STUDY ON ANTI-MICROBIAL ACTIVITY OF HERBS COATED ON NON-WOVEN FABRIC IN SANITARY NAPKINS FOR TOP LAYER FINISHING

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Abstract

The key property requirements of hygiene products are to soak up and retain menstrual fluid discharge which may be a complex viscous mixture of water, salts and cells; barrier performance for containment and absorption without leakage, comfort and breathability, wicking and wetting behaviour, mechanical properties, sterilization stability, antimicrobial properties without skin irritant tendencies which are achieved by suitable staple choices and style considerations (Jassal, 2011). Menstruation may be a process during which woman discharge blood and other material from the liner of the uterus at an interval of about 28 to 35 days from puberty until menopause. It causes serious problems to the ladies if not managed properly. This menstrual discharge are often absorbed by some absorbent . The functional requirement of a female hygiene product is to soak up and retain the menstrual fluid in order that back tracking of fluid doesn't happen and at an equivalent time it should be odor free. Commercially available menstrual hygiene pads are made from material which can seem innocuous but they're laced with dioxins, petrochemicals, artificial fragrances etc. These chemicals are available contact with sensitive skin tissue, can cause skin irritation. Cellulosic chlorine bleached pulp; rayon which is employed to extend absorbency of pad contains dioxin results in cervical cancer irregular growth in reproductive organs.

Keywords: Sanitary Napkins, absorbent pads, herbal extracts, Cytotoxicity assay, Persistence

1. Herbal Selection:

1.1. Neem(Azardirachta indica)

Neem is used in traditional medicine as a source of many therapeutic agents in the Indian culture and grows well in the tropical countries. Neem has showed that it contains active substances with multiple medicinal properties. Neem (Azadirachta indica A. Juss) is perhaps the most useful traditional medicinal plant in India. Every division of the neem tree has some medicinal property and is thus commercially utilizable. During the last five decades, spaced out from the chemistry of the neem compounds, substantial development has been accomplish on the subject of the biological activity and medicinal purpose of neem. It is now well thought-out as a precious source of distinctive natural products for expansion of medicines adjacent to various diseases and also for the expansion of industrial products. Oil from the leaves, seeds and bark possesses a wide variety of antibacterial action next to Gram-negative and Gram-positive micro organisms. Neem leaves has antibacterial property and could be used for controlling airborne bacterial contamination in the residential premise. The Neem seed oil also showed a wide spectrum activity against Gram-negative and Gram-positive microorganisms, including *M. tuberculosis* and streptomycin resistant strains. Natural herbal products are attractive alternative to synthetic agents for imparting antimicrobial properties to textiles since there is a tremendous source of medicinal plants with bioactive agents in India. Neem extract is one such type of product which is extracted from seed, bark or leaves of Neem tree (Azadarichta indica) belonging to Mahgony family and found abundantly in the Indian subcontinent

1.2 Evolvulus alsinoides (Vishnu kranti)

The most common maladies against which these plants are now considered as a remedy in Ayurveda and by nonprofessional villagers in India are various mental problems. Among these illnesses are epilepsy, insanity, nervous debility, and loss of memory. *Evolvulus alsinoides* is estimate a recollection enhancer and anti-amnesic. Even in Africa the herb is used to treat low spirits and depression. Several studies show that *Evolvulus alsinoides* plant extracts*also possess anticancer activity. E. alsinoides* methanolic abstract treatment caused momentous cytotoxicity in HepG2 cells in a concentration-dependent way. Dual staining assay established the occurrence of early and late apoptotic cells only in extract-treated groups. Plant extract treatment also caused nuclear fragmentation and chromatin condensation in HepG2 cells. The phytochemicals such as piperine, octadeconoic acids, hexadecanoic acid and squalene have been found in *E. alsinoides* . Interestingly, piperine from *E. alsinoides*, has been shown to induce growth inhibition and apoptosis in various cancer cell lines.

2. Extraction and Finishing

Extraction refers to separating the antimicrobial active substances by chemical means with the aid of a solvent (methanol). Antimicrobial active substances were extracted from the plant by Soxhlet extraction method. The powdered plant material was extracted by adding 20g of herbal powderin100ml of solvent and extracted continuously for 6 hours in a Soxhlet extractor. The herbal extracts were finished in top layers of sanitary napkin using dip and dry method. The herbal extract was applied on to the top layers of sanitary napkin by immersing in a dye bath containing the herbal extract at liquor ratio of 1:50ml herbal solution for each gram of the fibre sample at room temperature without heating. Then at 100°C sodium chloride was added under closed dyeing system. Finally, the herbal dyes were rinsed in water and dried at 40°C for 30 min.

Fig.1. Herbal extraction of (1) Evolvulusalsinoides (2) Azardirachtaindica (3) Caesalpiniabonducella

2.1 Selection of best Concentration of Herbal Extract

The one best concentration of the herbal extracts (*Azardirachtaindica, Evolvulusalsinoides, Caesalpiniabonducella*and *Pergulariadaemia*) were evaluated against the *Escherichiacoli* and *Staphylococcus aureus* strains by well diffusion method. Sterile Nutrient Agar (Composition g/L: Peptone: 5g; Yeast extract: 5g, Beef extract: 3g, Sodium chloride: 5g, Agar 15 g; Final pH (7.0 ± 0.2) plates were prepared and allowed to solidify. About 0.1% inoculum suspensions of each of the bacterial cultures (*Escherichiacoli* and *Staphylococcus aureus*)were streaked with the sterile cotton swab three times by turning the plate at 60º angle between each streaking. Under sterile conditions, 6mm wells were cut on the agar surface of each Nutrient Agar (NA) plates. About 50µl each of each plant extract at four different concentration (10µg/ml, 20µg/ml, 30µg/ml and 40µg/ml)in 5% dimethyl sulfoxide (DMSO) were loaded into the well and the plates were incubated at 37ºC for 24 - 48h. The antibacterial activity was evaluated in terms of zone of inhibition around the wells of each extract in all the inoculated NA plates. The inhibition clear zones were measured and recorded in millimeter.

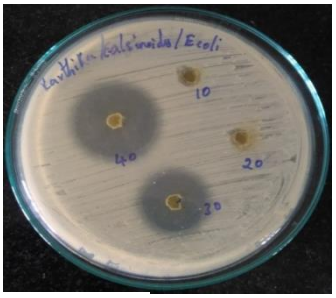
3.ANTI-MICROBIAL ACTIVITY OF HERBS FOR TOP LAYER FINISHING

The result of selected finishing herbs are evaluated by screening the Anti-Microbial activity of selected herbal plant, and Anti- bacterial efficiency of herbal combination by well diffusion method,cytotoxity and SEM. Selection of one best concentration of the medicinal herb extract for finishing on top layer of napkin was carried out by agar well diffusion method against two different organisms (*E. coli* and *S. aureus*) are exhibited in Table

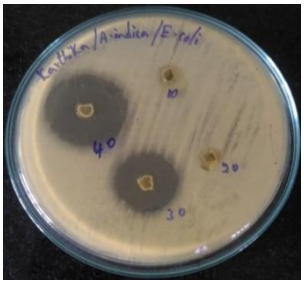
Table.1. Assessment of Antibacterial effect well diffusion method (different concentrations)

S.No	Herbal extract	Organisms	Zone of inhibition (mm)			
			Concentration (µg/ml)			
			10	20	30	40*
1	*Azardirachtaindica*	*E. coli*	0	0	15	18
		S. aureus	0	0	14	16
2	*Evolvulusalsinoides*	*E. coli*	0	0	17	19
		S. aureus	0	0	18	21
3	Caesalpiniabonducella	*E. coli*	0	0	11	18
		S. aureus	0	0	10	16
4	*Pergulariadaemia*	*E. coli*	0	0	11	16
		S. aureus	0	0	10	17

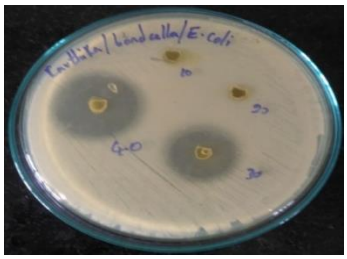
a. Activity against E.coli



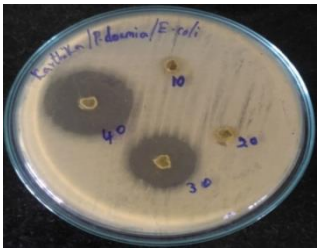
Evolvulusalsinoides



Azardirachtaindica



Caesalpiniabonducella



Pergulariadaemia

b. Activity against *S. aureus*

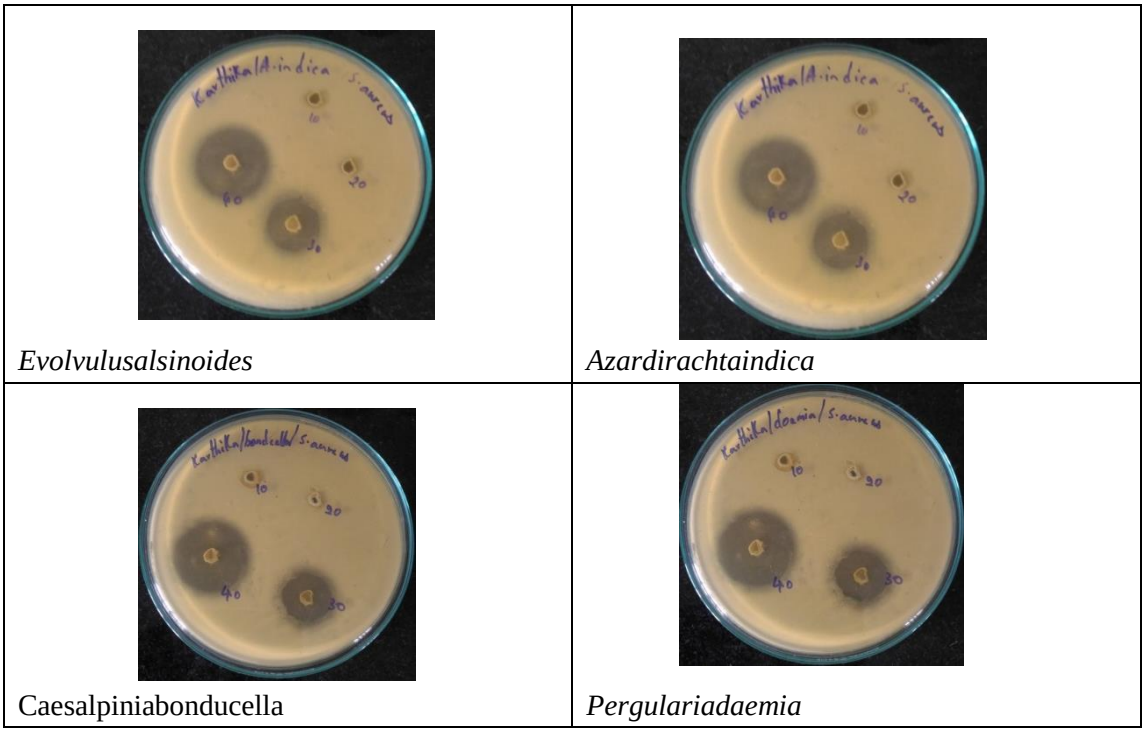


Fig 1.Screening of Anti Bacterial Efficiency of Selected Herbal Extracts by Well Diffusion

From the above table it is revealed that,

1. *Azardirachtaindica* extracts showed 15mm and 18mm against *E. coli* and 14mm and 16mm against *S. aureus* at 30µg/ml and 40 µg/ml concentrations.
2. No zones were observed for 10µg/ml and 20µg/ml. *Evolvulusalsinoides* extracts showed 17mm and 19mm against *E. coli* and 18mm and 21mm against *S. aureus* at 30µg/ml and 40µg/ml concentrations.
3. Similarly no zone of inhibition was observed for other two concentrations. Caesalpiniabonducella extracts showed 11mm and 10mm against *E. coli* and *S. aureus* at 30µg/ml concentration; and 18mm and 16mm at 40µg/ml concentration. *Pergulariadaemia* extracts showed 11mm and 10mm against *E. coli* and *S. aureus* at 30µl concentration; and 16mm and 17mm at 40µg/ml concentration.
4. All the medicinal extracts (*Azardirachtaindica*, *Evolvulusalsinoides*, Caesalpiniabonducella and *Pergulariadaemia*) showed higher inhibitory zones for 40µg/ml concentration and 40µg/ml concentration is selected for the finishing on non-woven cotton fabrics.

4. ANALYSIS OF ANTI- BACTERIAL ACTIVITY

The herbal extracts were finished on non-woven cotton fabrics and antibacterial activity was evaluated using AATCC-147 test method. The inhibitory zones were measured in millimeters (mm) are exhibited in Table.

Table 2 Antibacterial activity - Parallel streak method (AATCC 147 test method)

S. No.	Fabric finished with herbs	Inhibitory zones (mm)	
		Escherichia coli	Staphylococcus aureus
1	Azardirachtaindica	34	35
2	Evolvulusalsinoides	36	37
3	Caesalpiniabonducella	32	34
4	Pergulariadaemia	34	32

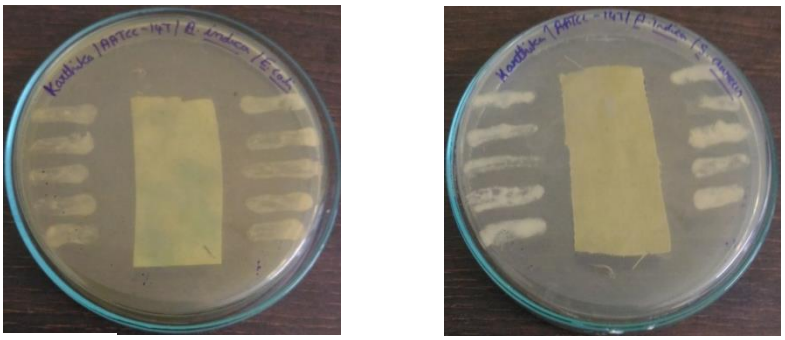


Fig.3Azardirachtaindica extracts finished fabric
(A)Escherchia coli (B)Staphylococcus aureus

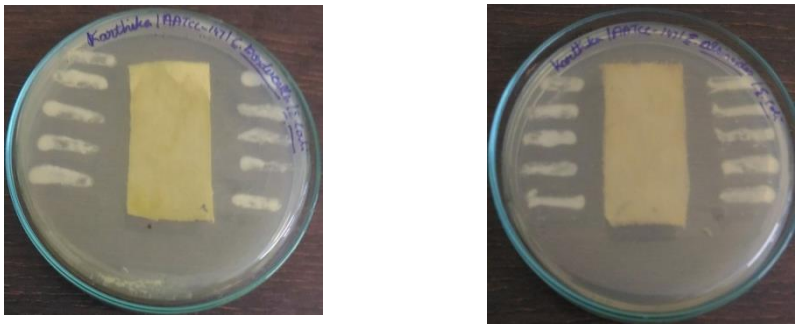


Fig.4 *Evolvulusalsinoides* extracts finished fabric (a)*Escherchia coli (b)Staphylococcus aureus*

Fig 5. Pergulariadaemia extracts finished fabric (A)Escherchia coli (B)Staphylococcus aureus

Fig.6 Caesalpiniabonducella extracts finished fabric (A) Escherchia coli (B) Staphylococcus aureus

From the above plate it is perceived that,

1. *Azardirachtaindica*and *Evolvulusalsinoides*showed 34mm and 35mm; and 36mm and 37mm of inhibitory zones against *E. coli* and *S. aureus.* Caesalpiniabonducellaand *Pergulariadaemia*showed 32mm and 34mm; and 34m and 32mm against *E. coli* and *S. aureus.*
2. The highest inhibitory zone was observed for *Evolvulusalsinoides*extract finished fabric.

5. ANALYSIS OF ANTI- FUNGAL ACTIVITY

The herbal extracts were finished on non-woven cotton fabrics and antifungal activity was evaluated using AATCC-30 test method are exhibited in Table 3.

Table 3 Antifungal activity (AATCC – 30)

S. No.	Fabric finished with herbs	Inhibitory zones (mm)	
		Candida albicans	*Candida tropicalis*
1	Azardirachtaindica(Neem)	57	59
2	Evolvulusalsinoides	59	58
3	Caesalpiniabonducella	57	56
4	Pergulariadaemia	56	55

From the above table it is perceived that,

1. *Azardirachtaindica*and *Evolvulusalsinoides*showed 57mm an59mm; and 59mm and 58mm of inhibitory zones against *C. albicans* and *C. tropicalis.* Caesalpiniabonducellaand *Pergulariadaemia*showed 57mm and 56mm; and 56mm and 55mm against *C. albicans* and *C. tropicalis.*
2. The highest inhibitory zone was observed for *Evolvulusalsinoides*and *Azardirachtaindica*extract finished fabric. Based on the higher inhibitory zones *Evolvulusalsinoides* and *Azardirachtaindica* plant extract is selected for preparation of top layer.
3. Generally, most of the tested organisms like *Pseudomonas aeruginosa, E. coli,Klebsiella sp., S. citrus and* S. aureus were found to be sensitive for the Caesalpiniabonducellaextracts. The strong activity of the plant extract may be due to its easily diffusible nature that permits to enter the cell wall of tested bacteria in the study without any permeable barriers. A new compound α-(2-hydroxy-2-methylpropyl)-ω-[2-hydroxy-3-methylbut-2-en-1-yl] polymethylene was identified from the extracts and the significant anti-bacterial and antifungal activity is due to the presence of the compound.
4. Flavonoids are hydroxylated phenolic material known to be synthesized by plants in response to microbial infection. Antimicrobial property of saponin is outstanding to its ability to cause leakage of proteins and certain enzymes from the cell. Tannins bind to proline rich proteins and interfere with the protein synthesis. The phyto chemical composition of the plant may be the reason for higher the antibacterial and antifungal activity against different test organisms.

6. FABRIC PROPERTIES OF ANTI- MICROBIAL FINISHED LAYER

Absorption Properties

The fabric properties of Absorption Properties,Persistence,and Comfortanti-microbial finished top layer material are presented in Table 4.4 and Figure 4.7.a. Absorption Properties of Top Layer Material. The Absorbency of the top layer (non-woven cotton) fabric was evaluated under ISO 17190-7/WSP 240.3 standard. Absorbency under

pressure is performed to find out the absorbency of the test sample with a specific pressure. The absorbency of the top layer was found to be 5.1g.

Persistence

Persistence of the fabric is determined by AATCC-147 test method. Reduction in zone of inhibition was noted from Day-1 to Day-3. About 36mm and 27mm inhibitory zones were obtained on Day-1 and Day-3. After 3rd day no inhibitory zone was observed indicating the durability of the top layer finished with plant extracts are exhibited in the Table

Table 4 Persistence of the fabric

S. No	Day	Zone of inhibition (mm)
1	1	36
2	2	27
3	3	-

Persistence		
		
Day 1	Day 2	Day 3

Fig. 4.7 Persistence of the fabric

Comfort

Comfortless of the test sample was determined by wetness or dryness of the fabric. After incubation the top layer swatch was found to be dried after 300s.

Analysis of Cytotoxicity assay

Cytotoxicity assay was carried out on mouse fibroblast (L_{929}) cell lines. The amount of formazan produced is directly proportional to the number of viable cells. The medium with MTT was then brushed off and the shaped Formosan crystals were solubilized in 100µl of DMSO and then restrained the absorbance at 570 nm by means of micro plate reader. The *Evolvulusalsinoides* plant extracts had 14.6% cell inhibition and 85.2% of cell viability whereas *Azardirachtaindica* had 13.2% cell inhibition and 86.5% of cell viability.

Table 5 Assessment of Biocompatibility of Herbal Extracts

S. No	Samples	Concentrations (µg/ml)	% cell inhibition (L_{929})	% viable cells (L_{929})
1	Control	10	10.9	88.6
2	*Evolvulusalsinoides*plant extract	40	14.6	85.2
3	*Azardirachtaindica*	40	13.2	86.5

Methanolic extracts of *Evolvulusalsinoides*also triggers apoptosis in HepG2 (Hepatacellular carcinoma cancer cells). *E. alsinoides* methanolic extract treatment caused important cytotoxicity in HepG2 cells in a concentration-dependent way. Dual staining assay established the existence of early and late apoptotic cells only in extract-treated groups. Plant extract action also caused nuclear disintegration and chromatin condensation in HepG2 cells. Mitochondrial membrane potential also reduced upon *E. alsinoides* treatments. This treatment also controlled the catenin – β 1 protein expression.

CONCLUSION

From the above study, we concluded that, In SEM analysis that foundas rough, irregular in shape and strongly bound to the fabrics. The herbal extracts were held together by van-der-Waals and electrostatic interaction. Under ambient conditions, herbal extracts can form aggregates or agglomerates in various forms leading to inhibition of bacterial and fungal microorganisms. The antibacterial activity was evaluated using AATCC-147 test method. The inhibitory zones were measured in millimeters (mm)and antifungal activity was evaluated using AATCC-30 test method.The absorbency of the top layer was found to be 5.1g. After incubation the top layer swatch was found to be dried after 300s.Mitochondrial membrane potential also reduced upon *E. alsinoides* treatments. This treatment also controlled the catenin – β 1 protein expression.

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