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Evaluation of Phytoconstituents and Antimicrobial Activity of Combinatorial Herbal Extract

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ABSTRACT

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 results recommend the use of selected plants at their best combination as a substitute to synthetic agents that can effectively prevent the microbial infestation.

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INTRODUCTION

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 synthetic antimicrobials (Iwu *et al*., 1999). 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 is 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.

The present study was initiated to evaluate the antimicrobial property of the combinatorial 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 37ºC. 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 combinatorial herbal extracts combination was added to the wells. After which the plates were incubated at 37ºC 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 27ºC for 5 days. After incubation, the antifungal activity was measured by the zone of mycostasis.

Phytochemical Analysis of Polyherbal 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 flavonoids.

Saponins: About 50 mg of extract was diluted in 10 ml of distilled water and made up to 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.

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

Sr. 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

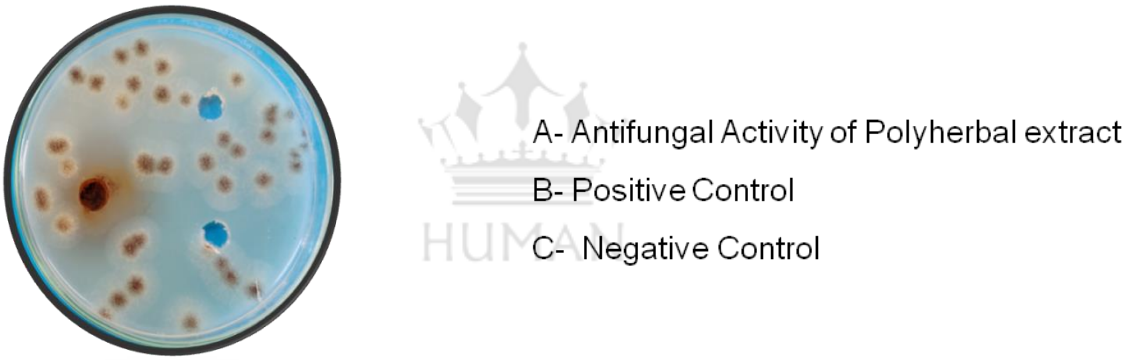

Figure 1: Antifungal Activity of Polyherbal Extract

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 antifungal activity about 11 mm zone of inhibition against *A. niger*.

Phytochemical Analysis of Selected Herbal Combination

Table 2: Phytochemical Analysis of Selected Herbal Combination

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

The above table showed the presence of alkaloids, flavonoids 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.

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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