results revealed that phytochemicals, viz., alkaloids, flavonoids, tannins, and terpenoids, were found in appreciable amount in the selected forest species. Methanolic extract of *C. fistula, P. pinnata,* and *T. grandis* exhibited maximum TPC and TFC content, while *J. curcas* showed higher amounts of TPC and TFC content in ethanolic extract. Therefore, we conclude that extracts from forest species such as *C. fistula, P. pinnata, T. grandis,* and *J. curcas* can be used for applying eco-friendly and healthy finishes to textile substrates.

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