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Antagonistic Property of Selected Herbal Combination for Substitute of Synthetic Antimicrobial Agents

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ABSTRACT

The emergence and spread of bacterial strains of multi- drug resistant continues to challenge the healthcare sector of both developed and developing countries. Owing to this problem and concerning the side effects of synthetic antimicrobial agents, the current work was done with medicinal plants. The plant materials such as *Terminalia bellerica, Withania somnifera, Madhuca longifolia and Sygyzium cumini* was selected based on its potent antimicrobial activity. The best combination of the selected plants was evaluated for their antimicrobial activity. The phytochemical analysis of the selected combination showed the presence of phytoconstituents that are responsible for their biocidal activity. The Minimum inhibitory concentration (MIC) was studied for the selected herbal combination. The results recommend the use of selected plants at their best combination as a substitute to synthetic agents that can effectively prevent the microbial infestation.

Keywords: Antimicrobial activity, Cross infection, Medicinal Plant, MIC, Phytochemical.

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INTRODUCTION

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 emergence and dissemination of multidrug resistant bacteria has consequence towards the increased cost of medicines (Harbottle et al., 2006). The risks associated with synthetic antimicrobial drugs are their inefficiency to overcome the strains of resistant bacteria towards antibiotics. The use of such drugs are now a threatening factor for the patients who may result with harmful side effects (Cosgrove and Carmeli, 2003). Predate the introduction of antibiotics there was an efficient way to treat diseases with medicinal plants (Braga et al., 2005).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.

Medicinal plant contains novel pharmaceutical compounds. One of the vital activities found in these plants are their 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, Jeyaseelan et al., 2012). Most of them offers broad spectrum of antimicrobial activity against microorganism 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 selected herbal extract in their effective combination and to analyze their phytochemical constituents.

Materials and Methods

Materials

The potent antimicrobial herbs *Terminalia bellerica, Withania somnifera, Madhuca longifolia and Sygyzium cumini* were collected in and around Coimbatore, India. All the chemicals and solvents were supplied by Himedia Chemicals Private Limited, Mumbai, India.

Herbal Extraction Method

The selected herbs were dried under shade. The dried herbs were ground to fine powder and used for extraction (Sathianarayanan et al., 2010). *Terminalia bellerica,*

Withania somnifera, Madhuca longifolia and Sygyzium cumini in the optimized combination 2:1:1:1 was used for further analysis. An ethanolic extract of the selected herbs in combination was done by maceration process at M: L ratio of 1:5 at room temperature for 48 h at 120 rpm (Perez et al., 1990). The ethanolic extracts were finally obtained.

Assessment of Antibacterial Testing

The reference bacterial strains of *S.aureus* and *E.coli* was inoculated in nutrient broth and incubated at 37°C. After incubation a sterile cotton swab was immersed into the bacterial suspension and swabbed aseptically on the sterile Muller-Hinton agar plates. Wells of 6 mm diameter were punctured on the agar medium. About 60µl of the selected herbal extracts combination was added to the wells. After which the plates were incubated at 37°C for 24 hrs. After incubation, the zone of inhibition was measured and recorded.

Assessment of Antifungal Activity

Potato dextrose agar plates were prepared and the spores of the fungi were inoculated into 50±2 ml of sterile distilled water containing few glass beads and shaken vigorously to bring the spores into suspension. The test specimens (3.8 ± 0.8 cm in diameter) were placed in contact with hardened agar medium over which 0.2±0.001 ml of the inoculums was evenly distributed by means of a sterile pipette. The plates were incubated at 27°C for 5 days. After incubation the antifungal activity was measured by the zone of mycostasis.

Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined for selected combination against test bacterial pathogens according to the micro broth dilution technique (Murray et al., 1999). The stock plant extract (0.1g/mL) were diluted to prepare the various concentration (20, 40, 60, 80, 100, 120, 140, 160, 180 µg/mL) by serial dilution.

Sterile nutrient broth about 100µl was dispensed into each well of 96 wells micro titre plate. About 100µl from each dilution was added to tubes containing 100µl broth and each well was inoculated with 5 µl (10^5 CFU/mL) of the test culture. Two control wells were maintained for each strain they are nutrient broth with extract (extract control) and nutrient broth with inoculums (organism control). Plates were then incubated at 37° C for 18-24 hours (Malabadi et al 2005). After incubation, the plate was analysed through the Micro plate reader (ELISA- Cyber lab). MIC was determined as the lowest concentration of the extracts permitting no visible growth (no turbidity) when compared with the control tubes.

Phytochemical Analysis of Poly Herbal Extract

Alkaloids (Mayer's test):

To 1 ml of the plant extract, few drops of Mayer's reagent was added along the sides of test tube. Appearance of white or pale yellow precipitate confirmed the presence of alkaloids.

Flavanoids:

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavanoids.

Saponins:

About 50 mg of extract was diluted in 10 ml of distilled water and made upto 20 ml. the suspension is shaken in a graduated cylinder for 15 min. a two cm layer of foam indicates the presence of saponins.

Phenols (Ferric chloride test):

About 50 mg of extract was diluted in 5 ml of distilled water followed by few drops of neutral 5% ferric chloride solution was added. Formation of dark green colour indicated the presence of phenols.

Steroids (Solkowshy's test):

To 2ml of chloroform extract, 1 ml of concentrated sulphuric acid was added along the sides of the test tube. The presence of steroids was confirmed by the presence of red colour in the chloroform layer.

Tannins (Gelatin test):

About 50 mg of extract was dissolved in 5 ml of distilled water and 2 ml of 1% solution of gelatin containing 10% NaCL is added to it. White precipitate indicates the presence of tannins.

Chemical Characterization of the Selected Herbal Combination

Fourier Transform Infra-Red Spectroscopy (FTIR)

The functional groups present in the poly herbal extract were determined using FTIR, a Shimadzu, IR Affinity 1, Japan. The dried powder of the poly herbal extract was ground with 2.5 mg of dry potassium bromide (KBr). The powder so obtained was filled in a 2 mm internal diameter micro-cup and loaded onto FT- IR at 26°C ± 1°C. The samples were scanned using infrared in the range of 4000–400 cm-1. Nearly, 100 scans were performed with the specified resolutions.

RESULTS AND DISCUSSIONS

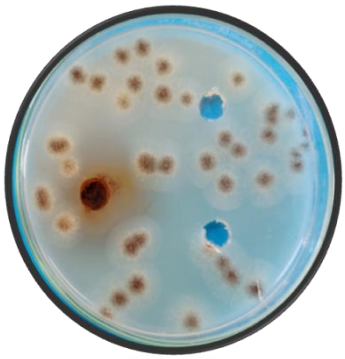
Assessment of Antibacterial Testing of Selected Herbal Ratio

The selected herbal ratio 2:1:1:1 was found to be more effective in inhibiting the clinical strains of test bacteria was represented in table 1. The ratio 2:1:1:1 exhibited 23 mm zone of inhibition against *A. baumannii* followed by *E. coli and S. aureus* (21 mm). Whereas the minimum zone of inhibition was found against *P. mirabilis* (18 mm). 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 the wound. It is evident that the selected herbal combination showed enhanced inhibitory activity against most of the wound pathogens listed by the reported author.

Table 1: Antibacterial Activity of Selected Herbal Combination

S.No	Test Organisms	Zone of Inhibition of Selected ratio 2:1:1:1 (in mm)
1	*A.baumannii*	23
2	*B.cereus*	20
3	*E.coli*	21
4	*K.pneumoniae*	19
5	*P.aeruginosa*	22
6	*P.mirabilis*	18
7	*S.aureus*	21
8	*S.epi*	19
9	*MRSA*	20

Assessment of Antifungal Activity of Selected Herbal Combination



A- Antifungal Activity of Polyherbal extract

B- Positive Control

C- Negative Control

Figure 1: Antifungal Activity of Polyherbal Extract

The above figure represented the antifungal activity of selected herbal combination against *A.niger.* It was clearly seen from the figure that the selected herbal combination exhibited e antifungal activity about 11 mm zone of inhibition against *A.niger.*

Minimum Inhibitory Concentration (MIC) of Selected Herbal Combinatorial

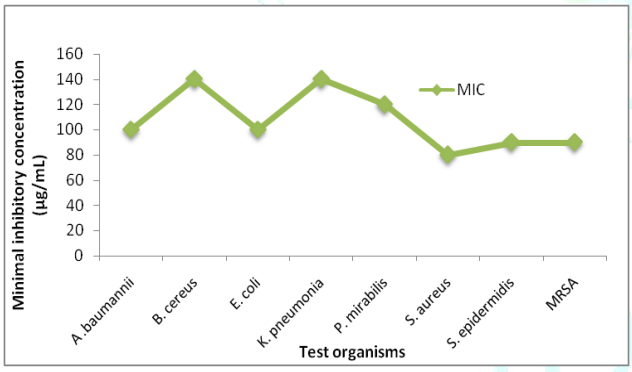


Figure 2: Minimum Inhibitory Concentration of Selected Herbal Combination

From the above figure it was clearly observed that MIC level ranges from 80µg/ml to 140µg/ml. The Minimum inhibition concentration was recorded against *P.aeruginosa and S.aureus* (80µg/ml). The Maximum inhibition concentration was observed against *B.cereus and K.pneumoniae* (140µg/ml).

Phytochemical Analysis of Selected Herbal Combination

Table 2: Phytochemical Analysis of Selected Herbal Combination

S.No	Phytochemicals	Selected Herbal Combination
1	Alkaloids	+
2	Flavonoides	+
3	Saponins	-
4	Phenols	+
5	Steroids	+
6	Tannins	+

The above table showed that the presence of alkaloids, flavanoids and phenolic compounds and absence of saponins in the selected herbal combination. The phytochemical constituents are responsible for the activities like antimicrobial, anti-inflammatory, anti-oxidant and various properties of selected herbal combination. The Phytochemical present in the plants were useful in their antibacterial activity against the pathogens.

Fourier Transform Infra-Red Spectroscopy (FTIR) of Selected Herbal Combination

The FTIR spectrum of the selected herbal combination 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. The spectrum sample obtained at 871- 763 cm^{-1} was attributed to presence of N-H wag i.e., primary and secondary amines. Thus it is reported that most of the functional groups present in the current study are the potential reason for the contribution of antimicrobial activity.

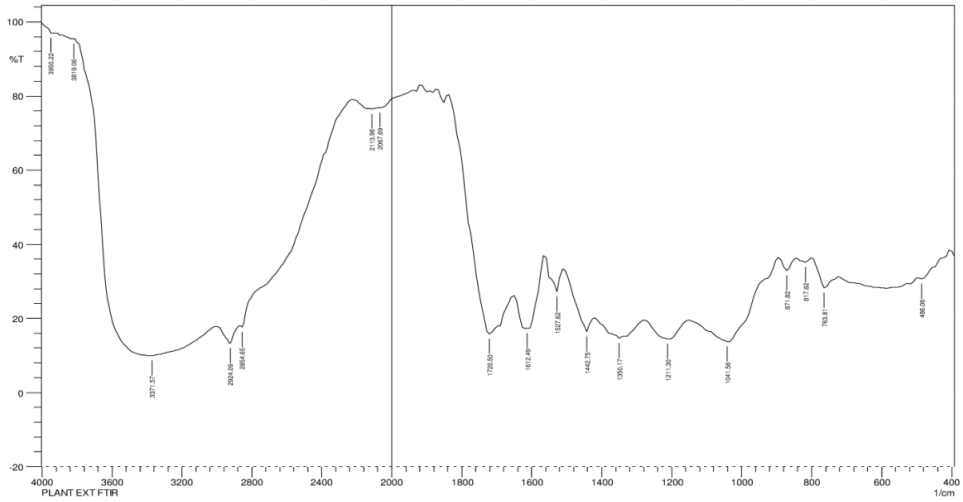


Fig 3: FT-IR analysis of Selected Herbal Combination

CONCLUSION

Availability of the selected herbal combination proves the possible implementation of the current study. Biologically active compounds found in the selected combination confirmed the presence of functional groups and their potent antimicrobial property. Thus the study initiates an alternative to expensive, synthetic and toxic antimicrobial agents.

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