

IMPROVED ANTIBACTERIAL EFFICACY OF A NOVEL FORMULATION - SYNERGISTIC EFFECT OF HERBS

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ABSTRACT

Herbal formulations have attained widespread acceptability as therapeutic agents being a part of traditional system of medicine. Synergistic effect of individual counterparts of formulations leads to the development of combination therapy. In the present study, we investigated the synergistic effect of two medicinal plants viz. *Murraya koenigii* and *Sesamum indicum* for their *in-vitro* antibacterial activity. *In-vitro* antibacterial activity was assessed for a range of varied concentrations of formulation against different bacterial strains and the results were compared with standard drug, ampicillin as well as with individual plants. The formulation was found to have significant efficacy in comparison to the individual plants. The minimum inhibitory concentration of formulation was 0.075mg/ml against *Klebsiella pneumoniae* and *Escherichia coli* which is very low as compared to individual plants. This formulation with enhanced potential to reduce bacterial infections could be developed as a novel therapeutic agent.

Keywords: Herbal formulation, *Murraya koenigii*, *Sesamum indicum*.

INTRODUCTION

The concept of phytotherapy efficacy is based on the synergistic effect of chemical constituents present in the medicinal plants and hence the polyherbal formulations composed of more than one herbal ingredient have specific therapeutic values (Rafeeuddin *et al.*, 2009). There are several polyherbal formulations in the Ayurvedic system of medicine which have been used to treat a wide variety of diseases. The formulations have the ability to act by various mechanisms and therefore it could be possible that different combinations of different plants would be more effective than the individual plants itself (Srivastava *et al.*, 2012).

A number of plants and their products have already been reported for their significant antimicrobial activities (Bylka *et al.*, 2004; Shimpi and Bendre, 2005; Kilani, 2006) and it has been anticipated that this antimicrobial trait of medicinal plants are mostly due to the presence of their secondary metabolites. Still, there is a need to discover new leads as antimicrobial agents with diverse chemical structures and novel mechanisms of action for new and emerging infectious diseases (Rojas *et*

al., 2003). Researchers are also turning their attention towards folklore medicines, looking for novel drugs with improved bacterial resistance against microbial infections (Bankeblia, 2004).

The plant, *Murraya koenigii* L. Spreng (Family: Rutaceae) is commonly known as 'Curry Leaf' in English and 'Meethi Neem' in Hindi. It is native to India and its leaves constitute an important part of the Indian diet especially of the southern region. It is very well known for its pharmacological activities as well and its leaves have already been scientifically evaluated for their antidiabetic (Kesari *et al.*, 2007), antilipidemic (Birari *et al.*, 2010) and antioxidant efficacies (Gupta and Prakash, 2009). Its antibacterial activity has also been reported against different microorganisms by using Minimum Inhibitory Concentration (MIC) studies (Handral *et al.*, 2012) thereby highlighting its antibacterial potential which could be further developed in combination therapy for rapid alleviation of the bacterial infections associated with it.

Another traditionally significant medicinal plant, *Sesamum indicum* L. (Family: Pedaliaceae)

commonly known as 'Sesame' in English, and 'Til' in Hindi, has been selected for formulation preparation in order to enhance its antibacterial efficacy. They are available in black, brown, red, and white varieties. Sesame has been found to possess antioxidant and health promoting activities (Sirato *et al.*, 2001). Its seeds consumption appears to increase plasma gamma-tocopherol and enhances vitamin E activity, which is believed to prevent cancer, and heart disease (Cooney *et al.*, 2001). Sesame fermented meals have been reported to exhibit antioxidant and antiinflammatory activities (Ching *et al.*, 2010). Seeds have also been reported to show antibacterial activity as well (Momoh *et al.*, 2013).

Since, each of the selected plants has significant antibacterial activity. Therefore, their formulation could definitely be developed as an agent with improved efficacy to control bacterial infections.

MATERIALS AND METHODS

Plant collection and identification

Fresh leaves of *M. koenigii* and black seeds of *S. indicum* were purchased from the local market of Allahabad, U. P., India, and got identified by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, Allahabad, U. P., India. A voucher specimen has been submitted to the University herbarium. The leaves and seeds were first washed well, shade dried, and powdered. They were then extracted in the ratio 1:1 (50/50 w/w) with hot distilled water using soxhlet apparatus till the colorless solvent was obtained. Extract obtained was allowed to dry till constant weight was obtained (10% w/w).

Bacterial strains, stocks and growth *in vitro*

Bacterial strains of Gram-positive viz. *Staphylococcus aureus*, *Enterococcus faecalis* and Gram-negative viz. *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* were clinical isolates obtained from the Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), New Delhi, India. The microbiologist of the department confirmed the identity based on microscopic examination, Gram's character, and biochemical test profile. Bacterial stocks were maintained, and stored as 1 ml aliquots at -80°C in Luria Bertani (LB) broth for all the five bacterial strains. Bacterial stocks were revived from -80°C and grown in LB broth for all five bacterial strains. Cultures were grown overnight at 37°C ± 0.5°C, pH 7.4 in a shaker incubator (190-220 rpm) for each one of them. Their sensitivity to the reference drug, Ampicillin (Sigma-Aldrich, New Delhi, India) was also checked (Shukla *et al.*, 2011).

Determination of *in-vitro* antimicrobial activity

Minimum Inhibitory Concentration (MIC) of the freshly prepared inocula of each strain was determined by the micro-dilution method using serially diluted (2-fold) plant extracts according to the NCCLS (National Committee for Clinical Laboratory Standards) (NCCLS, 2000). A final concentration from 0.075 to 2.4 mg/ml was used for the formulation. The effects were also compared with that of a standard antibiotic, ampicillin at the same concentration range. Finally, the test tubes closed with cotton plugs were incubated at 37°C for 24 h in a shaker incubator. Control was also run parallelly without formulation. All samples were tested in triplicates.

RESULTS

Table 1 represents the MIC values of the herbal formulation, *M. koenigii* leaves, *S. indicum* seeds and standard drug, ampicillin against four different bacterial strains. The results reveal that the herbal formulation exhibited considerably better antimicrobial activity as compared to the *M. koenigii* leaves, *S. indicum* seeds against all four bacterial strains. In the present study, the growth of *K. pneumoniae* and *S. aureus* strains was remarkably inhibited by the herbal formulation even at much lower concentration viz. 0.075 and 0.150 mg/ml, respectively. However, against the bacterial strains, *E. coli* and *P. aeruginosa* formulation showed MIC values 0.075 and 0.150 mg/ml, respectively with maximum inhibition.

DISCUSSION

It is evident from the MIC observation that the formulation of *M. koenigii* and *S. indicum* has much improved antibacterial activity against all four bacterial strains, *K. pneumoniae* (0.075 mg/ml), *S. aureus* (0.150 mg/ml), *P. aeruginosa* (0.150 mg/ml), *E. coli* (0.075 mg/ml) in comparison of the respected plants.

Interestingly, the MIC values of the aqueous extract of *M. koenigii* leaves against the bacterial strains, *S. aureus*, *P. aeruginosa*, *E. coli* and *K. pneumoniae* have already been reported to be enormously higher viz. 50, 25, 12.5 and 12.5 mg/ml respectively than the herbal formulation suggesting thereby that the enhanced antibacterial activity of *M. koenigii* leaves in the formulation are due to the synergistic effect of *S. indicum* seeds. On the other hand, aqueous extract of exclusively *S. indicum* seeds which has already been explored by our research group for their antibacterial activity, has revealed relatively higher MIC values than the

formulation itself against all four bacterial strains. The higher MIC values associated with the *S. indicum* aqueous extract were found to be 5.0 mg/ml for *E. coli*, *K. pneumoniae*, *S. aureus* and 2.5 mg/ml for *P. aeruginosa*. The present investigation therefore not only justifies the traditional use of these two medicinal plants viz. *M. koenigii* and *S. indicum* as antibacterial agents but also validates their synergistic effect with improved antibacterial potential in the formulation.

Numerous investigations have proved that medicinal plants contain diverse classes of bioactive compounds such as tannins, alkaloids, and flavonoids which exhibit various pharmacological properties including antibacterial activity (Emam, 2010). The known success of some traditional therapies against bacterial infections using plants has guided the search for new chemotherapeutic alternatives against various infections caused by drug-resistant bacteria.

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects,

whereas plants still hold their own unique place, by the way of having no side effects. Continued further exploration of plant-derived antimicrobials is needed today. Therefore, a systematic approach should be made to find out the efficacy of plants against bacterial infections in order to develop them as herbal formulation with improved antibacterial efficacy with minimal or no side effects.

CONCLUSIONS

The present study supports the traditional uses of the leaves of *M. koenigii* and seeds of *S. indicum* for the treatment of bacterial infections. It also proves that there is a sufficient possibility to develop this herbal formulation, with improved antibacterial efficacy, as a novel, potent, safe and cost effective therapeutic agent. Moreover, clinical trials are still warranted.

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Table 1: MIC values of formulation, *M. koenigii*, *S. indicum* and standard drug, ampicillin

Microorganism	MIC (mg/ml)			
	Formulation	<i>M. koenigii</i>	<i>S. indicum</i>	Standard drug
<i>K. pneumoniae</i> (Gram-negative)	0.075	12.5	5.0	0.150
<i>S. aureus</i> (Gram-positive)	0.150	50.0	5.0	0.300
<i>P. aeruginosa</i> (Gram-negative)	0.150	25.0	2.5	0.150
<i>E. coli</i> (Gram-negative)	0.075	12.5	5.0	0.075

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