ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *MELIA AZADIRACHTA* AND *MURRAYA KOENIGII*

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ABSTRACT

The *present* study was carried out to evaluate the antimicrobial and antioxidant effects of *Melia azadirachta* and *Murraya koenigii extracts.* Antimicrobial activities of the extracts were evaluated against various Bacterial and fungal strains using Agar well diffusion technique. For antioxidant effect, the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method described by Brand-Williams *et al.* (1995) with certain modifications. Different concentrations of extracts were prepared (25µg/ml, 50µg/ml, 75µg/ml, 100 µg/ml and 120µg/ml)for antioxidant activity. The antimicrobial activity of *M.azadirachta and M.koenigii were* determined and it was found that both the extracts at a concentration of 500 µg/ml produced significant zones of growth inhibition. The extracts were *Melia* and *Murraya* was more susceptible to *Enterococcus faecalis* followed by *Staphylococcus aureus, Klebsiella oxytoca,*and *Candida albicans*. *M.azadirachta* exhibited the highest radical scavenging activity with 60.23±0.03 % inhibition at 125 µg/ml and *M.koenigii* showed highest antioxidant activity (58.22±0.03) at the same concentration. Chloroform soluble partitionate of *Melia* showed the highest scavenging activity.

Keywords: *Melia azadirachta*, *Murraya koenigii*, Antimicrobial activity, Antioxidant activity

INTRODUCTION

Traditional medicine (TM) is a comprehensive term used to refer both to TM systems such as traditional Chinese medicine, Indian ayurveda and Arabic unani medicine, and to various forms of indigenous medicine. Natural products and their derivatives have been a successful source of bioactive molecules in medicines much before the advancement of other modern therapeutics in the post-genomic era. One of most studied natural products has been flavonoids which are found predominantly in several parts of daily food consumption. Herbal medicines have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects and believed to have better compatibility with the human body. Many plants are used as folk medicines to infectious diseases. Due to the indiscriminate use of antibacterial drugs, the microorganisms have developed resistance to many commercial antibiotics. Therefore, investigation of the chemical compounds within medicinal plants has become desirable (Ahmad *et al*., 1998). Some of the herbal plants traditionally used in formulations as antimicrobial agents.

Melia azidirachta Linn, commonly known as mahanimbin belongs to family Meliaceae. It is large evergreen tree found throughout India and very similar to neem. Traditionally it is used as anthelmintic, antilithic diuretic, antioxidant ,astringent and stomachic. Varios scientific studies reported the analgesic, anticancer, antiviral, antimalarial, antibacterial, antifeedent and antifertility activity of this plant (Rana 2008).

Murraya koenigii Linn. (Rutaceae), is commonly known as Curry patta and is widely used condiment and spice in India. Vaibhav *et al*., (2011) reported anti-inflammatory activity of *Murraya koenigii* leaves. The leaves and roots are bitter, acrid, cooling, anthelmintic, analgesic, it cures piles,allays heat of the body, reduces inflammation and itching. It is also useful in blood disorders. An infusion of the toasted leaves in used to stop vomiting. Crushed leaves are applied externally cures skin eruption and to relieves burn. The extract shows significant effects in anti-inflammatory activity. Previous studies had rationalized the ethano medicinal use of this plant for cut, injury and alignment of body temperature by tribal people.

Moreover, a proper scientific evaluation by screening of plants through pharmacological testing followed by chemical investigations is necessary and to make these herbal remedies more viable and also finding a single or combinational drugs can effectively control clinical disorder such as diabetes without side effects requires a real breakthrough research. In this context, the present study was undertaken to evaluate the antioxidant and antimicrobial potential of *Melia azadirachta* and *Murraya koenigii* which forms an integral part in Indian diet and medicine

MATERIALS AND METHODS

Plant materials

The leaves of *M. azadirachta* and *M. koenigii* were collected from Kristu Jayanti College campus, Kothanur (Bangalore, India) at an altitude of 949 meters (3113 ft.).The collected plant leaves were shade dried, powdered and stored in air tight containers. Plant samples were authenticated by Dr.M.D.Rajanna, Curator in-charge, Botanical garden, University of Agricultural Sciences, GKVK, Bangalore (GKVK/No.3/proj/B-Garden)

Crude extraction

Fresh plant material was collected; shade dried and powdered in a mixer. 100 g of plant material was put into 600 ml of chloroform, covered and kept standing for 5 hours. The solvent was then removed after squeezing the sample and filtered through Whatman filter paper No 1. The solvent was evaporated at low pressure by using a Buchi Rotavapor R-200 at 40^{o}C and stored in refrigerator for further studies (Bakus *et al*., 1981).

Phytochemical screening

The crude extracts were subjected to screening for phytochemical constituents using standard procedures described by Harbone (1973) and Sofowora (1993).

Alkaloids

About 0.2 g of the extracts was warned with 2% H_2S0_4 for two minutes. It was filtered and few drops of Dragencloffs reagent were added. Orange red precipitate indicates the presence of alkaloids.`

Flavonoids

Extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless, indicates the presence of flavonoids

Steroids

2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids

Terpenoids

0.2 g of the extract of the whole plant sample was mixed with 2ml of chloroform ($CHCl_4$) and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids

Tannins

Small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

Anthraquinones

About 0.5 g of the extracts was boiled with 10% Hcl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl3 was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heat. Formation of rose-pink colour indicates the presence of authraquinones

Glycosides

The extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate in indicates the presence of glycosides.

Reducing sugars

The extracts was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for minutes. An orange red precipitate indicates the presence of reducing sugars

Saponins

About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing shows the presence of saponins

The samples were then observed for the presence of turbidity or precipitation. A (+) score was recorded if the reagent produced only a slight opaqueness; a (2+) score was recorded if a definite turbidity, but no flocculation was observed; a (3+) score was recorded if a definite heavy precipitate or flocculation was produced and a (4+) score was recorded if a definite very heavy precipitate was produced.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of plant extracts were determined by using Folin-Ciocalteu method described by Kim *et al.* (2007). Reading samples on a UV-Vis Spectrophotometer at 650 nm. Results were expressed as catechol equivalents (μg/mg)

Evaluation of antioxidant activity

The antioxidant activity of the *M. azadirachta* and *M. koenigii* on the basis of the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method described by Brand-Williams *et al.* (1995) with certain modifications. Different concentrations of extracts were prepared (25µg/ml, 50µg/ml, 75µg/ml, 100 µg/ml and 120µg/ml). 5 ml of each concentration was mixed with 0.5ml of 1mM DPPH solution in chloroform. Experiment was done in triplicate. The test tubes were incubated for 30 min. at room temperature and the absorbance measured at 517 nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Vitamin C was used as a standard and the same concentrations were prepared as the test solutions. The difference in absorbance between the test and the control (DPPH) was calculated and Scavenging effect was expressed as %. The capability to scavenge the DPPH radical was calculated by using the following equation. Scavenging effect (%) = (1-As/Ac) ×100

As is the absorbance of the sample at t =0 min.

Ac is the absorbance of the control at t=30 min.

Determination of antimicrobial activity

Four bacterial [*Enterococcus faecalis, Klebsiella oxytoca, E.coli, Staphylococcus aureus*] and three fungal strains [*Candida albicans, Candida krusei, Candida tropicalis*] were used for assessing the antimicrobial activity of the test extracts. The microorganisms were obtained from Bioline laboratory, Coimbatore. Antimicrobial activity of extracts was determined by Agar well Diffusion method. The Muller Hinton agar was used as medium and well size was adjusted to 6 mm. The extract (10 mg/ml) was solublized with DMSO and about 50 µl of each extract was loaded in the respective wells. Later the plates were kept at room temperature for 30 minutes (pre diffusion time). The petridishes inoculated with the bacterial and fungal strains were incubated at 37±2ºCfor 24 hrs and 25±2ºC for 48 hrs respectively. The assessment of anti microbial activity was based on the measurement of diameter of inhibition zone formed. The experiment was done in triplicate (Sherad *et al*., 2008; Kumar raja *et al*., 2008).

RESULTS AND DISCUSSION

Percent yield of the plant extracts

Leaves of *M.azadirachta* and *M.koenigii* were extracted with chloroform and percentage yield was found to be 0.96 and 1.19 respectively (Table 1).

Table 1. Yield percentage of plant extracts

S No	Name of the solvent	Name of the plant material and percentage yield	
		M. azadirachta	***M. koeingii***
1	Chloroform	0.96	1.19

Phytochemical screening

The preliminary phytochemical studies were performed to screen the presence different phytoconstituents in both the chloroform extracts. The results revealed that the presence of nine different phytochemicals that include alkaloids, tannins, glycosides and saponins in chloroform extract of *M.azadirachta*. The results of phytochemical screening of *M.koenigii* leaf extract are shown in Table 2.

Table 2. Qualitative analysis of phytochemical constituents of *M. azadirachta and M.koenigii* extracts

S.No	Constituents	*M. azadirachta*	*M. koenigii*
1	Reducing sugar	+	-
2	Anthraquinone	+	+
3	Flavanoids	+	+
4	Saponins	+	-
5	Tannins	+	-
6	Alkaloids	+	+
7	Phenols	+	+
8	Glycosides	+	+
9	Terpenes	+	+

+ = Presence, - = Absence of phytochemical constituents

Total phenol content

The total phenolic content (TPC) varied significantly between the extracts of *M.azadirachta and M.koenigii.* The TPC was found to be higher in *M.koenigii* extract (300 catechol eqv.) than *M.azadirachta* (245 catechol eqv). The results of total phenolic contents were reported as catechol equivalents (μg/mg) in Table 3.

Table 3. TPC in *M.azadirachta* and *M. koenigii* leaves

Solvent	Phenol Content (μg/mg)	
	M. azadirachta	*M. koenigii*
Chloroform	245	300

TPC was expressed as Catechol equivalents (μg/mg)

Antioxidant activity of *M. azadirachta* and *M. koenigii*

It shows the results of the free radical (DPPH) scavenging activity of the extracts was increased as the concentration increase. *M.azadirachta* exhibited the highest radical scavenging activity with 60.23±0.03 % inhibition at 125 μg/ml and *M.koenigii* showed highest antioxidant activity (58.22±0.03) at the same concentration. Overall *M.azadirachta* extract showed the highest scavenging activity (Table 4)

Table 4. Antioxidant activities of *M.azadirachta* and *M.koenigii*

Conc. of extracts in μg/ml	Antioxidant activity (%)	
	M. azadirachta	*M. koenigii*
25	51.22±0.03 5	53.22±0.32
50	54.42±0.02	55.43±0.24
75	58.23±0.03	56.41±0.42
100	59.11±0.02	57.33±0.53
125	60.23±0.03	58.22±0.03

Each value is expressed as the mean ± SD (n = 3)

The antioxidant activity of *M.azadirachta and M.koenigii* extracts may be due to presence of significant amounts of the polyphenol content . This finding is similar to that of previous reports by Katsube *et al*. (2004).

Antimicrobial activity of *M.azadirachta* and *M.koenigii*

The antimicrobial activity of *M.azadirachta and M.koenigii were* determined in this study and it was found that both the extracts at a concentration of 500 μg/ml produced significant zones of growth inhibition

(Table 5 and Figure 3.1.a.b.c.d). Among tested microbial strains *Enterococcus faecalis* was more susceptible to the extracts followed by *Staphylococcus aureus*, *Klebsiella oxytoca*,and *Candida albicans*.

Table 5. Antimicrobial activity of *M.azadirachta* and *M.koenigii* extracts

S.No	Microbial strains	*M.azadirachta* (500µg/ml)	*M.Koenigii* (500µg/ml)
		Zone of Inhibition(mm)	
1	*Enterococcus faecalis*	**11.0**	**11.6**
2	*Klebsiella oxytoca*	**7.6**	**9.6**
3	*E.coli*	**6.0**	**6.0**
4	*Staphylococcus aureus*	**10.4**	**10**
5	*Candida albicans*	**6.0**	**9.0**
6	*Candida krusei*	**6.0**	**6.0**
7	*Candida tropicalis*	**6.0**	**6.0**

A number of antibacterial and anti-fungal drugs available in the market produce many side effects; hence to improve the status of therapy, various ailments of plant extracts like *M. koenigii and M.azadirachta* will be much useful. From the results obtained in this study, it is clear that the chloroform extracts, may be used as source to develop new drugs for treating microbial infections.

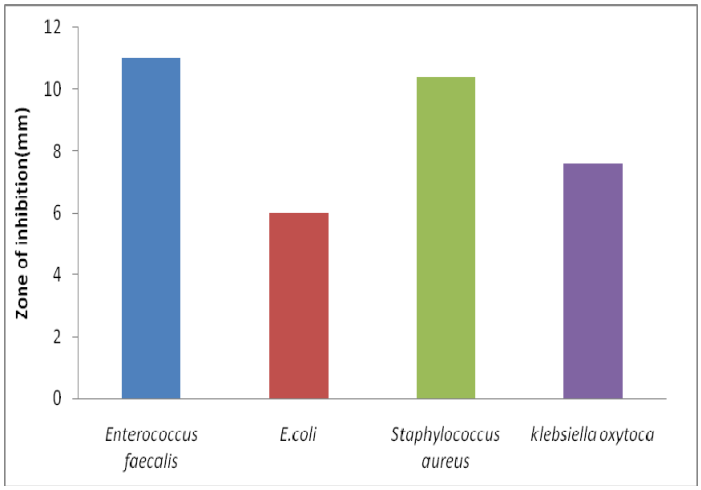

Figure.3.1.a.Effect of *M.azadirachta* on growth of bacterial strains

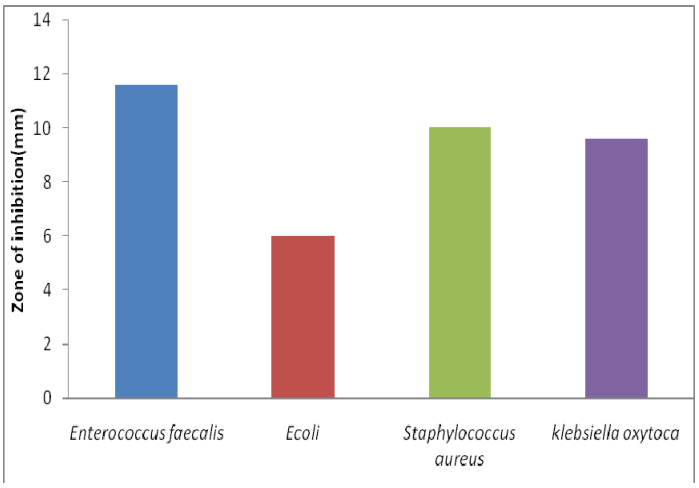

Figure.3.1.b. Effect of *M.koenigii* on growth of bacterial strains

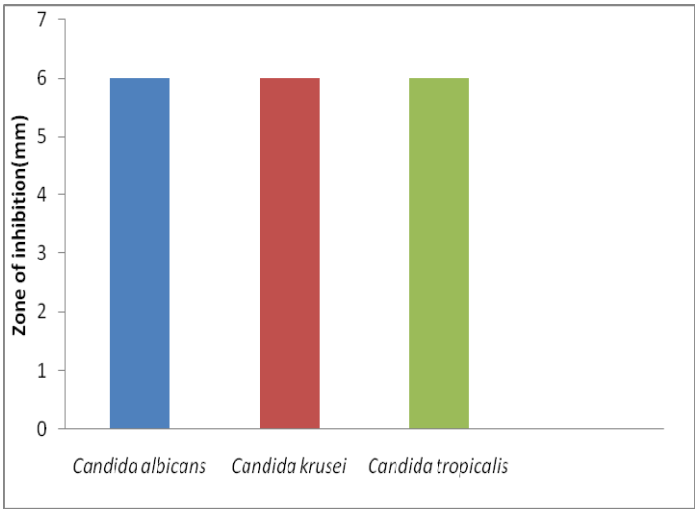

Figure.3.2.c.Effect of *M.azadirachta* on growth of fungal strains

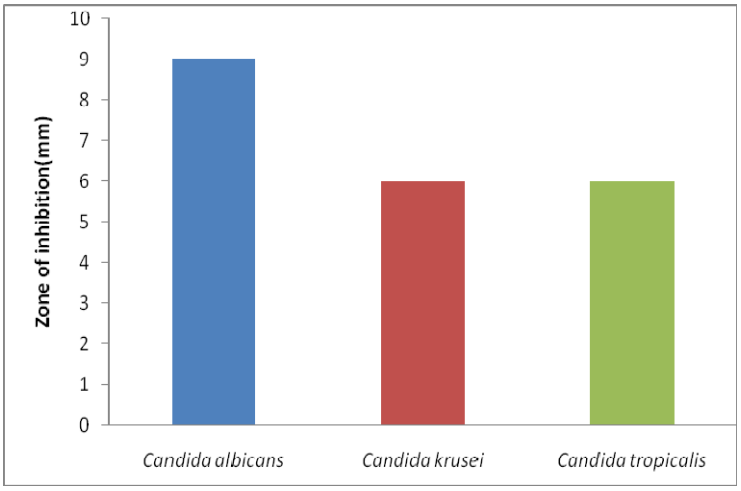

Figure.3.1.d. Effect of *M.koenigii* on growth of fungal strains

CONCLUSION

Qualitative phytochemical analysis of *Melia azadirachta* and *Murraya koenigii* extracts revealed the presence of nine different phytochemicals that include alkaloids, tannins, glycosides and saponins in chloroform extract of the plants. The extracts were further analysed for total phenolic content (TPC), the results varied significantly between the extracts and the plants also posses significant *in vitro* antioxidant activity. *M. azadirachta* and *M. koenigii* extracts also showed considerable antimicrobial activity against *Enterococcus faecalis, Klebsiella oxytoca, E.coli, Staphylococcus aureus, Candida albicans, Candida krusei, Candida tropicalis.* The results of these investigations indicated that the leaf extracts of *Melia azadirachta* and *Murraya koenigii* possess potent Antioxidant and Antimicrobial activity may be used as source to develop new drugs for treating bacterial and fungal infections.

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