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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *MURRAYA KOENIGII* (L.) AGAINST *ESCHERICHIA COLI, KLEBSIELLA PNEUMONIAE* AND *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

In developing countries like India, low income people such as farmers, isolated villagers and native communities are using folk medicines for the treatment of common infections. Herbs and spices are known to produce certain bioactive compounds which react with other organisms in the environment. Phytochemicals are known to be biologically active and they are formed to contain various biomolecules effective against many diseases and disorders. In the present work, dried leaf powered of *Murraya koenigii* was subjected to phytochemical screening in aqueous extract and antimicrobial activity using four different organic solvents like aqueous, acetone, isopropanol and methanol against three pathogenic bacteria such as *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* by disc diffusion method. The phytochemical compounds were qualitatively analysed and the antimicrobial activity of *Escherichia coli* and *Staphylococcus aureus* showed maximum zone of inhibition in acetone extract, whereas *Klebsiella pneumonia* showed maximum zone of inhibition in aqueous extract.

Keywords: *M. koenigii*, phytochemicals, disc diffusion method, antibacterial activity.

INTRODUCTION

Medicinal plants are naturally gifted with invaluable bioactive compounds which form the backbone of traditional medicine. There has been an increasing interest worldwide on therapeutic values of natural products from plants due to disenchantment with modern synthetic drug (Ahmad and Beg, 2001).

Herbs and spices are usually used to flavour dishes among the tremendous sources of phenolic compounds which are the good antioxidant activity (Zheng and Wang, 2001). Numerous aromatic spicy and medicinal plants have been examined for their antioxidant potential (Chan, *et al.,* 2007). Many plant components are now synthesized and analyzed in large laboratories. For example, vincristine (antitumor drug) and ephedrine (bronchodilator) used to decrease respiratory congestion were well originally discovered through research on medicinal plants (Hill, 1952).

In general low income people such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common infections (Fabricant and Farnsworth, 2001). They are also made into a poultice and applied directly on the infected wounds or burns. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases (Gonzalez, 1980). The beneficial aspects of plant derived drugs as good source of antibiotics, antioxidants and anti-inflammatory agents (Mathur, *et al.*, 2011).

M. koenigii belongs to the family Rutaceae and commonly known as curry leaf and native of India, Srilanka and South Asian countries. It is an aromatic, more or less deciduous shrub or small tree commonly in forest often gregarious

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under growth. Fresh juice of the root is taken to relive pain associated with kidney. Leaves are used internally for dysentery and diarrhoea cases. Roots and barks are stimulant and applied externally for skin eruptions and poisonous bites (Muthumani, *et al.,* 2009).

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Therefore, there has been a tremendous upsurge in the demand for the drug from natural sources.

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential indeed, thus determination of antimicrobial effectiveness of herbal plant against specific pathogen is essential to proper therapy. Testing can show which agents are most effective against the pathogen and give an estimate of the proper therapeutically dose. Hence, the present study has been chosen to determine the antimicrobial activity of *Murraya koenigii* against the microorganisms such as *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* which may pave the way to produce herbal medicine for pharmaceutical industries.

MATERIAL AND METHODS

The fresh curry leaves of *M. koenigii* plants were collected in and around Thanjavur, Tamil Nadu, India.

Preparation of extract

The plant material was shade dried for three days. After drying, plant material was powdered with the help of mixer grinder. Twenty gram of powdered plant material was mixed with 100 ml of various solvents like aqueous, acetone, isopropanol and methanol. The extracts prepared in succession from powdered leaf material by Soxhlet method (Basker and Thomsberry, 1983). The collected extracts were stored in a vial for further studies.

Phytochemical screening

The aqueous extracts were subjected to phytochemical screening for secondary plant metabolites according to the methods described by Edeoga, *et al.*,(2005), Banso and Adeyemo, (2006), Ayoola, *et al.,* (2008) and Anyasor, *et al.*, (2010).

Test microorganisms

Disease causing infectious bacteria in animals and human such as *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* were used in the present study. They were collected from the Microbial Type Culture Collection (MTCC) at Chandigarh, India.

Antibacterial activity of plant extract

Antibacterial assay was carried out by agar diffusion method. The sterile Muller-Hinton agar plates were prepared. The test organisms were spread over the Muller-Hinton agar plates by using separate sterile cotton swabs. The prepared sterile disc was placed on the surface of the medium at equal distances and then the plates were incubated at 37˚C for 24 hours to determine the antibacterial activity of the respective solvent extracts. Antibiotic (Ciprofloxacin) discs (15mg/disc) were used as positive control. Each extract was treated in triplicate for calculation of mean value.

Statistical analysis

Mean and standard deviation were calculated to facilitate the comparison of the data. The obtained data were computed by ANOVA test followed by the pos hoc Duncan's test. All the data analyses were significant at $P< 0.05$ (Zar, 1984).

RESULTS

The efficacy of *M. koenigii* was tested against three different pathogenic bacteria and the data were recorded and statistics were calculated. In phytochemical screening, the compounds like saponins, tannins, phenols, alkaloids, terpenoids, volatile oils and hydrolysable tannins are present whereas steroids, flavonoids, amino acids, carbohydrate, phylobatannis, glycosides, cardiac glycosides and vitamin-C were found to be absent (Table 1).

The antimicrobial activity of *M. koenigii* prepared in acetone extract shows significant zone of inhibition in *S. aureus* when compared to all other extracts (Figure 1). Methanol extract showed high zone of inhibition in *E coli* and isopropanol in *S. aureus* and aqueous in *K. pneumoniae* (Table 2).

Table 1. Preliminary phytochemical studies on the extract of *M. koenigii*

S. No.	Phytochemical tests	*M.koenigii*
1	Saponins	+
2	Tannins	+
3	Phenols	+
4	Alkaloids	+
5	Steroids	-
6	Terpenoids	+
7	Flavonoids	-
8	Amino acids	-
9	Carbohydrate	-
10	Phylobatannis	-
11	Volatile Oils	+
12	Hydrolysable Tannis	+
13	Glycosides	-
14	Cardiac Glycosides	-
15	Vitamin-C	-

\+ indicates the presence of phytochemicals

\- indicates the absence of phytochemicals

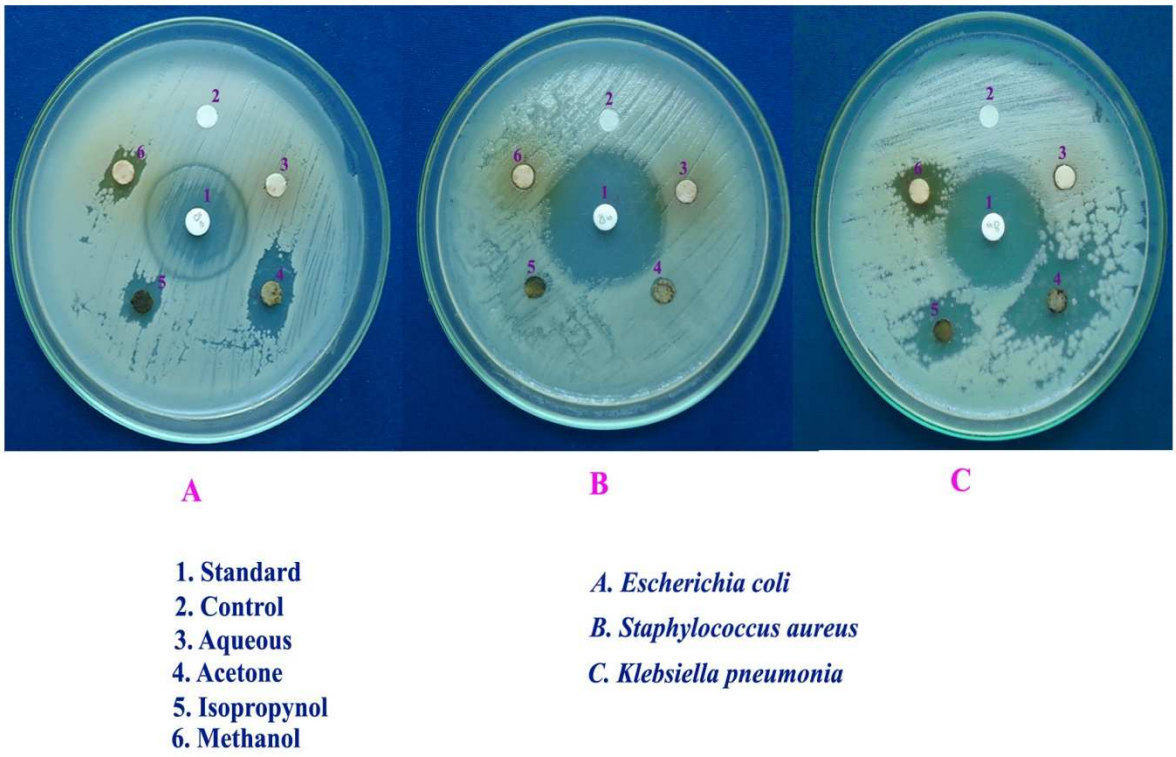

Figure 1. Antimicrobial activity of *M.koenigii* against pathogenic bacteria

Table 2. Antimicrobial activity of *M. koenigii* against pathogenic bacteria

S. No.	Name of the Bacteria	Zone of inhibition mm in diameter (M±SD) (n=6)					
		Stand S*	Control	Aqueous	Acetone	Isopropanol	Methanol
1.	*Escherichia coli*	27	-	-	17.4 ±0.15^{c}	13.1±0.24^{b}	14.3±0.17 ^{b}
2	*Klebsiella pneumoniae*	35	-	6.9±0.24 ^{b}	-	-	9.94 ±0.02^{c}
3	*Staphylococcus aureus*	29	-	7.0±0.16^{a}	20.13±0.25^{d}	15.13±0.14^{c}	13.9±0.15 ^{b}

S* - Ciprofloxacin (disc 15mg) Ref. Hi Media Standard value.
Values are expressed in Mean ± SE.
Dissimilar alphabets in horizontal rows are significantly different at P <0.05% level.

DISCUSSION

Preliminary phytochemical investigation of aqueous extract of *M. koenigii* leaves revealed the presence of saponins, tannins, phenols, alkaloids, terpenoids, volatile oils and hydrolysable tannins. This result is supported by Sharma *et al.,* (2011) that the presence of phenols, carbazole, alkaloids, flavonoids and tannins in *M.koenigii* aqueous leaf extract. Nurain, *et al.,* (2012) reported that the phytochemical screening of the ethanolic herb extract shows alkaloids, flavonoids, saponins, tannins, terpenoids and steroids in *M. koenigii*. These secondary metabolites exert antioxidant and antimicrobial properties through different mechanisms. Most of the secondary metabolites were identified in the polar extracts (Gonzalez-Guevara, *et al.,* 2004).

Alkaloids which are one of the largest groups of phytochemicals which has human based on toxicity against cells of foreign organisms and it may responsible for the antimicrobial activity of herbal extract. Saponin, which is one of the active constituents involved in plant disease resistance because of its antimicrobial activity (Barile, *et al.,* 2007). Traditionally, saponins are subdivided into triterpenoid and steroid glycoside. Tannins are phenolic compound which act as primary antioxidants or free radical scavengers (Ayoola, *et al.,* 2008).

The antimicrobial efficacy of *M. koenigii* was studied in four different extracts. Among the four *S. aureus* show maximum zone of inhibition inacetone extract followed by isopropanol, methanol and aqueous. This result was correlated with Malwal and Sarin (2011) that the methanolic extract pronounced antimicrobial activity than any other. Among the tested bacterial strain, the most susceptible bacterium is *S. aureus,* which plays a significant role in skin diseases (Basri and Fan, 2005). It indicates that root of *M. koenigii* may possess compounds with antimicrobial properties which are effective against infectious diseases. The aqueous extract shows no significant zone of inhibition in *E. coli* and *S. aureus* thus it was also supported by Malwal and Sarin (2011) that aqueous extract showed no inhibition effect against *Bacillus subtilis, E. coli, S. aureus* and *Salmonella typhii*

Klebsiella pneumonia showed maximum zone of inhibition in aqueous extract , this results was supported by Nair and Chanda, (2004) that the mechanism of the antimicrobial effects involved in the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the cells (Walsh *et al.,* 2003).

CONCLUSION

The present study demonstrates that *M. koenigii* showed that the presence of bioactive compounds like saponins, tannins, phenols, alkaloids, terpenoids, volatile oils and hydrolysable tannins. This plant exhibited good antibacterial activity against pathogenic bacteria

which indicating the potential of this plant as a source of functional ingredient that can be used in pharmaceutical industries so as to develop it as a potent antimicrobial drug and further studies on experimental animals are also needed.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest associated with this article.

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