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SYNERGISTIC EFFECT OF POLY HERBAL EXTRACT AGAINST MULTI-DRUG RESISTANT PATHOGEN

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ABSTRACT
On consideration of toxicity and the adverse side effects of synthetic antimicrobial agents, the study accords the broadcast of herbs such as *Terminalia bellerica, Withania somnifera, Madhuca longifolia and Sygyzium cumini* based on its persuasive antimicrobial activity. The potentially best combination was screened and tested against nine clinical bacterial strains. GC-MS analysis revealed the existence of n-Hexadecanoic acid, Dotriacontane and Oleic acid which is responsible for their antimicrobial properties. The results of FTIR spectra of the poly herbal extract showed the presence of functional groups as follows: primary and secondary amines, alkanes, carbonyls. Thus the study recommends the use of poly herbal extract as a substitute to synthetic agents which effectively prevent the microbial infestation.

KEYWORDS: Antibacterial Susceptibility, FT-IR, GC-MS, Poly herbal extract.

INTRODUCTION
The emergence and spread of Multi- Drug Resistant bacteria continues to challenge the global healthcare sector. In recent years, the global concerns in therapeutics are antibiotic resistance and morbidity due to the non-availability of new drugs and increased usage of antibiotics (Babu and Subhasree, 2009; Williams, 2000). The risk associated with synthetic antimicrobial drugs is its inefficiency to overcome antibiotic resistant bacteria.

Usage of these drugs is now a threatening factor for the patients resulted in harmful side effects (Cosgrove and Carmeli, 2003). The emergence and dissemination of multidrug resistant bacteria has consequence towards increased cost of medicines (Harbottle et al., 2006). To address this challenge the development of new antibiotics in pharmacology is vital (Cosgrove and Carmeli, 2003).

Researchers turned their attention to develop new drugs from traditional medicinal plants. Predate the introduction of antibiotics there was an efficient way to treat diseases with medicinal plants (Braga et al., 2005). Medicinal plant contains novel pharmaceutical compounds. One of the vital activities found in these plants is its antimicrobial nature (Demetrio et al., 2015).

Antimicrobial or biologically active compounds from plants origin are efficient in the treatment of cross infections (Chanda et al., 2013, Chanda et al., 2011; Jeyaseelan et al., 2012). Most of them offers broad spectrum of activity against pathogens due to their secondary metabolites. They also mitigate the side effects associated with the synthetic antimicrobials (Iwu et al., 1999). The present study was initiated to evaluate the antimicrobial property of the synergetic action of the poly herbal extracts.

MATERIALS
Terminalia bellerica, Withania somnifera, Madhuca longifolia and Sygyzium cumini were collected in and around Coimbatore, India. All the chemicals and solvents used in the study were procured by HIMEDIA Chemicals Private Limited, Mumbai, India.

METHODS
Poly Herbal Extraction Method
Selected herbs were shade dried, powdered and sieved separately was used for the extraction (Sathianarayanan et al., 2010). Five different combinations of the selected herbs such as *Terminalia bellerica, Withania somnifera, Madhuca longifolia and Sygyzium cumini* such as 1:1:1:1, 2:1:1:1, 1:2:1:1, 1:1:2:1 and 1:1:1:2 were used to optimize the concentration of the herbal powder ratio. Extraction was done using maceration process at room temperature for 48 h at 120 rpm (Perez et al., 1990).

Supernant filtrate was filtered twice using Whatman no 1 filter paper and the filtrate was mixed with Dimethyl Sulphoxide(100mg/ml).

Preliminary Assessment of Antibacterial Susceptibility Testing

The screening of the combinatorial plant extract was evaluated by the Well diffusion method (Perez et al., 1990). The prepared bacterial cultures were swabbed aseptically on the Muller-Hinton agar plates. Wells of 6 mm diameter were punctured using a sterile gel puncture. About 60µl of the crude extract were loaded into the wells and the plates were incubated at 37°C for 24 h (Pranay and Rishabh, 2011). Tetracycline and DMSO were used as positive and negative control respectively (Nithya et al. 2014). Finally, the best combinatorial ratio of the plant extract showing highest diameter zone of inhibition towards the tested bacteria was selected for the further study.

Gas Chromatography – Mass Spectrometer Analysis

The interpretation on mass-spectrometer of the poly herbal extract was done by GC-MS analysis using Hewlett Packard Model 5890A. The name, molecular weight and the activity of the compound responsible for it were ascertained by the comparison of both GC retention times and MS data against those of the data library.

Fourier Transform Infra-Red Spectroscopy-Attenuated Total Reflectance

The attenuated total reflection (ATR) principle operates on measuring the changes of the reflected infrared beams when it comes into contact with the sample. The Infrared beam is directed onto an optically dense crystal with high refractive index. This creates an evanescent wave that extends beyond the surface of the crystal into the sample which is held in contact. The ATR crystals were cleaned using solvents before the sample presentation. The selected herbal powder sample was placed onto the ATR plate ensuring the sample height is not more than few mm. The pressure arm was positioned over crystal /sample area and the force was applied to the sample. The sample absorbs the energy and attenuates an evanescent wave. The waves pass back to infrared beam and then to the detector. The generated infrared spectrum was collected. The test was scanned for 25 runs using infrared in the range of 400-4000 cm^{-1} with the specified resolution of 4 cm^{-1}.

RESULTS

Antibacterial activity of the combinatorial ratios (1:1:1:1, 2:1:1:1, 1:2:1:1, 1:1:2:1 and 1:1:1:2) were recorded for the zone of inhibition (Figure 1). Among all the combinatorial ratio, the poly herbal extract ratio 2:1:1:1 exhibited significant activity against *A. baumannii* (23 mm) followed by *P. aeruginosa* (22 mm) and *E. coli and S. aureus* (21 mm). Herbal extract was also compared with the positive control (Tetracycline) and the negative control (DMSO).

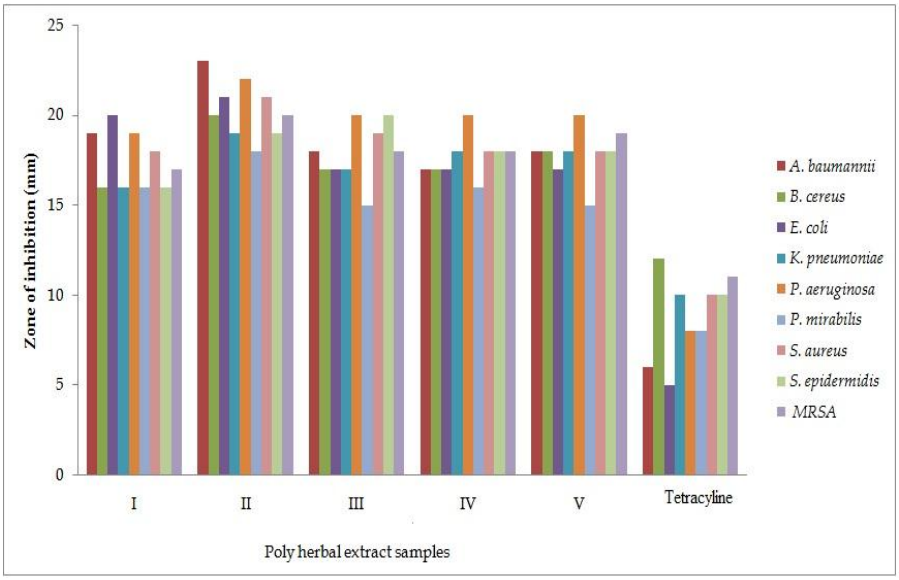

Fig 1: Assessment of Antibacterial Susceptibility of Poly herbal extract.

According to Figure 2 and Table I, poly herbal extract showed major 44 compounds. The chromatogram exhibited 48 peaks in the retention time ranging 5.2 min to 45.9 min. whereas, the major compounds were identified as n-Hexadecanoic acid (15.8%), Dotriacontane (13.0%) and Oleic acid (13.7%).

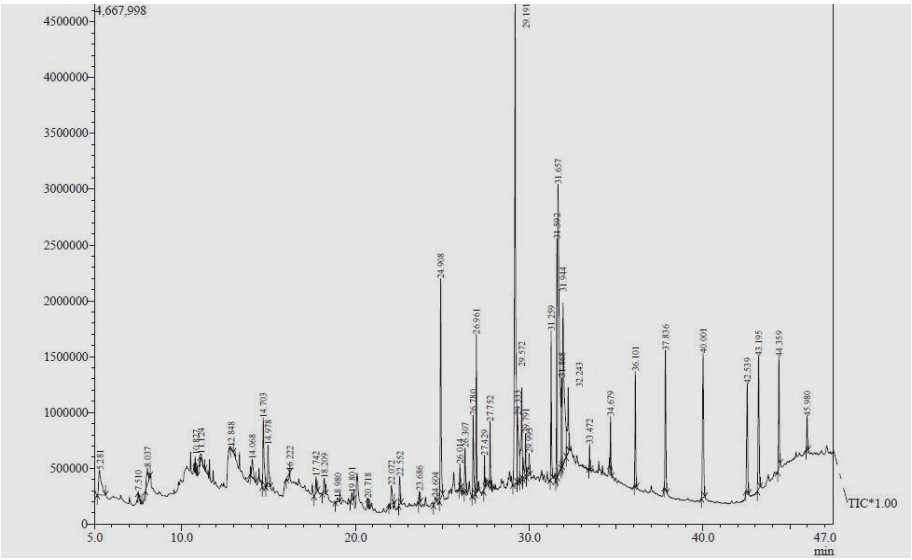

Fig 2: GC-MS interpretation of the Poly Herbal extract.

The FTIR spectrum of the poly herbal extract was represented in fig.3. In the spectra of herbal ethanolic extract, the broadband at 3950 cm^{-1} represented the presence of primary and secondary amines. The absorption band at 2924, 2854 cm^{-1} and 1720 cm^{-1} corresponded to alkanes, carbonyls. The peak in the region of 1612 cm^{-1} was caused by COOH stretching. The peaks at 1720 cm^{-1} essentially substantiated the presence of carbonyl (–C=O) groups.

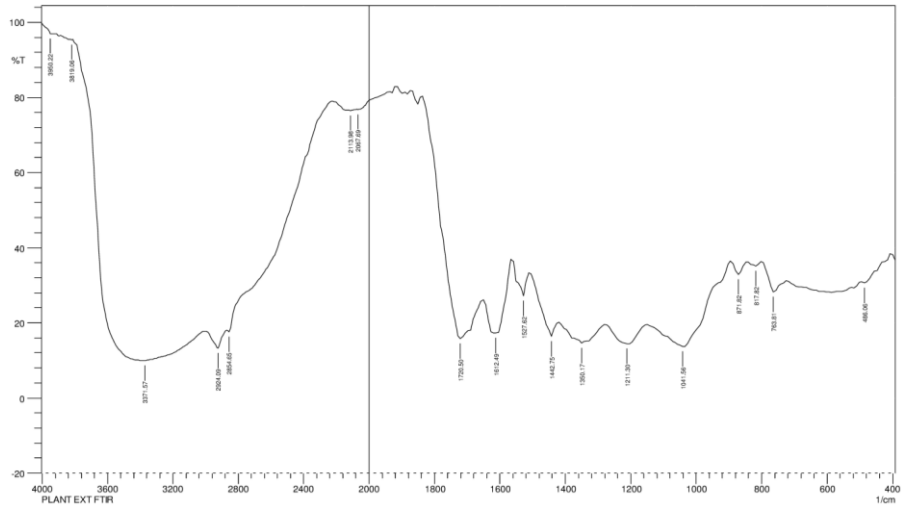

Fig.3. FT-IR analysis of Poly Herbal Extract.

DISCUSSION

Rathinamoorthy *et al.*, 2014 reported *Klebsiella sp, Proteus sp, Pseudomonas sp, Bacillus sp, Staphylococcus sp and Escherichia sp* were the consolidated list of most found bacterial pathogens in wound. It is evident that the combinatorial herbal extract showed enhanced inhibitory activity against most of the wound pathogens listed by the reported author. It was also found that all the combinatorial herbal ratios proved to show antibacterial activity equivalent to that of positive control against the test pathogens. However the combinatorial ratio (II) was selected for the further study due to their maximum bacterial inhibition.

In the GCMS analysis of poly herbal extract, a study reported that n-Hexadecanoic acid is a palmitic acid responsible for anti-fungal, anti-inflammatory and anti-oxidant property (Rajeshwari & Rani, 2014). The second peak found to be Dotriacontane (13.0%) was reported to be an effective anti-microbial agent (Ngassoum et al., 2000). The third prominent peak at 31.6 min with 13.7% area corresponded to Oleic acid with anti-inflammatory property (Gopalakrishnan, 2011). The peak at 31.5 min was observed as 9, 12 – Octadecadienoic acid (4.6%) is a linoleic acid which was responsible for the anti-fungal and anti-bacterial properties (Sermakkani and Thangapandian, 2012). 2, 6, 10-Trimethyl, 14-ethylene-1 (3.3%) was said to be olefins which was reported to have anti-proliferative properties (Selvamangai and Anusha, 2012). The peak (3.0%) was found to be Hexadecanoic acid (palmitic acid).

Table I: GC-MS analysis of the Poly Herbal extract.

Properties	Compound name	Area %	Mol. Formula	IUPAC/Common name
Antiseptic, Flavor, Fungicide	Furfural	1.8	$C_5H_4O_2$	Furan-2-carbaldehyde
Antifungal & Antibacterial	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydr	0.2	$C_6H_6O_4$	Kojic acid
Antioxidant Antiseptic	Phenol	0.8	C_4H_8O	Phenol
Antibacterial, anti-inflammatory, fungicide	Naphthalene, 1,2,3,5,6,8A - hexahydro-4	0.3	$C_{15}H_{24}$	Sesquiterpene
Antifungal and Antibacterial	Pentadecanoic acid	0.1	$CH_3(CH_2)_{13}COOH$	Pentadecanoic acid
Antimicrobial, Anti -inflammatory	Tau.-Cadinol	0.3	$C_{15}H_{26}O$	Sesquiterpene alcohol
Antiproliferative	2,6,10-Trimethyl,14-Ethylene-1	3.2	$C_{20}H_{38}$	Olefins
Antimicrobial and Anti-inflammatory	3,7,11,15-Tetramethyl-2-hexadecen-1-o	1.0	$C_{20}H_{40}O$	Terpene alcohol
Antifungal, Antioxidant	N-Hexadecanoic acid	15.8	$C_{16}H_{32}O_2$	Palmitic acid
Anti-inflammatory, Antioxidant	Hexadecanoic Acid, Ethyl Ester	3.0	$C_{18}H_{36}O_2$	Palmitic acid
Antimicrobial Antiinflammatory, Anti-cancer	Phytol	2.6	$C_{20}H_{40}O$	Diterpene
Anti-inflammatory	9,12-Octadecadienoic acid	4.6	$C_{18}H_{32}O_2$	Linoleic acid
Anti-inflammatory	Oleic Acid	13.7	$C_{18}H_{34}O_2$	Oleic acid
Antifungal, Antitumour, Antibacterial	Octadecanoic Acid	2.0	$C_{20}H_{36}O_2$	Stearic acid
Antioxidant	Eicosane	0.3	$C_{20}H_{42}$	Eicosane
Antimicrobial agent	Dotriacontane	13.0	$C_{32}H_{66}$	Dotriacontane

The minor active compounds identified were Phytol (2.6%) is a diterpene responsible for the anti-inflammatory, anti-cancer, and anti-microbial (Arokia and Veerabahu, 2014). Octadecanoic acid (2.0%) is a stearic acid which was reported as anti-microbial, anti-fungal and anti-tumour (Eugin and Jeyaraj, 2014). Furfural (1.8%) was identified and reported to have a fungicidal activity (Rajeshwari & Rani, 2014). 3, 7, 11, 15-Tetramethyl-2-hexadecen-1 (1.0%) is an olefin compound which was reported to be an anti-microbial and anti-inflammatory agent (Selvamangai and Anusha, 2012).

The poly herbal extract has many other phytoconstituents like sesquiterpene, pentadecanoic acid, flavanoids, kojic acid, eicosane and phenol. These compounds were reported to have therapeutic properties such as anti-fungal, anti-bacterial, anti-oxidant, antiseptic, anti-inflammatory (Gopalakrishnan, 2011).

The poly herbal extract has similar results that were produced by Poonkothai et al., (2014) with the ethanolic extract of T. bellerica which confirmed that the functional groups in the extract corresponded to their therapeutic properties at the observed peak. Paramjyothi Swamy et al., (2012) confirmed the similar absorption peak in the methanolic extract of Madhuca longifolia indicated the presence of aromatic stretching with phenolic ring structure. In the present study the spectrum sample obtained at 871- 763 cm^{-1} was related in an earlier report of Ramachandran et al., (2014) confirmed the presence of N-H wag i.e., primary and secondary amines in the extract of Withania spp. Thus, from the previous literatures reported it is evident that most of the functional groups present in the current study are the potential reason for the contribution of antimicrobial activity. Moreover the combinatorial herbal extract treated fabric may improve the rate of inhibition than that is treated individually on the fabric.

CONCLUSION

Availability of the selected poly herbs proves the practical implementation of the current study. Biologically active compounds found in the poly herbal extract confirms the presence of functional groups and their potent antimicrobial property. Also the poly herbal extract is more active than the commercial antimicrobial agent when tested against multi-drug resistant pathogens. The treated non woven fabric showed significant zone of inhibition against all the test pathogens which indicated that the poly herbal extract is both bactericidal and bacteriostatic. Thus the study initiates an alternative to expensive, synthetic and toxic antimicrobial agents for the antimicrobial finishing of textile fabrics.

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REFERENCE

1. Arockia Jenecius, Veerabahu Ramasamy Mohan. GC-MS Analysis of Bioactive Components on The Stem Extract of Bacolepis Nervosa (Wight & Arn.) Decne. Ex Moq. (periplocaceae). World J of Pharmacy and Pharmaceutical Sciences, 2014; 3(4): 1044-1059.

2. Babu PD, Subhasree RS. Antimicrobial activities of Lawsonia inermis - a review. Acad. J. Plant Sci., 2009; 2(4): 231-232.
3. Braga LC, Leite AAM, Xavier KGS, Takahashi JA, Bemquerer MP, Chartone-Souza E, Nascimento AMA. Synergic interaction between pomegranate extracts and antibiotics against Staphylococcus aureus. Can. J. Microbiol, 2005; 51: 541-547.
4. Chanda S, Rakholiya K, Dholakia K, Baravalia Y. Antimicrobial, antioxidant, and synergistic properties of two nutraceutical plants: Terminalia catappa L. and Colocasia esculenta L. Turk J Biol., 2013; 37: 81-91.
5. Chanda S, Rakholiya K, Nair R. Antimicrobial activity of Terminalia catappa L. leaf extracts against some clinically important pathogenic microbial strains. Chinese Med., 2011; 2: 171-177.
6. Chinta SK, Landage SM, Abhishek, Sonawane KD, Jalkate CB. Medical Textiles – Application of Essential Oil as Antimicrobial Agent on Nonwoven, 2012; 1(1): 75-80.
7. Cosgrove SE, Carmeli Y. The impact of antimicrobial resistance on health and economic outcomes. Clin Infect Dis., 2003; 36(11): 1433-1437.
8. Deepti Gupta, Ankur Laha. Antimicrobial Activity of Cotton Fabric Treated with Quercus infectoria extract. Indian J of Fibre and Textile Research, 2007; 32: 88-92.
9. Demetrio Valle Jr L, Jeannie Andrade I, Juliana Janet Puzon M, Esperanza Cabrera C, Windell Rivera L. Asian Pacific Journal of Tropical Biomedicine, 2015; 5(7): 532-540.
10. Eugin Amala V, Jeyaraj M. Comparative Evaluation Of Phytocomponents Present In The Methanolic Extract Of Terminalia Chebula Retz., Terminalia Bellirica Roxb., And Phyllanthus Emblica L., Fruit Extracts Using Gc-Ms Analysis. Int J of Pharma and Bio Sciences, 2014; 5(4): 927-934.
11. Gopalakrishnan S. GC-MS Analysis of Some Bioactive Constituents of Mussaenda Frondosa Linn. Int J of Pharma and Bio Sciences, 2011; 2(1): 314.
12. Harbottle H, Thakur S, Zhao S, White DG. Genetics of Antimicrobial Resistance. Anim. Biotechnol, 2006; 17: 111-124.
13. Helmut Mucha, Dirk Hofer, Sigrid Abfalg, Maximilian Swerev. Melliand Int, 2002; 83(5): 148.
14. Jeyaseelan EC, Jenothiny S, Pathmanathan MK, Jeyadevan JP. Antibacterial activity of sequentially extracted organic solvent extracts of fruits, flowers and leaves of Lawsonia inermis L. from Jaffna. Asian Pac J Trop Biomed, 2012; 2: 798-802.
15. New antimicrobials of plant origin In: Perspectives on new Crops and new Uses. Iwu MW, Duncan AR, Okunji CO, eds. J. Janick, ASHS Press, Alexandria VA, 1999; 457-462.
16. Ngassoum MB, Jirovetz L, Buchbauer G, Fleischhacker W. J. Ess. Oil Res, 2000; 12: 345.
17. Nithya Devi P, Kaleeswari S, Poonkothai M. Antimicrobial Activity and Phytochemical Analysis of Fruit Extracts of Terminalia Bellerica. Int J of Pharmacy and Pharmaceutical Sciences, 2014; 6(5): 639-642.
18. Perez C, Pauli M, Bazerque P. An Antibiotic Assay by Agar Well Diffusion Method. Acta Biol Med Exp, 1990; 15(13): 115.
19. Pranay Jain, Rishabh Varshney. Antimicrobial activity of aqueous and methanolic extracts of Withania somnifera (Ashwagandha). J. Chem. Pharm. Res., 2011; 3(3): 260-263.
20. Rajendran R, Hasobo Mohammad Ahamed, Radhai R, Balakumar C. Proceedings. Int Conference on Nanostructures (ICNS4) Kish Island. I.R. Iran, 2012; 1(1): 75.
21. Rajeshwari J, Rani S. GC-MS Analysis of Phytochemical Compounds in the Ethanolic Extract of Root of Lawsonia inermis Linn. Int J chemtech Research, 2014; 7(1): 389-399.
22. Rathinamoorthy R, Thilagavathi G. Characterisation And In-Vitro Evaluation of Terminalia Chebula Extract For Antibacterial Potential. Int J of Pharmacy and Pharmaceutical Sciences, 2014; 6(2): 932.
23. Sathianarayanan MP, Bhat NV, Kokate SS, Walunj VE. Antibacterial Finish for Cotton Fabric from Herbal Products. Indian J Fibre & Textile Res., 2010; 32: 50-58.
24. Selvamangai G, Anusha Bhaskar. GC-MS analysis of phytocomponents in the methanolic extract of Eupatorium triplinerve. Asian Pacific J of Tropical Biomedicine, 2012; S1329-S1332.
25. Sermakkani M, Thangapandian V. GC-Ms Analysis of Cassia Italica Leaf Methanol Extract. Asian J of Pharmaceutical and Clinical Research, 2012; 5(2): 90.
26. Smart Textiles - Their production and Marketing strategies. Edited by S Gupta (National Institute of Fashion Technology. New Delhi, 2000.
27. Stedfan Zikeli. Text Asia, 2003; 1: 35.
28. Teresa Wierzbowska, Marianna stachowiak-Nagacka, Asian Text J., 2002; 12: 95.
29. Thilagavathi G, Rajendrakumar K, Rajendran R. Development of ecofriendly antimicrobial textile finishes using herbs. Indian J. Fibre Text. Res., 2005; 30: 431-436.
30. Williams R. Antimicrobial resistance a global threat. Essential Drug Monitor, 2000; 1: 28-29.