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Evaluation of Antibacterial and Antioxidant activities of Essential oil from *Michelia champaka*

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ABSTRACT

Antibiotic resistance has been called one of the world's most pressing public health problems. Irrational use of antibiotics promotes development of resistance against bacteria. The antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world as they found to have lesser side effects and devoid of drug resistance. In an effort to expand the spectrum of antibacterial agents from natural sources the present study aimed to evaluate antibacterial and antioxidant activity of essential oil from the flowers of *Michelia champaka* (*M. champaka*). Essential oil was extracted from flowers of *M. champaka* by hydro distillation method using Clevenger's apparatus and screened for its antibacterial activity against gram positive *(Micrococcus luteus, Streptococcus mutans*) and gram negative (*Escherichia coli, Salmonella typhi)* strains. The minimum Inhibitory Concentration (MIC) of oil was determined using an agar diffusion method. Nevertheless the peroxide antioxidant assay was also performed. It was observed that essential oil has shown remarkable antibacterial activity against *Streptococcus mutans* (the causative agent for dental caries) with a zone of inhibition of 30 mm. A zone of inhibition was compared with standard antibiotic gentamicin. The MIC was reported as 25 mg/ml. The % inhibition of peroxide free radical was found to be 63.7% at 200mg/ml concentration. The antibacterial and antioxidant activity of *M. champaka* essential oil (MCEO) was due to various phytoconstituents. Hence MCEO can be used as an antibacterial source and phytoconstituents responsible for this activity can be explored and utilized in discovery of leads in the area of pharmacy research.

INTRODUCTION

Microbial diseases are a major cause of death globally and new emerging infections are rapidly increasing in incidence geographically day by day rather than cancer. Antibiotics have been one of the pillars allowing us to treat microbial diseases (Westh *et al.,* 2004; IOM, 1992). However, drug resistance has become a major problem in the health care system for the treatment of microbial diseases. Fortunately phytomedicines extracted from plants have been shown antimicrobial effects for centuries. Nowadays this is the promising field of medicine with fewer side effects and no drug resistance (Maurice *et al.*, 1999; Idu *et al.*, 2007). Data on antimicrobial properties of various medicinal plants were well explored (Avato *et al.*, 2006).

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Simultaneously now many diseases are linked to oxidative stress due to free radicals (Sharma *et al.*, 1998). In the past few years, there has been growing interest in the role of free radicals in several diseases like cancer, chronic pain, cardiovascular diseases, gouty arthritis and liver injury (Liao *et al.*, 2000). Due to adverse side effects of synthetic antioxidants the search for effective and natural antioxidants has become essential. Essential Oils [EOs] produced by plants have been used therapeutically from long time, but there is little published research on many of them. So there has been an increased interest in searching for antimicrobial property of aromatic plant essential oils. EOs can be obtained from various parts of plant materials by expression and distillation. Flower essential oils are particularly used in aromatherapy and fragrance industries (Van de Braak *et al.*, 1999). But traditionally they have been used for respiratory tract infections and nowadays using as ethical medicine for cold (Federspil *et al.*, 1997; Shigeharu *et al.*, 2001).

Recent studies explored antimicrobial and antioxidant properties of essential oils (Burt *et al.,* 2004; Kordali *et al.,* 2005). The essential oils have been reported to possess the antibacterial activity by targeting extracellular membranes, cytoplasmic proteins and cell walls of bacteria (Tiwari *et al.,* 2009). Most EOs have a more powerful effect on gram positive bacteria than a gram negative species due to differences in cell membrane composition (Gutierrez *et al.,* 2008). *Michelia champaka* belongs to the family Magnoliaceae is locally known as Swarna champaka, Sampangi is a tree with golden yellow fragrant flowers and aggregate fruits. Champa oil or champaka oil extracted from the flowers of *M. champaka* is widely used in preparation of attars and perfumed hair oils. However the literature survey reveals the antimicrobial, anti-inflammatory, analgesic, cytotoxic, antioxidant, antidiabetic properties of various parts of *M. champaka* (Vimala *et al.,* 1997; Khan *et al.,* 2002; Takahashi *et al.,* 2004; Jarald *et al.,* 2008; Kumar *et al.,* 2011). But antimicrobial and antioxidant activity of flower EO have not been fully explored in India. This is the first report of comprising of antibacterial and antioxidant activities of *M. champaka* essential oil. The aim of present study is to find out the efficacy of *M. champaka* essential oil as an antibacterial agent and also to evaluate its antioxidant activity (peroxide free radical scavenging activity).

MATERIALS AND METHODS

All solvents and chemicals that were used in the present study were of AR grade and purchased from Finar chemicals limited (Hyderabad, India). The nutrient agar was obtained from Hi-media (Hyderabad, India).

Collection of flowers of *M. champaka*

Fresh flowers of *M. champaka* were collected in the first week of January in full flowering stage from pragathi resorts (Hyderabad, India). The flowers were identified by taxonomist, Department of Botany, Osmania university, Hyderabad.

Test organisms

The antibacterial activity was tested against two gram positive bacteria, i.e *Streptococcus mutans* MTCC497 *(S.mutans*), *Micrococcus luteus* MTCC106 (*M.luteus*) and two gram negative bacteria, i.e *Escherichia coli* MTCC40 *(E.coli), Salmonella typhi* MTCC733 *(S.typhi).* The collected bacterial strains were maintained in the form of nutrient agar slants at 4^{o}c.

Extraction of essential oil

The essential oil was extracted from fresh flowers of *M. champaka* by hydrodistillation using clevenger apparatus according to the modified method (Singh *et al.,* 2008).

An amount of 500gms of fresh flowers was used for extraction with 1000ml distilled water for 4hrs until no more essential oil was obtained. The collected oil was stored in an amber colored glass bottle under refrigeration and protected from light for further analysis.

Preparation of Inocula

The suspension of inoculum was prepared from preserved slant cultures using saline solution. The turbidity of the suspension was adjusted to 0.5 Macfarland standard spectrophotometrically which contains a cell density of approximately 1.5×10^{8} cfu/ml.

Antibacterial assay

The antibacterial activity of essential oil from *M. champaka* was determined against test organisms by the modified agar well diffusion method (Mbata *et al.,* 2008). The nutrient agar medium was prepared and sterilized. One ml of inoculum was mixed with 20 ml of nutrient agar medium and poured into petri plates aseptically and allowed to solidify and wells of 6 mm diameter were aseptically punched on the solidified agar using sterile stainless steel borer. Meanwhile, the essential oil was diluted using dimethyl formamide (DMF) to prepare a stock solution. Serial dilutions of stock solution were made using DMF to obtain different concentrations (10mg/ml, 25mg/ml, 50mg/ml, 75mg/ml, 100mg/ml, 200mg/ml) and 100µl of each concentration was poured into the wells. The control and standard wells were also prepared simultaneously. Then the plates were incubated at 37^{o}c for 24hrs to measure the zone of inhibition. Gentamicin was used as reference standard (Singleton, 2004).

Determination of Minimum inhibition concentration

The Minimum inhibition concentration (MIC) was determined by the broth dilution method (Courvallin *et al.,* 2006). Gentamicin was used as reference antibiotic control. In this method 0.1ml of each concentration of essential oil was added to 2.9ml of nutrient broth, controls were prepared without sample. Culture tubes were incubated at 37^{o}c for 24hrs. MIC was calculated as the lowest concentration of the essential oil inhibiting the visible growth of bacteria.

Antioxidant assay

The antioxidant activity of essential oil was evaluated by peroxide scavenging activity. Hydrogen peroxide (H_2O_2) is the major free radical generating in the various biochemical reactions of the human body. The free radical scavenging activity of EO was determined according to the method Ruch *et al.,* 1989. A solution of H_2O_2 (40mM) was prepared using phosphate buffer (p^{H} 7.4). A sample of EO/standard (3.4ml) was added to an H_2O_2 solution (0.6ml) and absorbance of the mixture was determined (sample). Absorbance of H_2O_2 (control) was determined at 230nm against a phosphate buffer (blank). Ascorbic acid was used as reference antioxidant. The percentage of hydrogen peroxide scavenging was calculated using the following formula:

$$\% \text{ scavenged } (H_2O_2) = \frac{A_c - A_s}{A_c} \times 100$$

A_c = Absorbance of control

A_s = Absorbance of sample (test & standard)

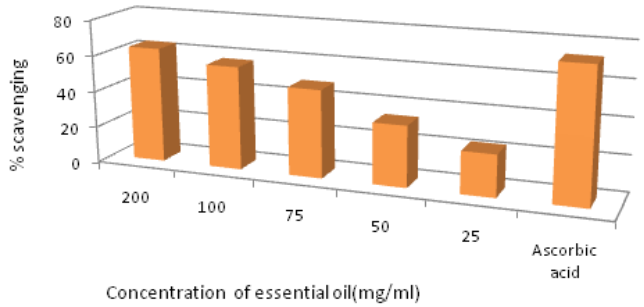

Fig. 1: Free-radical scavenging capacities of different concentrations of MCEO measured by the Peroxide scavenging method.

Table 1: Antibacterial activity (zone of inhibition, mm) of MCEO against gram positive and gram negative strains.

Concentration of MCEO (mg/ml)	Zone of inhibition, mm			
	E. coli	***S. typhi***	***M. luteus***	***S. mutans***
10	NR	NR	NR	NR
25	NR	NR	14.5±1.00	12.83±0.577
50	18 ±1.32	14.5±0.86	18 ±1.00	17.66±1.04
75	17.83±0.76	17.66±1.04	19 ±0.50	20.16±0.577
100	18.16±1.04	18.33±0.76	20.83±0.57	21.33±1.25
200	19.96±0.55	20.05±0.5	24.83±0.76	30.33±1.04
Gentamicin (R)	27±1.73	24.66±0.57	32 ± 2	28.5±0.5
MIC Values of MCEO against bacterial strains, mg/ml				
	50	50	25	25

R –Reference antibiotic, NR- No Result; ***** Values are expressed in mean ±SD where n=3.

RESULTS AND DISCUSSION

The present study has reported the extraction of essential oil from *M. champaka* by hydrodistillation method. This is the first report on extraction of essential oil from *M. champaka* by hydrodistillation method. The % Yield of essential oil from flowers of *M. champaka* was found to be 0.2% w/w. This method is inexpensive and widely used method of extraction. And the % yield of essential oil was found to be high compared to other methods of extraction (steam distillation & solvent extraction) (Denys *et al.*, 1999).

Antibacterial assay

The antibacterial activity of different concentrations of essential oil against different bacterial strains was assessed quantitatively by measuring the diameter of the zone of inhibition. The assay was carried out triplicate. The results were expressed as mean±SD and were given in table 1. Moreover MIC has been determined to assess the potency of essential oil against test organisms and results were given in table 1. The results of antibacterial activity of essential oil were promising and mean zone of inhibition was found to be high against *S.mutans* (30.33±1.04) at a concentration of 200mg/ml with MIC of 25mg/ml and low against *E.coli* (19.96±0.55) at a concentration of 200mg/ml but with MIC of 50mg/ml. Simultaneously the mean zone of inhibition of standard antibiotic was determined and compared to essential oil against *S.mutans.* The results revealed that MCEO has significant antibacterial activity against *S.mutans* compared to standard antibiotic (Gentamicin). It was also observed that EO was not showing significant variation in the inhibitory zone at different concentrations against *E.coli* reveal the fact that its activity against *E.coli* is not dose dependant. The results of antibacterial activity of essential oil were compared with previous studies where the inhibitory zones of methanol, ethanol, water, ethyl acetate and hexane extracts of flower were 16.4mm, 12.3mm, 13.1mm, 14mm and 13mm respectively against the growth of *S. typhi* (Umadevi *et al.*, 2012; Vivek Kumar *et al.*, 2011). As well as the inhibitory zones of methanol, ethanol, water, hexane extracts of flower were 12.64mm, 10.48mm, 10.32mm and zero respectively against the growth of *E.coli* (Vivek Kumar *et al.*, 2011;Dalvi Sanjay *et al.*, 2015). In contrast to these MCEO had a reproducible antibacterial activity against *E.coli* and *S.typhi*. This study also reported first time the antibacterial activity against *S.mutans* (Causative agent for dental caries) and promising results were observed. This suggests the importance of MCEO in the treatment of dental caries caused by *S.mutans.*

Antioxidant assay

The antioxidant capacity of MCEO was evaluated by peroxide scavenging activity. H_2O_2 generated in biochemical reactions was inactive itself, but might be toxic to the cell by a generation of hydroxyl radical in the cell. It has a characteristic absorption at 230nm and also used to study the radical scavenging activity of essential oil. As antioxidant MCEO added to H_2O_2 solution the absorbance decreases. The decrease in absorbance is taken as a measure of radical scavenging capacity. The peroxide scavenging activity of MCEO was significant and comparable with standard antioxidant ascorbic acid. The results of % scavenging activity at concentrations of 25,50,75,100 and 200mg/ml were shown in figure 1. It was observed that inhibition values of essential oil were dose dependent and significant at a concentration of 200mg/ml which is comparable to the % scavenging of ascorbic acid. Previous studies have reported the antioxidant activity of flower extracts of *M. champaka* by DPPH and reducing power assay (Umadevi *et al.*, 2012; Vivek Kumar *et al.*, 2011). In contrast, this could be the first study of peroxide scavenging activity of MCEO.

CONCLUSION

The present study concludes that the MCEO possess significant antibacterial and antioxidant activity. This study reported the antibacterial activity of MCEO against *S.mutans (*Causative agent for dental caries) and results suggested the importance of MCEO in the treatment of dental caries caused by *S.mutans*. This was the first report on the antibacterial and antioxidant activity of essential oil from the flowers of *M. champaka* extracted by hydrodistillation method. This result justifies the traditional use of *M. champaka* in the health care

system. Further studies recommended exploring the phytoconstituents responsible for its activity and using MCEO in the discovery of leads in the area of pharmaceutical research.

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