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A STUDY ON THE ANTIMICROBIAL PROPERTY OF THE COTTON FABRIC IMPARTED WITH *MICHAELIA CHAMPACA* LEAF EXTRACT LOADED NANOPARTICLES

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ABSTRACT: In the present work ethanol, methanol, ethyl acetate, chloroform and benzene extract of the *Michaelia champaca* leaves were screened for their antimicrobial activity. The ethanolic extracts of *M. champaca* leaves were proved to have the maximum antimicrobial activity; thus the ethanolic leaf extract was selected and the nanoparticles were synthesized using ionic gelification method. The nanoparticles were characterized physically and chemically using TEM and FTIR respectively. The herbal extract loaded nanopartricles were coated on to the non woven cotton fabric using the pad dry cure method. The antimicrobial activity of the untreated and treated (*M. champaca* leaf extracts coated fabric and leaf extract loaded nanoparticles coated fabric) fabric were assessed by the standard AATCC 147, AATCC 30 and laundering durability. The herbal leaf extract loaded nanoparticles coated fabric showed the better antimicrobial activity and particularly highest wash durability when compared with other treated fabric. The controlled and sustained releasing property of the herb extract loaded nanoparticles could be attributed to its very effective antimicrobial activity. These particles could further have numerous applications in the field of medical textiles. The study revealed that the fabric coated with herbal extract loaded nanoparticles could act against microorganism in the fabric.

INTRODUCTION: The medical textile industries have always played an important role in the protective aspects of fabrics. The fabrics have long been recognized as a good support medium for the growth of microbes [1]. A microbe on textile causes the unwanted effects to both the wearers and textile itself [2]. Where the protective aspects, mainly rely on the presence of microorganisms and the effects they cause on the fabrics. Due to the negative factor of the microbes has resulted in the development of innovative and hygienic finishes on textiles. The consumers are also demanding for the hygienic clothing has created antimicrobial textile products [3]. Antimicrobial finish prevents the growth of bacteria; health protects and prevents diseases [4].

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There are numerous synthetic antimicrobial agents and quite a few agents are also commercially available [1]. The synthetic antimicrobial agents and metal oxides are very effective against a range of microbes, but it was also associated with side effects could not be used for medical application.

Hence, there is a great demand for eco-friendly antimicrobial finishes on textiles. The herbal extract finished fabrics were considered as significant for the medical textile application. The finishing of natural compounds would be a good alternative for the synthetic antimicrobial agents and heavy metals on the medical fabric. Among the natural compounds, plants and its compounds are widely known for the antimicrobial properties [5, 6].

Many studies have reported herbs are used for the inhibition of the growth of microorganisms on textiles [7]. The plant compounds serve as the defence mechanisms against predation by insects, herbivores and also microorganisms.

Hence, the herbal extract and its herbs derived compounds are still promising for the application of medical and health care textiles. The major problem associated with the herbal antimicrobial finishing is that they are not durable. To overcome this problem, finishing the nanocapuslated drug on the fabrics provides a slow and controlled release of the active antimicrobial agents [8]. Considering the significant characteristics of this technique, in the present study, *M. champaca* leaf extract and leaf extract loaded nanoparticles were prepared and were finished on the cotton fabrics. The efficiency of the antimicrobial activity, wash durability and physical properties was found out for the leaf extract and extract loaded nanoparticles finished cotton fabrics.

MATERIALS AND METHODS:

Fabrics used in the study: Fabric material selected for the study was plain weave 40s medical grade cotton with 60 ends per inch (EPI) and 56 picks per inch (PPI). The leaves of *M. champaca* were obtained from in and around Coimbatore. The solvent methanol (Sigma – Aldrich, Mumbai), ethanol (Changshu yangyuan, China), ethyl acetate (Loba cheme, Mumbai), chloroform (Nice, Cochin) and benzene (Qualigens, Mumbai) were used for extract preparation and Chitosan (95% deacetylated) from India Seafoods, Kerala. Nutrient broth, Muller Hinton agar and agars purchased from Himedia (Mumbai) were used as the culture media

Medicinal plant: *M. champaca* was collected in and around Coimbatore, India. The sterile leaves of the plants were used in the study.

Test cultures: The test bacterial cultures are *Acinetobacte*r *baumaanii* (MTCC 11451), *Bacillus cereus* (MTCC 8733), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC 741), *Proteus vulgaris* (MTCC 1771), *Staphylococcus aureus* (MTCC 3160) and *Serratia marcescens* (MTCC 86). The fungi cultures are *Aspergillus niger* (MTCC 282) was obtained from IMTECH, Chandigar, India.

Preparation of *M. Champaca* leaf extracts: Fresh leaves of *M. champaca* leaf were shadow dried at 37˚C. Collected leaves of the plant were allowed to Shadow dry in room temperature to reduce the moisture content. The dried leaves were ground into fine powder by mechanical shearing, and sieving. The fine powder obtained after grinding was used for extraction. About 20 grams of the dried *M. champaca* leaf powder was weighed and added into different flasks containing 100 ml of solvents (Methanol, Ethanol, Ethyl acetate, Chloroform, Benzene) each and was kept in a shaker for 48 hours, the extracts were then filtered using Whatmann no 1 filter paper. The residue obtained was mixed with Dimethyl sulfoxide (DMSO) [9]. The extracts were then used for the determination of bacterial susceptibility testing and minimal inhibitory concentration

Assessment of antimicrobial activity of *M. Champaca* leaf extracts:

Preparation of test inoculum: Test organisms were first revived in nutrient broth at 37 °C for 1-2 hours before it was swabbed onto Muller Hinton agar.

Bacterial susceptibility test: The antibacterial assay was carried out by the well diffusion method. The inoculum was spread on the sterile Muller Hinton agar; about 10µl of the different solvent leaf extracts were loaded on to the wells and the plates were then incubated at 37 °C for 24 hours [10]. And finally, the zone of inhibition around the well was measured. Streptomycin was used as reference to evaluate the susceptibility of test organisms.

Assessment of antifungal activity: The plant extracts that showed better antibacterial activity were subjected to antifungal assessment. The activity of the one short listed plant extract among different solvents on various fungal strains was assayed by the agar plug method [11]. A fungal plug was placed on the center of the plate. Sterile discs immersed in plant extracts were also placed on the plates. Nystatin was used as antifungal control. The antifungal effect was seen as crescent shaped zones of inhibition.

Determination of Minimum Inhibitory Concentration (MIC): MICs were determined by broth dilution methods, where it was serially diluted to obtain a narrower MIC range [12]. After incubation at 37°C for 18-24 hours, the MIC was determined in UV-Visible Spectrophotometer

(Elico SL 244 double beam) using uninoculated broths as test controls.

Phytochemical screening: In order to determine the presence of alkaloids, flavonoids, saponins, carbohydrates, proteins, phenol, steroids, glycosides, tannin, thiols and thus, a preliminary phytochemical study (qualitatively) was performed with plant extracts, using the standard procedure [13].

Preparation of herbal extract loaded nanoparticles: Alginate nanoparticles were prepared by the principle involving cation induced controlled gelification of alginate [14]. Briefly, calcium chloride (0.5 ml, 18 mM) was added to 9.5 ml of sodium alginate solution (0.06% w/v) containing 1ml of herbal extracts. Two ml of chitosan solution (0.05% w/v) was added, followed by stirring for 30 min and the mixture was kept at room temperature overnight [15]. The samples were centrifuged at 1500 rpm for 10 min to remove any large aggregates prior to analysis. Centrifugation under these conditions allowed aggregates to form pellet, leaving nanoparticles suspended in the supernatant. The particle suspension was then centrifuged at 25°C in the centrifuge tube at 6000 rpm for 15 min to separate free polymers from nanoparticles. The pellet was then washed five times and was suspended in the distilled water and stored at 4°C for further analysis.

Physical and chemical characterization of nanoparticles: The physical and chemical characterization study was done by Transmission Electron Microscopy (TEM) and Fourier Transform Infra-Red (FTIR) respectively.

Transmission electron microscopy: A small amount of liquid sample of the nanoparticles redispersed by sonication was placed on a copper grid and then air dried. The samples were viewed at high magnification using TEM, JEOL-JEM 2100.

Fourier transform infra-red: IR-spectra were obtained using a Bruker tensor 27, Germany. Samples obtained by centrifugation were used for FTIR characterization. Sodium alginate nanoparticles were recorded from 4000-400 as scanning range between wave number (cm^{-1}) and % Transmittance. The samples were run in triplicate and the data presented were the average of the three measurements.

Preparation and coating of cotton fabric: The adsorbent sterile cotton fabric was purchased and was then subjected to desizing. The desized fabric was used for disc diffusion assay and quantitative bacterial reduction methods respectively. The desized fabric was primarily coated with citric acid to ensure better binding of the prepared formulation using pad-dry-cure method [16]. For 1 gm of the fabric 20 ml of the nanoparticles and about 1.6 gms of citric acid was used as a binder, the fabric was kept immersed in the treatment solution for 20 minutes. The fabric was then passed through a padding mangle (R. B. Electronics and Engineering, Mumbai), running at a speed of 15m/min with a pressure of 2 kg f/cm^2 to remove excess solution. A 100% wet pick-up was maintained for all of the treatments. After padding, the fabric was air-dried and then cured for 3 min at 140 ^{0} C and immersed for 5 min in 2 g/l of sodium lauryl sulfate to remove unbound solutions and rinsed to remove the soap solution followed by air-drying.

Assessment of antimicrobial activity of coated fabric: The antimicrobial activity of the herb extract loaded nanoparticles against the test organisms was done using the following methods.

Assessment of antibacterial activity (AATCC 147): The antibacterial activity of the herb extract loaded nanoparticles coated cotton fabric was tested according to AATCC 147 (Parallel streak method) against the test bacterial culture [17]. The coated cotton fabric were taken and cut into rectangular strips of 2.5cm x 5 cm. The test organisms were inoculated with sterile nutrient broth to retrieve a log phase culture which was used to determine the zone of inhibition. Sterile AATCC Bacteriostasis agar was dispensed in sterile petri dishes. The log phase culture of the test organisms was used as inoculum.

One loop full of culture was loaded and transferred to the surface of an agar plate by making five parallel inoculum streaks approximately 60mm in length and spaced 10mm covering the central area of petri dish without refilling the loop. The test specimen was gently pressed transversely, across the five inoculums of streak to ensure intimate contact with agar surface. The plates were incubated at 37°C for 18-24 hours.

The incubated plates were examined for the interruption of growth over the inoculum. The size of the clear zone was used to evaluate the inhibitory effect of the test sample and the average width of the zone along the streak on either side of the test specimen can be calculated by the following formula,

$$W = T - D/2 \quad \text{..... (1)}$$

Where, W is the width of the clear zone of inhibition in mm; T is the total diameter of test specimens and clear zone in mm; D is the diameter of the test specimen in mm.

Assessment of antifungal activity (AATCC 30): The antifungal activity was carried out by the humidity jar method for nanoparticles treated fabric and untreated (control) fabric. About 100ml of distilled water was taken in 1000 ml conical flask and autoclaved. *A. niger* was used as test cultures. About 0.5ml of the spore suspension was inoculated onto the test and control samples were prepared by cutting 2.5 ± 0.5 cm × 7.5 ± 0.5 cm; which was tied to a long thread and suspended into the conical flask just above the water level [18]. The flasks were then incubated at 27° C for 15 days. A record of the percent of surface area covered with fungal growth for each strip is made at weekly intervals, or until heavy growth occurs on each sample replicate.

Wash durability of the finished fabric: The wash durability testing of the finished fabrics was carried out using a neutral soap at 40˚C (± 2˚C) for 30 minutes, keeping the material: liquor ratio at 1:50, followed by rinsing washing and drying. After drying the test fabrics and the control were assessed for antimicrobial activity [19]. Washing was carried up to 30 laundering cycles and results were evaluated by after counting the bacterial colonies upon incubation and calculated for the bacterial reduction using the formula.

$$R = 100\frac{B - A}{B} \quad \text{..... (2)}$$

Where, R - % reduction; A - The number of bacteria recovered from the inoculated treated swatch; **B -** The number of bacteria recovered from the inoculated untreated swatch.

Physical characterization of the treated fabrics: Differences in the physical properties of two sets of coated fabric (herbal extract and herbal extract loaded nanoparticles coated fabric) were compared with untreated control cotton samples. The physical properties such as air permeability test (ASTM D737-96), Tensile strength (ASTM D 5035) and stiffness tests (ASTM D6828 – 02, 2007) were analyzed by standard methods [20].

RESULTS AND DISCUSSION:

Assessment of antibacterial activity of *M. champaca* leaf extracts: *M. champaca* leaf extract prepared from ethanol, methanol, ethyl acetate, chloroform and benzene solvent were assayed for their antimicrobial activity using the well diffusion method. The zone of inhibition around the well represented the antibacterial activity of the above mentioned solvent extracts and zone was measured and tabulated (**Table 1**). The maximum zone of inhibition against all the test organisms was observed in the ethanolic extract than the other four extracts. The ethanolic extract of *M. champaca* leaf extract showed a higher level of activity against *A. baumannii*, and *B. cereus* with an inhibitory zone of 10 mm, 8 mm for *S. aureus* and 6 mm for both *K. pneumoniae* and *S. marcescens*. The extracts were effective against all the test organisms.

TABLE 1: ASSESSMENT OF ANTIBACTERIAL ACTIVITY AND MIC OF *M. CHAMPACA* LEAF EXTRACTS

S.No	Test organisms	Antibacterial Activity (Zone of inhibition mm) 1mg/ml					
		Ethanol	Methanol	Ethyl acetate	Chloroform	Benzene	Standard
1	*A. baumannii*	10	9	8	10	7	23
2	*B. cereus*	10	9	8	11	10	30
3	*E. coli*	8	7	7	-	-	14
4	*K. pneumoniae*	6	7	6	-	-	33
5	*P. aeruginosa*	7	8	6	-	-	17
6	*P. vulgaris*	9	8	7	-	-	9
7	*S. aureus*	8	8	7	8	-	19
8	*S. marcescens*	6	6	7	-	-	12

The standard antibiotic disc (streptomycin) was used for comparison which was effective against all the test organisms and the zone of inhibition ranged from 8 mm to 33 mm against the test organisms. The antibacterial activity of *M. champaca* is because of the bioactive compounds. The plant contains active constituents such as alkaloids, saponins, tannins, sterols, flavonids and triterpenoids [21]. This active Phytochemical is basically acting against the microorganisms. An another study has reported that the ethanolic extract of *M. champaca* exhibited pronounced activity against several Gram positive and Gram negative bacteria's [22]. The ethanolic extracts of *M. champaca* which showed higher antimicrobial activity were selected for further study.

Assessment of antifungal activity of the *M. champaca* leaf extract: The methanolic extracts of the *M.champaca* leaves were screened for antifungal activity by Agar well plug method against *A. niger*. The antifungal activity was evaluated by measuring zone of inhibition of fungal growth surrounding the well in mm. The zone obtained for *A. niger* is about 3 mm. Whereas the Nystatin produced 5 mm against *A. niger*. Hence the leaf extract showed significant broad spectrum antimicrobial action against test bacteria and fungi.

Minimum inhibitory concentration: The minimum inhibitory concentrations of ethanolic extract against eight test organisms were shown in the **Table 2**. The MIC range was observed for the plant extracts from 10 mg/ml to 90 mg/ml. The ethanolic extract of *M. champaca* inhibited the *K. pneumoniae, Acinetobacter* sp., and *B. cereus*, with a MIC of 50µg/ml. Which was the lowest MIC recorded. For *S. aureus*, the MIC was 60 µg/ml and for *S. marcescens*., the MIC was 90 µg/ml, which implies that the inhibition rate was very low when compared to other organisms. Although, there are many studies revealed the antimicrobial properties of *M. champaca*, but there was no study reported the MIC of the ethanolic leaf extract. Therefore, the present study provided the MIC values of *M. champaca* leaf extracts against the test bacteria. From the above results it was confirmed that the ethanolic extract showed a better inhibitory rate. Ethanolic extract of *M. champaca* which showed higher inhibition was used for the nanoparticles preparation.

TABLE 2: DETERMINATION OF MIC FOR ETHANOLIC LEAF EXTRACT OF *M. CHAMPACA*

S. no	Test organism	Concentration of methanolic leaf extract of *M. champaca* (µg/ml)								
		10	20	30	40	50	60	70	80	90
1	*A. baumannii*	0.0693	0.0503	0.0535	0	0	0	0	0	0
2	*B. cereus*	0.0227	0.0555	0.0010	0	0	0	0	0	0
3	*E. coli*	0.3160	0.2674	0.2769	0.2677	0.2597	0.2314	0.1433	0.0394	0.0219
4	*K. pneumoniae*	0.3765	0.3290	0.3126	0.1864	0.1349	0.1024	0.0241	0.0593	0.0465
5	*P. aeruainosa*	1.4676	1.4604	1.4556	1.4459	1.4348	1.4368	1.4287	1.4229	1.4128
6	*P. vulgaris*	1.9808	1.8767	1.8723	1.8673	1.8650	1.8430	1.8354	1.823	1.8096
7	*S. aureus*	0.2944	0.2333	0.1552	0.1463	0.1395	0.1387	0.1127	0.1159	0.1030
8	*S. marcescens*	0.0616	0.0433	0.0172	0.0161	0.0142	0.0118	0.0059	0	0

Phytochemical analysis: The *M. champaca* leaf extract was tested for the presence of phytochemicals using standard protocol. The phytochemicals like alkaloids, flavonoids, saponins, carbohydrates, phenols, steroids, glycosides and tannins were present in the leaf extract. The phytochemicals like proteins and thiols were absent in *M. champaca* leaf extract. Several compounds like alkaloids, saponins, tannins, sterols, flavonoids and triterpenoids were already identified and reported [21]. Another study has reported that flavonoids posses a wide range of antifungal, antiviral and antibacterial activity [23]. Flavonoids also posses antioxidant, cytotoxic, chemoprevention and possesses strong anti-proliferative activities [24]. The basic structure of flavonoids is derived from C_{15} body of flavones and isoflavonoids were found to be toxic to fungi [25]. And thus, due to the presence of flavonoids and other phytochemicals the leaf extract of *M. champaca* exhibited antimicrobial activity.

Physical characterization of *M. champaca* leaf extract loaded nanoparticles - Transmission Electron Microscopy: The topography of the *M. champaca* leaf extract loaded chitosan alginate nanoparticles were observed by transmission

electron microscope (TEM, JEOL-TEM 2100) and represented in **Fig. 1**. The topographical study stated that the particles were found to be roughly spherical in shape with an average particle size of 20 nm. The sizes of the nanoparticles were found to be approximately in the range of 20 – 40 nm. The particles were well dispersed as well. The previous study also proved that the chitosan sodium alginate nanoparticles were found to be spherical, distinct and regular and the size of these particles ranged between 20 – 50 nm [27, 28].

It was also reported that the surface of the nanoparticles were not smooth and they had a fluffy appearance. Electron microscopic images of the *M. champaca* leaf extract loaded chitosan alginate nanoparticles revealed that the nanoparticles surface showed morphology related to a generic spherical shape with fluffy appearance. It could also be inferred from the figure that the nanoparticles were single distinctive particles with a clear boundary which were spherical in shape.

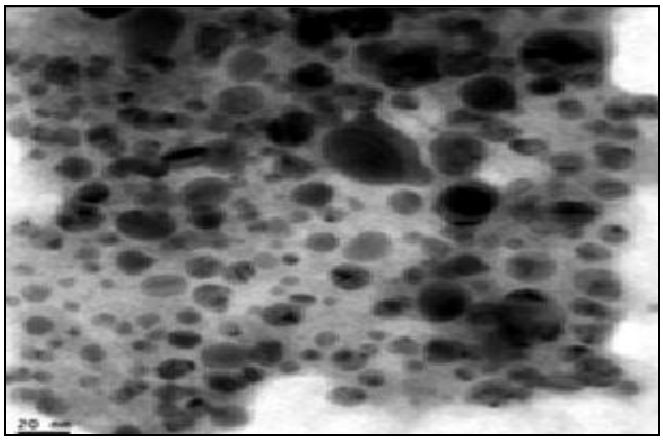

FIG. 1: TEM MICROGRAPH OF THE *M. CHAMPACA* LEAF EXTRACT LOADED ALGINATE CHITOSAN NANOPARTICLES

Chemical characterization of M. *champaca* leaf extract loaded nanoparticles – FTIR analysis: The FTIR analysis of the herbal extract and herbal extract loaded nanoparticles and were shown in **Fig. 2 a** and **b**. The broadband at 3442 cm^{-1} in the spectrum at peak 1 of herb loaded nanoparticles corresponded to the amines and hydroxyl groups. The peak at 1159 cm^{-1} at peak 5 was caused by C-O-C stretching in the saccharide structure of sodium alginate. In addition, the characteristic absorption band at 1646 cm^{-1} at peak 3 of chitosan was shifted to 1383 cm^{-1} at peak 4 after reaction with alginate, the stretching vibration of -OH and -NH_2 at 3438 cm^{-1} shifts to 3442 cm^{-1} and becomes broad which illustrates the interaction between chitosan and alginate. The characteristic absorption band of ethanolic extracts of *M. champaca* at 1072 cm^{-1} and 814 cm^{-1} which corresponded to aromatic and aliphatic amines appeared in *M. champaca* leaf extract loaded nanoparticles at 1159 cm^{-1} at peak 5 and 673 cm^{-1} at peak 6. This probably indicates that the ethanolic leaf extract of *M. champaca* was found in the polymer network.

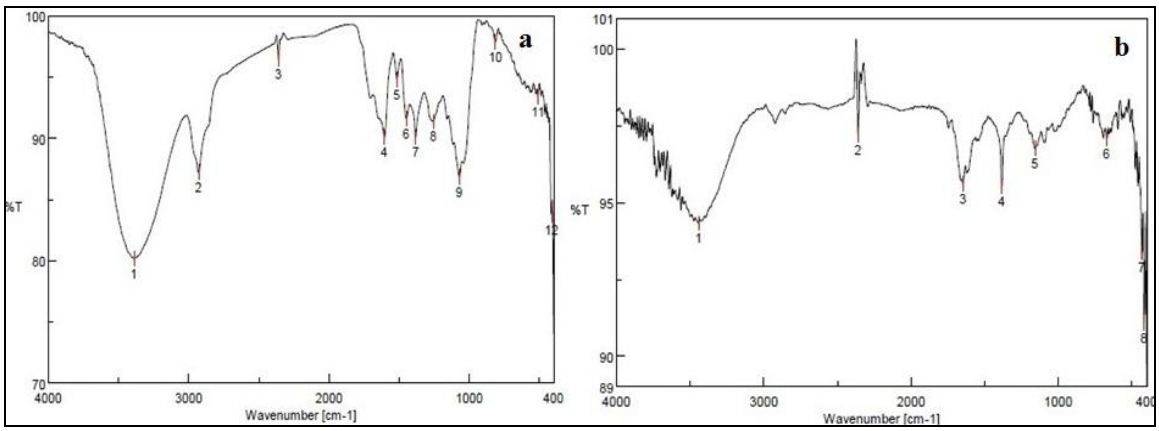

FIG. 2: FTIR SPECTRA a) *M. CHAMPACA* LEAF EXTRACT b) *M. CHAMPACA* LEAF EXTRACT LOADED NANOPARTICLES

Assessment of antibacterial activity of *M. champaca* leaf extract loaded nanoparticles coated cotton fabric: The cotton fabric was coated with *M. champaca* leaf extract loaded nanoparticles by using the pad dry cure method [28]. The antimicrobial efficacy of the fabric was determined by the parallel streak method (AATCC 147). The *M. champaca* leaf extract loaded nanoparticles coated fabric showed a maximum antibacterial activity against the test organisms. Another study reported that herbal encapsulated nanoparticle treated fabrics inhibited microorganisms than crude extract [28]. The highest zone of inhibition was recorded for *P. vulgaris* (20mm), *B. cereus* (13.5) followed by *E. coli*, *S. aureus* and *S. marcescens* (12mm). *P. aeruginosa* and *A. baumannii* exhibited a zone of 11.5mm and 10.5mm respectively. The lowest zone of inhibition was observed for *K. pneumonia* with 7 mm. whereas the *M. champaca* leaf extract coated cotton fabric showed the antibacterial activity, but comparatively less than the nanoparticles coated fabric (**Fig. 3**). The nanoparticles coated fabrics produced a greater antibacterial activity than the crude extracts due to the size of the nanoparticles which provided an enhanced affinity of the nanoparticles to the cotton fabric [29].

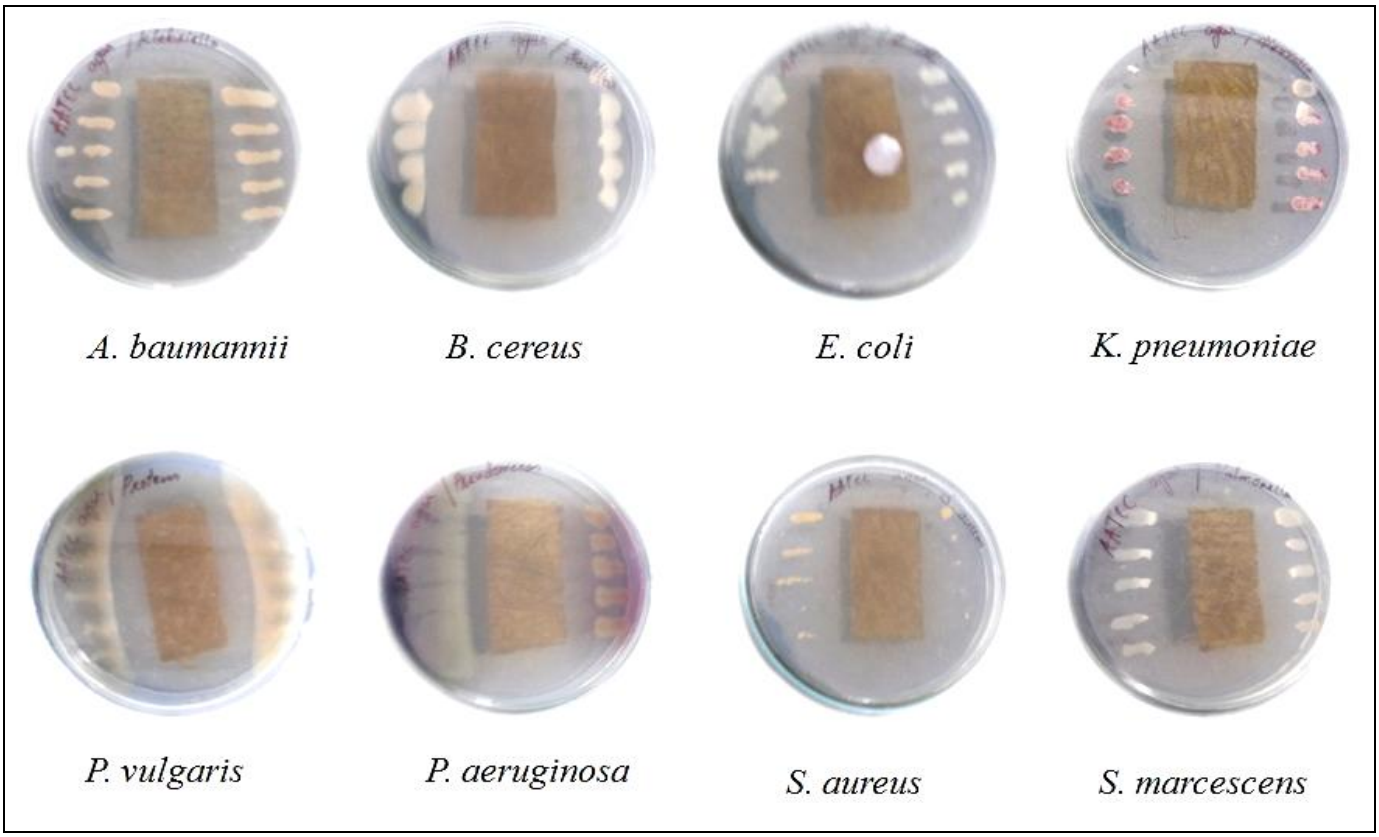

FIG. 3: ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF M. CHAMPACA LEAF EXTRACT COATED COTTON FABRIC

Assessment of antifungal activity of *M. champaca* leaf extract loaded nanoparticles coated cotton fabric (AATCC 30-2004 – Humidity jar method): The antifungal activity of *M. champaca* leaf extract loaded nanoparticles coated cotton fabrics were assessed using humidity jar experiment and showed in **Fig. 4 a, b** and **c**. The antifungal activity of *M. champaca* leaf extract loaded nanoparticles was found to exhibit maximum days of fungicidal activity for about 15 days, while the control fabric showed the growth of *A. niger* in 3 days after inoculation. Thus the *M. champaca* leaf extract loaded nanoparticles had a better fungicidal activity. *M. champaca* showed antifungal activity against pathogenic fungi viz., *A. vitis*, *A. versicolor* and *T. tronsurum* [22]. The alkaloid, liriodenine was the active constituent and it provokes the broader activity when compared to the some of the standard antibiotics at lower concentration.

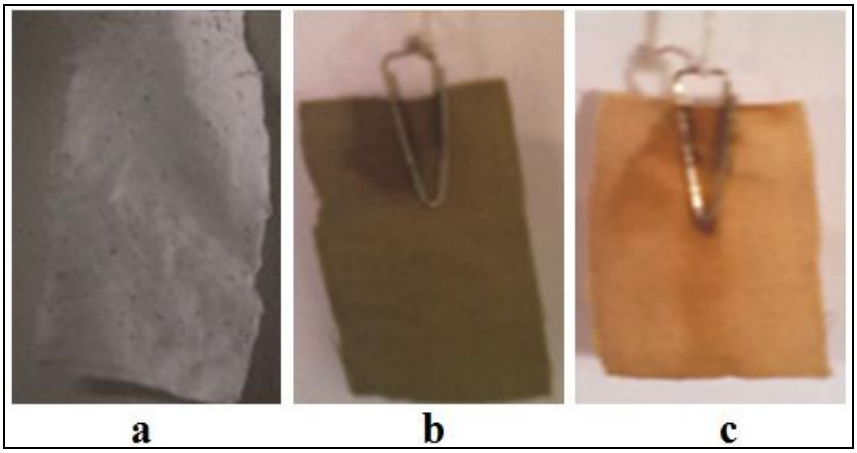

FIG. 5: ASSESSMENT OF ANTIFUNGAL ACTIVITY AGAINST *A. NIGER* a) UNTREATED FABRIC b) *M. CHAMPACA* LEAF EXTRACT COATED FABRIC c) *M. CHAMPACA* LEAF EXTRACT LOADED NANOPARTICLES COATED COTTON FABRIC

Wash durability test: The bacterial reduction percentage until 30 laundering cycles were determined in untreated fabric and treated fabric (the fabric coated with leaf extract, the fabric coated with herbal leaf extract loaded nanoparticles) using the laundry test. The fabrics were then tested for the percentage reduction of bacterial growth after every 5 cycles [16]. The difference in the durability properties between the different treatments of fabrics was analyzed by repeated washing. The results were calculated and tabulated in **Table 3**. The fabric coated with herbal leaf extract loaded nanoparticles showed the bactericidal activity against the test bacteria until 30 washes effectively due to the sustained release of the nanoparticles when compared to the untreated fabric coated with the herbal leaf extract. It could be conferred from the table that the herbal leaf extract loaded nanoparticles treated fabric sustained their maximum antibacterial activity against both the test bacteria *S. aureus* and *E. coli* until 30 washes. Another study had reported that the nanoparticles have the higher laundering durability to the small particle size, uniform coating and controlled release [16, 28]. However, the meagre reduction of bacteria during the initial laundering cycles of the fabric treated with the bulk extract could be attributed to the presence of the binding agent (citric acid) in the fabrics. Hence it could be inferred that the fabric coated with herbal leaf extract loaded nanoparticles was durable to 30 washes with a detergent in the provided process conditions of the test. As expected, the untreated fabrics did not show any antibacterial activity.

TABLE 3: WASH DURABILITY OF HERBAL LEAF EXTRACT LOADED NANOPARTICLES COATED COTTON FABRIC

S.No	Test samples	Number of laundering cycles	Percentage of Bacterial reduction (%)	
			E.coli	***S.aureus***
1	Herbal leaf extract coated on the fabric	5	95	94
		10	73	71
		15	62	59
		20	47	48
		25	39	35
		30	17	11
2	Herbal leaf extract loaded nanoparticles coated on the fabric	5	100	100
		10	97	95
		15	90	91
		20	87	86
		25	82	83
		30	79	80

Physical characterization of the treated fabrics: The physical characterization was done for the untreated and treated (*M. champaca* leaf extract loaded nanoparticles) fabrics. The tensile strength for control fabric was found to be 8000 grams; it was recorded as 7990 grams for *M. champaca* leaf extract loaded nanoparticles coated fabric. The air permeability of untreated and treated fabrics was 24.5 and 24.1cm^3/cm^2/sec 23.7cm^3/cm^2/sec respectively.

For the treated and untreated fabrics the air permeability was merely same. Thus, the nanoparticles coated on the fabric does not cause any interruptions in the air permeability [29]. The stiffness of the cotton fabric was 2.1 cm, whereas for the treated fabric 2.3 cm. The previous study also reported that the stiffness of the fabrics before and after nanoparticles treatment and opined that a slight increase in the stiffness was observed in the nanoparticles treated fabric [30]. It was found from the above results that there was no significant difference between the different fabric treatments with respect to the various physical tests (tensile strength, air permeability and stiffness). Hence it can be substantiated from the results there is no loss in comfort property of the nanoparticles coated fabrics.

CONCLUSION: The *M. champaca* leaf extract loaded nanoparticles coated cotton fabric showed a better antimicrobial activity. The extract loaded nanoparticles have paved way for the better antimicrobial activity which was evident from the antimicrobial assessment test of the treated fabrics. Although, the present study focused on the development of the *M. champaca* leaf extract loaded nanoparticles coated cotton fabric with remarkable antimicrobial activity, more work is needed for an understanding the laundering durability and the mode of action of the nanoparticles on the cotton fabric. The physical parameters proved that there was no significant difference between treated and untreated fabric. Thus the herbal extract loaded nanoparticles does not alter the comfort properties of the fabric. Hence this kind of antimicrobial cotton fabric could also influence its wide applications in the hospitals for the workers and patients as a better alternative to the commercially available chemical antimicrobial textiles.

CONFLICT OF INTERST: The authors declare that there is no conflict of interests regarding the publication of this paper.

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