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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL POTENTIAL OF *MELIA AZEDARACH* BARK EXTRACTS FOR DEVELOPING HEALTH CARE TEXTILE MATERIALS

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ABSTRACT

The textiles are not only the carrier but also an excellent media for the development of microorganisms, due to side effects and water pollution caused by synthetic antimicrobial agents there is a great need of antimicrobial textiles in particular for healthcare products from natural sources. The *Melia azedarach* was prepared using solvents namely methanol, ethanol, ethyl acetate, aqueous, acetone and chloroform.The present study was designed to check the preliminary phytochemical screening and to identify the antibacterial activity of *Melia azedarach* bark extracts. The prepared solvent extracts showed the presence of various phyotochemicals specifically terpenoids, alkaloids, glycosides, tannins, phenols. The *M.azedarach* bark extracts are tested against two test bacteria's *Staphylococcus aureus and Escherichia coli* using disc diffusion method. The concentration study was carried out to selected best concentration withhigher zone of inhibition. The optimized parameters were used for fabric finishing and tested against two bacteria's *Staphylococcus aureus and Escherichia coli* and two fungus *Aspergillusniger* and *Malassezia furfur*. The cotton linen blended fabric treated with *Melia azedarach* bark extract shows32mm and 29mm zone of inhibition against *s.aureus* and *E.coli* and 57mm and 55mm zone of inhibition against *A.niger* and *M.furfur* respectively.

KEYWORDS: *Melia azedarach*, solvents, phytochemicals, secondary metabolites, antimicrobial, cotton linen blended fabric.

INTRODUCTION

The health care industries increasingly concerned with the various microorganisms that are commonly present in the atmosphere. Microbial infection poses threat to both living and nonliving matter. Growing awareness of health and hygiene has increased the demand for bioactive or antimicrobial textiles.[1] Antimicrobial fabrics are significant not only in medical applications but also in terms of daily life usage.[2] Recently, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents. Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections.[3] And for now, researchers have paid attention to safer phytomedicines and biologically active compounds isolated from plant specious used in herbal medicines for the development of novel drugs. Drugs used by traditional healers are mostly prepared by some form of extraction with water, as the healers do not usually have access to other more lipophilic solvents. This is of concern, as it is possible that healers do not extract all the active compounds that might be present in the plant and consequently the prepared drug would not contain all the pharmacologically active compounds.[4] Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers used primarily aqueous as a solvent.

Melia azedarach is commonly known as bead-tree or cape lilac. The generic name is derived from the Greek word "*Melia*" means "the ash" and the specific name comes from the Persian "azzadirackt" i.e. the noble tree.[5] Its bark is dark brown in color, relatively smooth, and fissured. The leaves are alternate, leaflets are short stalked and thin, hairless, dark green and relatively pale. Flowers are white with purple stripes and are characterized by the presence of a typical fragrance. Fruits or berries are yellow, round, smooth, and fleshy. This is a species of deciduous tree in the *Meliaceae* family, that is native to India, Pakistan, Indochina, Southeast Asia and Australia is a species of deciduous tree.[6] Various scientific studies reported the antimalarial,[7,8,10] antibacterial,[9,10,13,19,20] wound healing,[10] anticancer, antifungal,[12,18] antiflammatory activity, analgesic,[8] and antioxidant activity.[1,5,12,14] Commercially oil of *M. azedarach* is used in preparation of soap and cosmetics.[5]

Traditionally different parts of *Melia azedarach* such as leaf, flower, seed, fruit, and young branches have been used for the treatment of malaria, diabetes, purgative, cough, skin disease, anthelmintic, astringent, antidiuretic, stomach-ache and so on.[3,17] The bark

decoction used in paroxysmal fever and skin diseases.[11] Active compounds reported and antiplasmodial activity: Contains triterpenoids (e.g., amoorastatone)[23] and quinoids (e.g., 1-8-dihydroxy2-methyanthraquinone) in stem bark[24], and flavones (e.g., apigenin-5-O-bD-galactoside) in roots.[22,23] The present research is to evaluate the phyto compounds of the plant and to study the antimicrobial activity of *Melia azedarach* plant extracts on cotton linen blended single jersey fabric.

MATERIALS AND METHODS

Collection and preparation of plant material

Mature bark of *Melia azedarach* were collected from the Thalavadi, village in India, near Sathyamangalam, Erode District, Tamilnadu. The barks of plant *Melia azedarach were* separated manually using sharp steel knife. The collected barks were washed with distilled water and were dried under the sun until it is fully dried for 45 days and were grounded into fine powder.

Preparation of Extracts

The extracts were prepared from six solvents according to its polarity from low to high. The various solvents used for the extraction are Aqueous, Ethanol, Methanol, Ethyl acetate, Acetone and Chloroform. The bark sample measuring 10 grams were dispensed in 100 ml of solvent and kept in shaker for 24 hours. All the extracts were filtered using whatman No. 1 filter paperand the extracts were collected and used for further analysis.

Selection of fabric

Cotton linen single jersey fabric with 70:30 blend of count was selected for the study. This particular blend was selected to add the strength and wickability property into the fabric.

Photochemical Analysis[15]

The crude extracts of *Melia azedarach* were analysed for the preliminary qualitative screening of phyotochemicals such as saponins, alkaloids, Phlobatannins, terpenoids, phenols, tannins, glycosides, and flavonoids.

Saponin: Added 2ml of filtrate with 5ml of distilled water and shaken well. Presence of frothing indicates the presence of saponin.

Alkaloids: Hager's test: The extract was treated with a few ml of Hager's reagent. Yellow precipitation indicated the presence of alkaloids.

Wagner's test: The extract was treated with few ml of Wagner's reagent. The reddish brown precipitation indicated the presence of alkaloids.

Terpenoids (salkowski test): The extract was mixed with 2ml of chloroform. Then 2ml of concentrated hydrochloric acid is added and shaken gently. A deep red colour indicates the presence of terpenoids.

Phenols and Tannins: 2ml of extract was mixed with 2ml of 2% solution of ferric chloride. Formation of blue-green or black colouration indicates the presence of phenols and tannins.

Glycosides: 2ml extract was mixed with 2ml of glacial acetic acid containing 2 drops of 2% of Fecl3. The mixture was poured into another tube containing 2ml of concentrated sulphuric acid. A brown ring at the interphase indicates the presence of glycosides.

Flavonoids: 2ml extract was mixed with zinc dust, and concentrated hydrochloric acid was added dropwise and heated. Formation red colour indicates the presence of flavonoids.

Antibacterial screening

Disc diffusion method- Screening of best Solvents

The extracted plant solvents with methanol, ethanol, ethyl acetate, acetone, chloroform, and aqueous were qualitatively assessed for antibacterial activity. The test organisms used were gram + bacteria *Staphylococcus aurous* and *gram – bacteria Escherichia coli.*

Experiment

The standardized inoculums is inoculated in the plates prepared earlier by dipping a sterile in the inoculums removing the excess of inoculums by passing by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60º C after each application. Round the edge of the agar surface pass the swab finally. Leave the inoculums to dry at room temperature with the lid closed. Each Petri dish is divided into 3 parts, in each part samples disc such as methanol, ethanol, ethyl acetate, acetone, chloroform and aqueous (100µg) disc (discs are soaked overnight in sample solution) are placed in the plate with the help of sterile forceps. Then Petri dishes are placed at 4º C in the refrigerator or at room temperature. Incubate for 24 hours at 37 º C. Observe the zone of inhibition produced by different samples. Measure it using a scale and record the average of two diameters of each zone of inhibition[16]. The best solvent extract was selected

from the six solvents by considering the maximum zone of inhibition and used for further analysis.

Optimization of concentration for selected solvent extracts

The selected solvent Ethyl acetate having maximum zone of inhibition is further analysed for its concentration study with 10%, 15%, 20%, 25%, and 30%. The selected five concentrations samples were kept for overnight shaking and the filtrate were evaporated and collected. They were analysed for antibacterial test with the same disc diffusion method as discussed above and the concentration with the maximum zone is selected for further finishing.

Fabric finishing

The selected cotton linen blended (70:30) single jersey fabricwas finished with prepared ethyl acetate extract. The method used for fabric finishing is pad-dry-cure method with material-to-liquor ratio of 1:20 using 4% citric acid concentration. After soaking and padding for 30min, the each of the samples were taken and dried at 100-120ºC for 5min and cured at180ºC for 3 mins.

Qualitative analysis of Antibacterial activity- parallel streak method (AATCC 147 test method)

The cotton linen blended single jersey finished fabricwasanalysed for antibacterial activity. The test fabrics were cut into pieces (25mm x 50mm). A 50mm length permits the specimen to lay across 5 parallel inoculums streaks each of diminishing width from both 8mm to 4mm wide. Sterile AATCC bacteriostasis agar plates were prepared. Test cultures used were *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* ATCC 6538).

Procedure

The test culture used was loaded and transferred to the surface of the agar plate. Inoculum streaks were made with five parallel lines. They are made with 10mm spaced covering the central area of the petridis without refilling the loopwas made. The test specimen was smoothly pressed transversely, across the five inoculums of streaks to ensure intimate get in touch with agar surface. The plates were incubated at 37°C for 18-24 hours. The inoculated plates were examined for the interruption of growth along the streaks of inoculum beneath the fabric and for a clear zone of inhibition beyond the fabric edge. The average width of the zone of inhibition around the test specimen measured in mm.

Qualitative analysis of Antifungal activity- (AATCC 30 method)

The finished fabric was analyzed to test their antifungal activity by AATCC-30 test methods. The test specimens were cut into pieces (50mm in diameter). Sterile Potato Dextrose agar plates were prepared. Test cultures used are *Aspergillusniger* and *Malassezia furfur.*

Procedure

Using sterile cotton swab the test fungal cultures was transferred to the upper area of the agar plate by swabbing all around and also covering the middle area of the petridish. The plates were incubated at 30°C for 30 hours. The inoculated plates were examined for the interruption of growth along the swabs of inoculum beneath the fabric and for a clear zone of inhibition beyond the fabric edge. The average width of the zone of inhibition around the test specimen measured in mm.

RESULTS AND DISCUSSION

Phytochemical analysis

Many sources on the *M. Azedarach* revealed that this plant contains many phytochemical constituents including phenols,[12] flavonoids,[12,19] tannins,[2,12,19] alkaloids,[12,19] terpinoids,[18,19] saponins,[18,19] glycosides,[18] phenolic compounds,[18,19] and rutins.[18] Phytochemicals such as phenol and flavonoids are well known for their antioxidant potential.From the table 1, it is clear that all the extracts showed showed considerable amount of phytocompounds present in the extracts. The result shows the presence of Terpenoids, phenols, tannins, glycosides, and flavonoids. Ethylacetate showed good amount of presence of phyto compounds compared to aqueous, ethanol and methanol which showed considerable presence of compounds respectively. The observed antibacterial effects on the isolates is believed to be due to the presence of alkaloids, tannins and flavonoids which have been shown to possess antibacterial properties.[21]

Table 1: Results of phytochemical analysis of *Melia azedarach.*

Phytochemicals	Solvents				
	Ethyl acetate	Acetone	Ethanol	Methanol	Aqueous
Saponin	+	++	++	++	++
Alkaloids	+	+	+	+	+++
Terpenoids	+++	+++	+++	+++	++
Phenol and Tannins	+++	+	++	++	++
Glycoside	+++	++	++	+++	+
Flavonoids	++	++	++	++	+++

+++ highly present ++ moderately present + low -absent

Antibacterial screening- Disc diffusion method

The table 2, fig 1-4tells the disc diffusion result of solvent extracts against the AATCC test bacteria's. From the table, ethyl acetate showed the higher zone of Inhibition, ethanol, methanol and aqueous showed good zone, acetone and chloroform extracts showed the moderate zone of Inhibition against the test organisms.

Table 2: Antibacterial activity of various plant extracts.

Test Organisms	Zone of Inhibition(mm)					
	Melia azedarach bark extracts(100µg/disc)					
	Methanol	Ethanol	Ethylacetate	Acetone	Chloroform	Aqueous
Staphylococcus aureus	12	14	17	10	13	13
Escherichia coli	14	13	14	11	10	12

Figure 1.

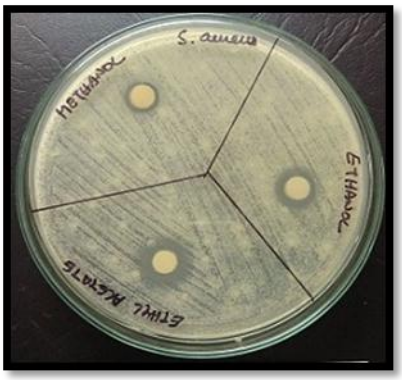

Figure 2.

Antibacterial activity of solvent extracts of *Melia azedarach bark - Staphylococcus aureus.*

Figure 3.

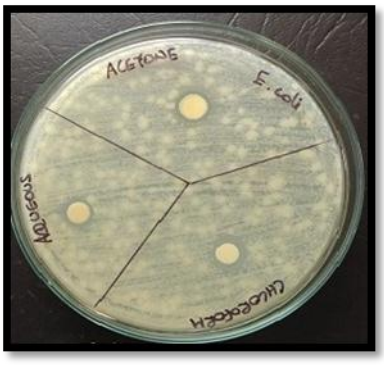

Figure 4.

Antibacterial activity of solvent extracts of *Melia azedarach bark- Escherichia Coli.*

Concentration study

The table 3, fig 5 & 6 shows the disc diffusion results of various concentrations tested against the AATCC test bacteria's. From the table, ethyl acetate extract with 20% concentration showed maximum zone of inhibition of 18mm and 14mm against the test organisms.

Table 3: Optimization of concentrations of Ethyl acetate extracts for antibacterial activity.

Test bacteria's	Zone of Inhibition(mm)				
	Ethyl acetate extract of *Melia azedarach* (100µg/disc)				
	10%	15%	20%	25%	30%
Staphylococcus aureus	16	16	18	16	14
Escherichia coli	13	13	14	12	10

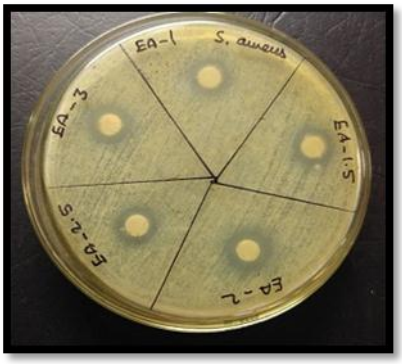

Figure 5-Staphylococcus aureus.

Figure 6-Escherichia coli.

Antibacterial activity of Ethyl acetate extract

Antibacterial activity- Parallel streak method (AATCC 147 test method)

The table 4, fig 7 & 8 shows the results of antibacterial activity of cotton linen knitted fabric finished with direct solvent extract.The zone of Inhibition in the direct solvent extract finished fabric is 30mm and 32mm, against the test organisms *Escherichia coli* and *Staphylococcus aureus* respectively.

Table 4: Antibacterial activity of cotton linen blended single jersey fabric.

Ethyl acetate extract treated fabric	Zone of inhibition (mm)	
	Test bacteria's	
	Escherichia coli	*Staphylococcus aureus*
Cotton/Linen70/30 blended single jersey fabric	30	32

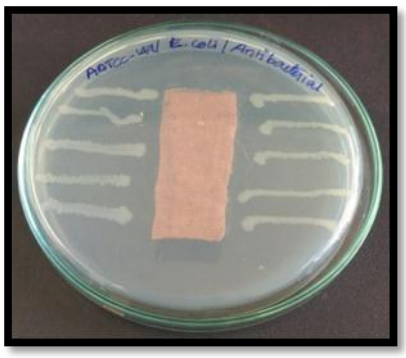

Figure 7: Escherichia coli.

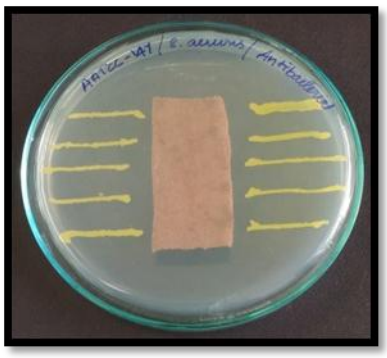

Figure 8: Staphylococcus aureus

Antibacterial activity of Ethyl acetate extract treated fabric

Antifungal activity- Parallel streak method (AATCC 30 test method)

The table 5, fig 9 & 10 shows the results of antifungal activity of cotton linen knitted fabric finished with direct solvent extract. The zone of Inhibition in the direct solvent extract treated fabric is 57mm and 55mm, against the two test organisms *Aspergillusniger* and *Malassezia furfur* respectively. The two test organisms was selected based on the required end use of the product. *A.niger* is an organism which affects textile materials; *M.furfur* is an organism which is a cause of creating dandruff in human scalp.

Table 5: Antifungal activity of cotton linen blended single jersey fabric.

Ethyl acetate extract treated fabric	**Zone of inhibition (mm)**	
	Test fungus	
	Aspergillus niger	***Malassezia furfur***
Cotton/linen 70/30 blended single jersey fabric	57	55

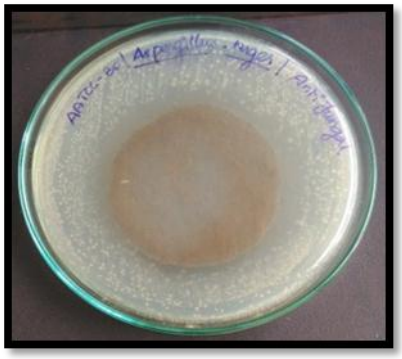

Figure 9: Aspergillusniger.

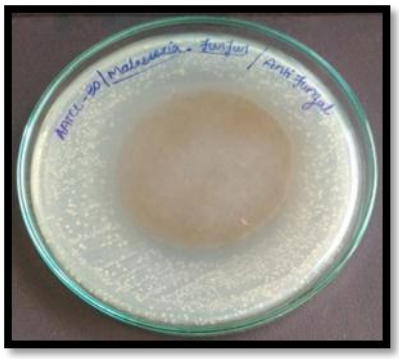

Figure 10: Malasseziafurfur.

Antifungal activity of Ethyl acetate extract treated fabric

CONCLUSION

The *Melia azedarach* treated cotton linen blended single jersey fabric has the potential to face the today's health care market demand. The fabric has good antibacterial and antifungal properties which is required for development of health care materials. So, the ethyl acetate extract prepared with *Melia azedarach* will be suitable for development of health care products such as hospital linens, cloths/wipes, medical clothing's etc. Since, the fabric is treated against *Malassezia fungi*, an etiological factor for developing scalp disorder and dandruff in human scalp; this can be more suitable for skull cap that inhibits sweat odour in environmentally benign. Skull cap is a product, which is worn inside the helmet by sports personal and two-wheeler drivers. These kind of specific selection of organisms like bacteria and fungi will help in developing the specific end product. So, cotton linen blended fabric was suggested for developing skull cap.

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