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Antimicrobial Activity of *Gloriosa superba*, *Cyperus rotundus* and *Pithecellobium dulce* with Different Solvents

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Abstract
Nowadays, antimicrobial finishing is a primary requirement for cotton apparel products. Many synthetic antimicrobial agents were used previously, but due to the non-ecofriendly nature of synthetic antimicrobial agents, natural herbal based antimicrobial agents are used these days. The various herbs used in Indian ayurveda medicines, which possess antimicrobial properties, are often applied to textile materials to impart functional properties required for microbicidal effects. In this work, Gloriosa superba, Cyperus rotundus and Pithecellobium dulce were used as antimicrobial agents. The herbs were extracted with different solvent sincluding methanol, ethanol, benzene and water to analyse the influence of these solvents on the efficacy of antimicrobial activity. These extracts were applied on cotton fabrics by the exhaust method and the degree of antimicrobial activity analysed by the zone inhibition method. All the herbs listed in this paper showed good antimicrobial properties against various microbes. However, not all the solvents used in the extraction showed good results in antimicrobial activity against all the microbes. Methanol extracts showed better antimicrobial activity in all three herbs due to their inherent antimicrobial properties. Thus, it is proposed to use methanol as a solvent for the extraction of these herbs to obtain concentrates suitable for antimicrobial activity in cotton fabrics.

Key words: *antimicrobial activity, herbs, exhaust method, cotton fabric, parallel streak method, Soxhlet apparatus.*

Introduction

Today, consumers are very particular about healthcare and hygiene in textile products, especially in innerwear and children's wear [1]. Consumers are ready to buy textiles that possess good antibacterial activity even by paying higher prices.

Cotton is the most widely used fibre in the apparel industry. Around 33% of cotton fibre is used in the apparel industry as per the world apparel fibre consumption survey in 2013, due to its comfort properties [2]. Although cotton fibres provide good comfort properties, it has certain disadvantages like it being easy to crease, shrinkage, and susceptibility towards microbial attack.

The cellulose present in cotton provides food for microbes, which can grow at a faster rate under favourable conditions [3]. Unlike bamboo and polyester, cotton fabrics are easily attacked by microbes. Pathogenic microbes can cause problems like skin diseases, unpleasant odour, stains, colour fading, strength loss, clammy feel, cross infections, and discomfort to the wearer [4]. Synthetic fibres are free from these issues, but in tropical countries they are not comfortable to wear. Cotton is the best choice for use in apparels along with finishing treatments to overcome such issues. Various finishes such as anti-shrink, crease recovery, durable press, antimicrobial and soft finishes are applied to cotton fabrics either by the exhaust method or pad-dry-cure method [5].

Antimicrobial finish is applied to cotton fabrics to overcome the issue of microbial attacks. Initially, chemicals used for sanitary purposes were applied as antimicrobial agents by either the exhaust method or pad- dry method [6-9]. Later, to increase durability, antimicrobial agents were applied along with crosslinking agents by the pad-dry-cure method. Various antimicrobial agents were used to improve the degree of antimicrobial activity and durability over a period of time. Some agents showed good antimicrobial effect and durability to some extent. However, none of them are ecologically safe. Then attempts were made to use environmentally safe antimicrobial agents based on plant extracts. Various plant species, such as *Punica granatum* [10], *Pithecellobium dulce* [11], *Neem* [12-14], *Aloe vera* [14], *Tulsi* [12], *Gloriosa superba* [15], *Cyperus rotundus* [16], *Coleus amboinicus* [17, 18], *Clitoria ternatea* [19] and *Vitex negundo* [20], which have antimicrobial activity have been identified, showing different antimicrobial activity levels.

Various methods are widely used, including aqueous extraction [21] and solvent extraction [3], in the extraction of medicinal plants from, for example, *Gloriosa superba*, which is a semi-woody perennial climber plant belonging to the *Liliaceae* family, native to tropical Africa and found throughout India [22]. It has a hollow stem of 6 meters length. *G. lutea, G. planti, G. sudanica, G. grandiflora, G. simplex, G. rotheschildiana* and *G. longifolia* are some of the species associated with *Gloriosa superba*.

Cyperus rotundus, a nut-grass, is a perennial plant of the *Cyperaceae* family, growing up to 140 centimetres. *Cyperus rotundus* is an edible brown tubers. The plant is native to Africa, but it is distributed and grown in tropical and subtropical regions [25]. In Indian folk medicine, the rhizomes of *Cyperus rotundus* are used to cure fever, pain, vomiting, dysentery and various blood disorders [26]. *Cyperus rotundus* contains alkaloids, oils, glycosides, flabonoids, saponins, tannins, carbohydrates, starch, and proteins. It is possesses traces of Mg, Cr, V, Co and Mn [25].

Pithecellobium dulce is a tree of the *Fabaceae* family, a native of the Pacific coast and Mexico, growing up to 10-15 metres. Its fragrant, sessile and greenish-white flowers can reach 12 centimetres in length. Due to coiling, the flowers appear shorter in length. The shell turns pink and opens an edible pulp, once it is ripe. Circular, flat and shiny seeds are

Table 1. Specifications of fabric sample.

S. No	Fabric parameters	
1	Linear density – warp, tex	15
2	Linear density – weft, tex	15
3	Ends per centimeter – warp	32
4	Picks per centimeter – weft	32
5	Areal density, g/m^2	115
6	Weave type	Plain

Table 2. Herbal sources used for antimicrobial finish.

S. No.	Common name	Botanical name	Parts of plant	Botanical family
1	*Kantal*	*Gloriosa superba*	Flower	*Colchicaceae*
2	*Nutgrass*	*Cyperus rotundus*	Seed	*Cyperaceae*
3	*Kodukkappuli*	*Pithecellobium dulce*	Leaf	*Fabaceae*

found inside the pulp. Anti-inflammatory, anti-venom and antimicrobial properties are possessed by *Pithecellobium dulce* [11], which *Pithecellobium dulce* was extracted with distilled water, acetone, chloroform and methanol. The antimicrobial activity of the extracts for twenty different microorganisms was evaluated, in which leaf extracts of *Pithecellobium dulce* exhibited a variable zone of inhibition ranging from 7 to 27 mm. Extracts made from solvents are more protective than those form an aqueous medium. In this work, three herbs: *Gloriosa superba, Cyperus rotundus* and *Pithecellobium dulce* were subjected to antimicrobial activity with different solvents by the exhaust method.

Materials and methods

Fabric

100% grey cotton woven fabrics were purchased from M/s Khadi and Village Industries in the Erode District of Tamil Nadu. ***Table 1*** provides the specifications of fabric samples used in this research work. The ends per centimetre and picks per centimetre of the fabrics were measured as per ASTM D 3775-96, and the linear density of the warp and weft yarns was calculated as per ASTM D 1059-2001. The areal density of the fabric was calculated as per ASTM D 3776-96, using a standard cutter, and the mean value of five samples was recorded and taken for analysis. The cotton fabric was desized, scoured and bleached with a standard recipe [27]. The bleached fabric then underwent the application of herbal extracts using the exhaust method.

Herbals used

In this research, three medicinal herbal sources, available in the nearby regions of Sathyamangalam of Tamil Nadu, India, with traditional medicinal properties were identified and used for the assessment of antimicrobial activity on the cotton fabrics. These herbal samples were procured (from farmers) in and around the Erode District. ***Table 2*** displays a list of the herbs, and the botanical name, family, and part of the plants used in this research.

Preparation of herbal sources for the extraction process

The manually cleaned (to remove other unwanted parts of the plants) herbal samples were dried at room temperature in the shade for a period of one week to reduce the moisture, making them suitable for the solvent extraction process. The dried herbal parts were mixed thoroughly, crushed into small pieces, and then made into powder using a mixer grinder. The ground herbal powder was filtered (sieved) using a fine mesh fabric to remove unground coarse portions. The resultant filtered fine herbal powder was the used in the extraction process as described below.

Extraction of herbal products

Soxhlet apparatus was used to extract the sieved fine herbal powders (20 grams) using the solvents, as specified in ASTM D2257 – 2004, at a rate of six extractions per hour for a total duration of four hours. Approximately 20 g of fine herbal powder was stuffed in a polypropylene nonwoven pack, and then the herbal stuffed nonwoven pack was placed in Soxhlet apparatus as a precaution to avoid degradation of the samples during the extraction process. Extractions were carried out using 200 ml of methanol (at 65 °C), ethanol (80 °C), benzene (80 °C) and water (100 °C) separately. The hot extracts are left overnight at room temperature to evaporate the solvents, and the concentrated herbal extracts were centrifuged, and a rotary vacuum was used to dry them. The herbal powders were mixed with water in the required proportion and applied on the cotton fabrics by the exhaust method. A laboratory model shaker water bath was used for this application. The bath was set at a material-to-liquor ratio of 1:20. The temperature of the bath was maintained at 55 °C for 30 minutes. Then the samples were washed and dried at 80-85 °C in an oven. The finished fabric samples were subjected to various testing and characterisation.

Antimicrobial activity of herbs

A qualitative test, Zone of Inhibition, was used to find out the antimicrobial activity of the sample. Standard broth/agar media were prepared and applied on the gar plate using a sterile swab. The specimens were cut into a circular shape (disc) using a standard die, placed gently on the petri plates, and then flooded with the agar medium inoculated with the microbes. Then the samples were incubated at 37+/– 2 °C for 24 hours. *S. simulans*, *S. xylosus*, *B. subtilis* and *S. epidermidis* microbes (obtained from soil cultures and subsequent separation and purification) were used for this test. 12 discs (3 petriplates) were used for every microbial incubation to ensure the consistency and reproducibility of the results. On completion of the inhibition, the petri dishes were examined visually for the interruption of growth of inoculum around the specimen and for the zone of inhibition beyond its edge.

The average zone of inhibition of 12 discs on either side of the test specimen was calculated using the following equation,

$$W = (T - D)/2,$$

Where, W = width of clear zone of inhibition in mm, T = total diameter of test specimen and clear zone in mm, and D = diameter of test specimen in mm.

Four samples were placed on each petri plate, and the average values of the four samples, rounded off to the full integer, were taken for reporting purposes.

Results and discussion

Antimicrobial activity of *Gloriosa superba*

The herbs extracted in the different solvents were used to finish the cotton sam-

ples using the exhaust method, and the finished samples were evaluated for the zone of inhibition. Antimicrobial activities of the extracts obtained from the *Gloriosa superba* herb in different solvents: methanol, ethanol, benzene and water are shown in ***Table 3***. The antimicrobial activity of *Gloriosa superba* herb extracted with methanol, ethanol, benzene and water showed (***Figure 1***) almost equal protection against *S. simulans* microbes. In the case of antimicrobial activity against *S. xylosus* and *B. subtilis*, the methanol extracted samples displayed better protection, whereas other solvents showed poor resistance in terms of zone inhibition, which confirms the results reported by earlier researches [10]. Overall, the methanol extracts of *Gloriosa superba* displayed good protection in all microbes, whereas the other solvents are not so in all of them.

S.epidermidis, a Gram-positive microbe, is a human skin bacterium that causes skin diseases and acne vulgaris. An effective agent for antimicrobial activity has not been reported thus far. *Galangin* is the most effective species to inhibit *S. epidermidis,* and very few agents have it [28]. The absence of *galangin* in *Gloriosa superba,* makes for very poor antimicrobial activity against *S. epidermidis* [28], whereas antimicrobial activity against other microbes are good.

Antimicrobial activity of *Cyperous rotandus*

The antimicrobial activity of the *Cyperus rotundus* herb against microbes *S. simulans*, *S. xylosus*, *B. subtilis*, and *S. epidermidis* is shown in ***Table 4***. *Cyperus rotundus* extract displayed very good antimicrobial activity against *S. simulans* microbe in all the solvents (***Figure 2***). The highest antimicrobial activity, a zone of inhibition of 10 mm, was obtained for benzene extracts; methanol and, water extracts also exhibited good antimicrobial activity (zone of inhibition is 9 mm). The ethanol extracts exhibited the least antimicrobial activity against *S. simulans* (zone of inhibition of mm 8).

In the case of *Cyperus rotundus*, the antimicrobial activity of methanol extracts against *S. xylosus* was very good, with a zone of inhibition of 12 mm, whereas for the ethanol extracts it was 9 mm, benzene extracts 7 mm and water extracts 5 mm, lower than for the methanol extracts. Regarding the antimicrobi-

Table 3. *Antimicrobial activity of Gloriosa superba.*

Solvent	Antimicrobial Activity – Zone of Inhibition, mm (fabric sample diameter – 10 mm)			
	S. simulans	***S. xylosus***	***B. subtilis***	***S. epidermidis***
Methanol (1)	10	9	8	5
Ethanol (2)	10	8	7	4
Benzene (3)	10	6	8	0
Water (4)	10	14	9	0

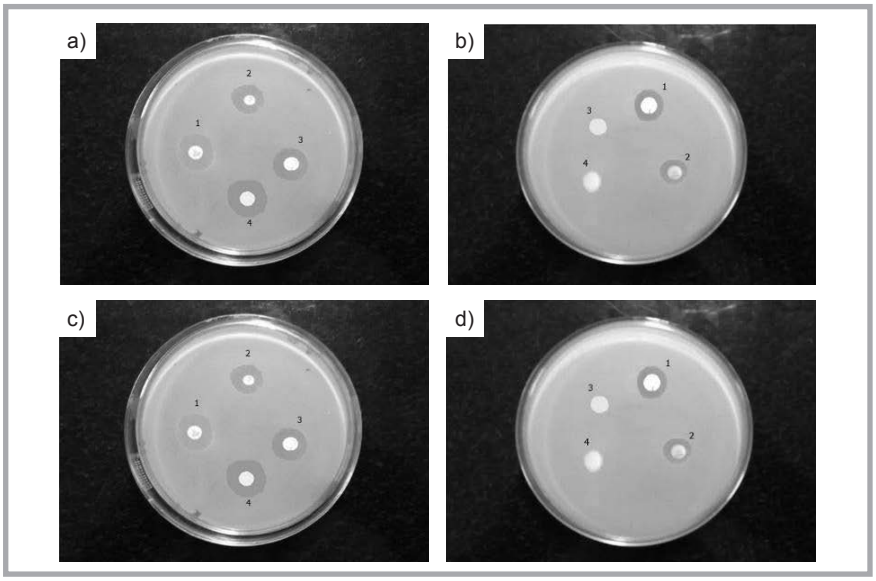

Figure 1. *Zone of Inhibition of the Gloriosa superba herb with different solvents: methanol (1), ethanol (2), benzene (3) and water (4) against microbes: a) S. simulans, b) S. xylosus, c) B. subtilis & d) S. epidermidis.*

Table 4. *Antimicrobial activity of the Cyperus rotundus herb.*

Solvent	Antimicrobial Activity – Zone of Inhibition, mm (fabric sample diameter 10 mm)			
	S. simulans	***S. xylosus***	***B. subtilis***	***S. epidermidis***
Methanol (1)	9	12	9	3
Ethanol (2)	8	9	7	3
Benzene (3)	10	7	8	2
Water (4)	9	5	9	0

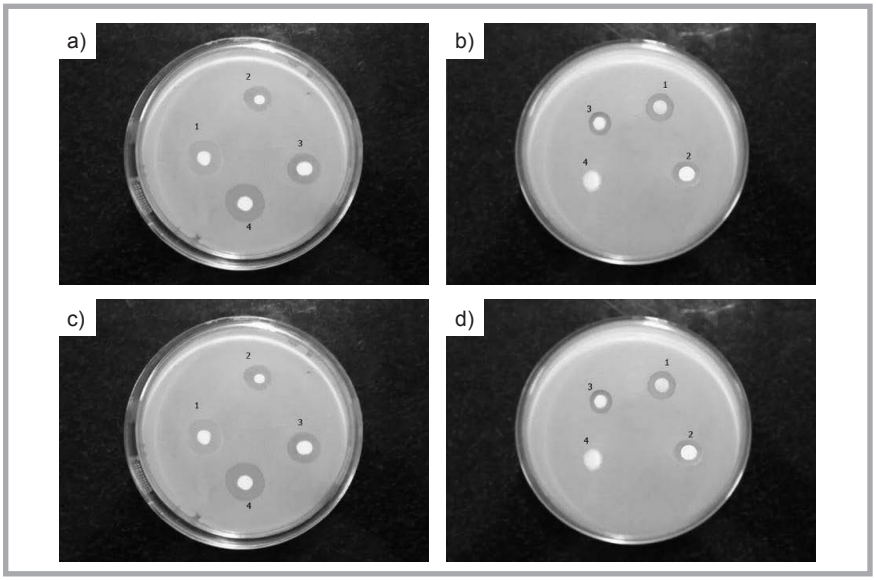

Figure 2. *Zone of Inhibition of Cyperus rotundus herb with different solvents: methanol (1), ethanol (2), benzene (3) and water (4) against the microbes: a) S. simulans, b) S. xylosus, c) B. subtilis & d) S. epidermidis.*

Table 5. *Antimicrobial activity of Pithecellobium dulce.*

Solvent	Antimicrobial Activity – Zone of Inhibition, mm (fabric sample diameter 10 mm)			
	S. simulans	***S. xylosus***	***B. subtilis***	***S. epidermidis***
Methanol (1)	10	4	6	2
Ethanol (2)	10	6	7	2
Benzene (3)	9	4	5	0
Water (4)	12	3	4	0

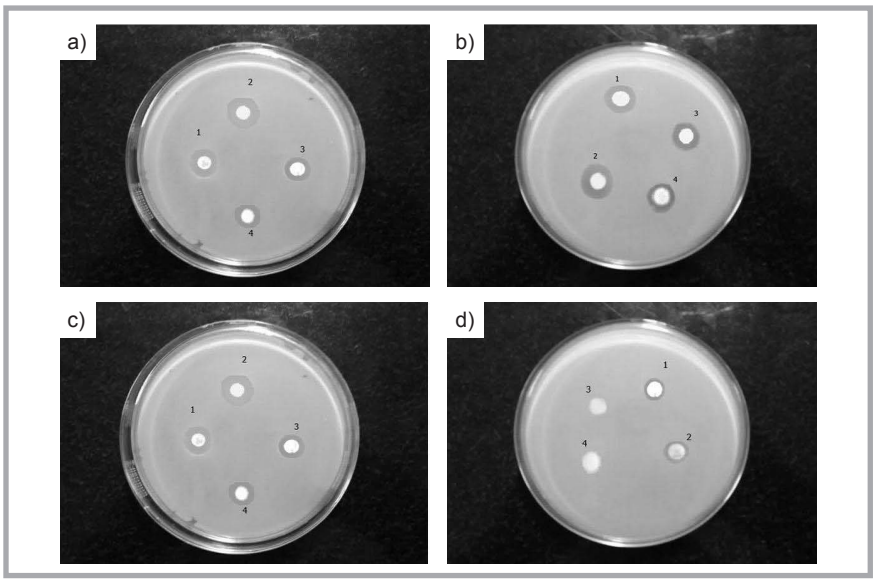

Figure 3. *Zone of Inhibition of the Pithecellobium dulce herb with different solvents: methanol (1), ethanol (2), benzene (3) and water (3) against the microbes: a) S. simulans, b) S. xylosus, c) B. subtilis & d) S. epidermidis.*

al activity of *Cyperus rotundus* against *B. subtilis* microbes, methanol and water extracts showed the highest activity with 9 mm, and the other extracts, benzene and ethanol, showed only 8 and 7 mm, respectively. With respect to the antimicrobial activity of *Cyperus rotundus* against *S. epidermidis* microbes, the ethanol and methanol extracts showed only moderate antimicrobial activity with a zone of 3 mm each, benzene only 2 mm, and there was no activity against water extracts. Overall, the methanol extracts showed good antimicrobial activity against all the four microbes, and the other extracts – ethanol, and water were poor in *S. epidermidis* microbes.

Kilani et al. reported the presence of tannins, coumarins and flavonoids in aqueous and methanol extracts in *Cyperus rotundus* by the photochemical method [29, 30]. The presence of these flavonoids and tannins in the extracts exhibits good antimicrobial activity [31, 32]. The absence of *Galangin* in *Cyperus rotundus* exhibits very poor antimicrobial activity against *S. epidermidis* [28], whereas that against the other microbes is good.

Antimicrobial activity of *Pithecellobium dulce*

The antimicrobial activity of *Pithecellobium dulce* against microbes *S. simulans*, *S. xylosus*, *B. subtilis*, and *S. epidermidis* is shown in ***Table 5***. The water extracts of *Pithecellobium dulce* showed the highest antimicrobial activity with a zone of 12 mm, when compared with methanol and ethanol extracts (10 mm each) and benzene extracts (9 mm) against *S. simulans* (***Figure 3***). Regarding antimicrobial activity against *S. xylosus*, methanol and benzene extracts showed moderate activity (4 mm), similar to that of ethanol extracts (6 mm) and water extracts (3 mm).

With respect to the antimicrobial activity of the *Pithecellobium dulce* herb against *B. subtilis*, the methanol ethanol extracts showed the highest – 6 mm and 7 mm, respectively, whereas benzene and water showed the lowest. Only methanol and ethanol extracts showed little antimicrobial activity against *S. epidermidis* microbe. These results confirm earlier reports published, where organic solvent extracts were reported to show better efficacy compared to aqueous extracts [10]. Photochemical studies of *Pithecellobium dulce* reported the presence of anthraquinones, alkaloids, proteins, terpenoids, cardiac glycosides and tannins. The presence of these constituents in *Pithecellobium dulce* means higher antimicrobial activity [33, 34].

Conclusions

Cotton fabric samples treated with antimicrobial extracts obtained from *Gloriosa superba*, *Cyperus rotundus* and *Pithecellobium dulce* showed consistent results, even though multiple samples were used in every incubation for a given microbe. However, significant differences were observed with respect to the microbial source used in the present study. Among the microbes, *S. simulans*, *S. xylosus*, *B. subtilis* and *S. epidermidis*, the sample finished with different solvents against *S. simulans* showed better antimicrobial activity than other microbes in all three herbs. The herbs extracted with different solvents exhibited lower microbial activity against the *S. epidermidis* microbes. The same trend is noticed in *Gloriosa superba*, *Cyperus rotundus* and *Pithecellobium dulce.* The natural inherent antimicrobial properties present in the methanol extracts of *Gloriosa superba*, *Pithecellobium dulce* and *Cyperus rotundus* showed better antimicrobial activity against all four microbes, and it is proposed to use methanol as a solvent for the extraction of these herbs.

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