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Antioxidant And Antimicrobial Activities Of Various Extracts Of *Michelia champaca Linn* Flowers

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ABSTRACT

Michelia champaca (Magnoliaceae) is a large medicinal plant which is traditionally used against a number of diseases including inflammatory conditions. Antioxidant capacity and antimicrobial activities of various extracts of *Michelia champaca* Linn flowers were investigated in this study. The study was aimed at determining the antioxidant activity (reducing powers, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities) total flavonoid concentration, and antimicrobial activities of pet. Ether, benzene, chloroform, ethanol, methanol and aqueous extracts of *Michelia champaca* Linn flowers. The antioxidant activity of extracts increases with increase in amount of extract (5-20mg). DPPH free radical-scavenging activity of methanol, ethanol and aqueous extracts of *Michelia champaca* Linn flowers, gallic and ascorbic acid standards were found to be 80.56%, 90.20%, 81.32%, 91.34% and 93.64% respectively, at a concentration of 200µg/ ml and total flavonoid were in the range of 20.4 ± 0.22 g/100 g, 12.2 ± 0.12 g/100 g, 14.4 ± 0.22 g/100 g, crude extract of Champaca, respectively determined by using aluminum nitrate colorimetric method. *M. Champaca* showed narrow antibacterial activity against Gram-negative bacteria and Gram-negative bacteria tested. The crude extract exhibited high anticandidal activity on Candida albicans. Results of the present study suggest that *M. Champaca* flower extract possesses strong antioxidant and antimicrobial activities.

Introduction:

There is growing interest in the use of natural antioxidants for expanding the shelf life of food without the need for synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyhydroquinone (TBHQ).These food additives used by the food industry to prevent lipid peroxidation have been reported to possess possible toxic and carcinogenic effects on health.

In the past few years, there has been growing interest in the involvement of reactive oxygen species (ROS) in several pathological situations like cancer, chronic pain, cardiovascular diseases and arthritis. The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or

propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease[1].

Antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated[2]. Restriction is being imposed on the use of synthetic antioxidants because of their carcinogenicity, the need for natural antioxidants therefore become imperative and desirable[3,4].

Therefore as sources of natural antioxidants much attention is being paid to plants and other organisms. Thus interest in natural antioxidant, especially of plant origin, has greatly increased in recent years[5]. Thus, efforts have been made to search for novel natural antioxidants from various plant sources.

Michelia champaca (Family: Magnoliaceae), locally known as Swarna Champa, is a tree with golden-yellow fragrant flowers and aggregate fruits and this species is probably native to India, globally distributed in the Indo-Malesian region. Within India it is distributed in the Eastern Sub-Himalayan zone of West Bengal, Assam, Bihar and in the Western Ghats. Flowers on short, axillary brachyblast, solitary or rarely in pairs, large, tepals 6-21, in 3-6 usually subequal whorls, white to yellow; stamens many, anthers with a short to prominently elongated connective; gynoecium stipitate, with spirally arranged, free or connate carpels containing many ovules.

Previous investigations on the plant have revealed that it possesses anti-inflammatory, antimicrobial and leishmanicidal activity[6,7,8]. Recently a sesquiterpene lactone, parthenolide having anticancer activity has been isolated from ethanol extract of bark of *M. champaca*[9]. Flowers and fruit contain essential oil; bark contains alkaloids and beta-sitosterol[10, 11, 12, 13.]

In this paper we are reporting Antioxidant capacity and antimicrobial activities of various extracts of *Michelia champaca* Linn flowers.

MATERIAL AND METHODS:

Chemicals

Pet. Ether, benzene, chloroform, ethanol, Methanol, aluminium nitrate,potassium acetate, gallic acid,2,2-diphenyl-1-picrylhydrazyl (DPPH), the other chemicals and reagents were purchased from Merck (Bangalore).All other unlabelled chemicals and reagents were of analytical grade.

Plant material

Flowers of M. champaca were collected from Bangalore during June 2010.Immediately after collection, the flowers were thoroughly washed with water and dried under shade at room temperature.

Extraction

The dried flowers were coarsely powdered. 50 gm powder of flowers was subjected to successive solvent extract using Pet. Ether, benzene, chloroform, ethanol, methanol and water using a Soxhlet apparatus at 65^0 C. The solvent was completely removed dried crude extract. This crude extracts was used for investigation.

Antioxidant activity

DPPH radical scavenging activity[14, 15.]

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable

1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical was determined by the method described by Braca et al. (2001). Plant extract (0.1 ml) was added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517nm was determined after 30 min, and the percentage inhibition activity was calculated from [(A0–A1)/A0] x100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/ standard. A blank is the absorbance of the control reaction (containing all reagents except the test compound). The % scavenging activity and EC50 value of various extracts of *M.Champaca* flowers were calculated for the various

concentrations and compared with standard gallic acid.

Reducing Power method[16.]

Pet. Ether, benzene, chloroform, ethanol, methanol and aqueous extracts of *Michelia champaca Linn* flowers (5mg,10mg,15mg, and 20mg) in 1 mL of appropriate solvents were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%), and then the mixture was incubated at 50°C for 30 min. Afterward, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of the upper layer solution was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.

Determination of total flavonoid concentration[17]

Flavonoid concentration was determined as follows:

Various extract of *Michelia champaca Linn* flowers (1 ml) was diluted with 4.3 ml of 80% aqueous ethanol containing 0.1 ml of 10% aluminum nitrate and 0.1 ml of 1 M aqueous potassium acetate. After 40 min at room temperature, the absorbance was determined spectro photo metrically at 415 nm.

Total flavonoid concentration was calculated using quercetin as standard (Park, Koo, Ikegaki, & Contado, 1997):

Absorbance = (0:002108 µg quercetin-0.01089) x R^2:0.999.

Antimicrobial activity[18, 19.]

Microorganisms:

The following strains of bacteria were used: Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25923, Staphylococcus aureus ATCC 25922, B. subtilis, Salmonella typhosa, S. paratyphi, and Candida albicans.

The bacteria and fungi were obtained from the culture collection of the Microbiology St.John hospitals Bangalore and the culture maintained in microbiology lab in Government College of pharmacy Bangalore.

Screening of antimicrobial activity:

Antimicrobial activity of Various extract of *Michelia champaca Linn* flowers was determined by the agar-well diffusion method(cup plate method).All the microorganisms mentioned above were

incubated at 37 ± 0.1ºC for 24 h by inoculation into Nutrient agar.

C. albicans was incubated sabouraud dextrose agar at 28 ± 0.1 ºC for 48 h.Nutrient Agar (NA) and sabouraud dextrose agar(80 ml) were poured into each sterilized Petri dish(10 · 100 mm diameter) after injecting cultures (100 µl) of bacteria and yeast and distributing medium in Petri dishes homogeneously. For the investigation of the antibacterial and anticandidal activity, the dried various extracts were dissolved in dimethyl foramide (DMF) and dimethylsulfoxide (DMSO) correspondingly to a required concentration.

Each sample (100 µl) was filled into the wells of agar plates directly. Plates injected with the yeast cultures were incubated at 28 ºC for 48 h, and the bacteria were incubation at 37 ºC For 24 h. At the end of the incubated period, inhibition zones formed on the medium were evaluated in mm. Studies were performed in duplicate and the inhibition zones were compared with those of reference discs. Inhibitory activity of DMF and DMSO was also tested. Reference discs used for control were as follows: ciprofloxacin (100µg/0.1ml), ketoconazole (100µg/0.1ml).

Penicillin (5µg) was added to fungi media to provent bacteria growth. All determinations were done duplicate.

Statistical Analysis. Each datum represents the mean of three different experiments in each of which two measurements were made. Values of $P < 0.05$ were considered to be significant and values of $P < 0.01$ very significant.

Results and discussion:

Antioxidant activity of extracts:

Pet. Ether, benzene, chloroform, ethanol, methanol and aqueous extracts of *Michelia champaca* Linn flowers were subjected to screening for their possible antioxidant activity. Three complementary test systems, namely DPPH free radical-scavenging, reducing power, and total flavonoid concentration, were used for the analysis.

DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extracts. As antioxidants donate protons to these radicals, the absorbance decreases. The decrease in absorbance is taken as a measure of the extent of radical scavenging. Free radical-scavenging capacities of the extracts and standard (gallic acid), measured by DPPH assay, are shown in **Fig. 1.**

It was observed, that in line with the increase seen in the amount of ethanol, methanol, aqueous extracts and standard, an increase in DPPH free radical-scavenging occurred. Inhibition values in the concentrations of 50, 100,150, 200,250, and 500µg/ml were, respectively given in

Table no 1.

100µg of ethanolic extract has an inhibition value equivalent to 50 µg gallic acid, 50µg of methanolic extract has an inhibition value equivalent to 50 µg gallic acid, 100µg of aqueous extract has an inhibition value equivalent to 50 µg gallic acid and the extract shows less inhibition value. The inhibition value increases with concentration.

Reducing Power

The reducing power of methanolic extract of *Michelia champaca* Linn flowers was high compare to ethanol and aqueous extract and it is increased as the amount of extract increased (Figure 2). This difference was statistically significant, $P < 0.05$.

The amount of total flavonoid concentration was the high in methanolic extract compare to other extracts similar results were obtained in reducing power activities. Hence, combining these two results, we can suggest that there may be a relationship between the amount of total flavonoid concentration and reducing powers. In fact, there is a statistically significant correlation between these two, r) 0.99, $P < 0.01$.To be able to compare the reducing powers of these extracts with a known reducing reagent, the reducing powers of 5000 µg of each of extract and gallic acid were measured. As can be seen in Table 3, the reducing powers of methanolic extracts were markedly higher than that of gallic acid.

Amount of Total Flavonoids concentration:

In aluminum nitrate colorimetric method, aluminum nitrate forms acid stable complex with the keto group and either the hydroxyl group in A or C ring of flavonoids, in addition it forms acid labile complexes with orthodihydroxyl groups in the A or B ring of flavonoids. The aluminum nitrate complexes of flavonoid compounds show strong absorbance at 415 nm and flavonoids with more functional groups absorb stronger at 415 nm (Chang et al., 2002). We used quercetin as a standard compound because it is one of the widely spread flavonoids and has strong absorbance at concentrations

lower than 100 ppm at 415 nm because of its more functional

hydroxyl groups. Total flavonoid contents of crude methanolic extracts varied from 1.22 ± 0.33 to7.79 ± 0.39 g/100 g crude extract (Table 3) with the lowest amount for Ethanolic ext **Antimicrobial Activity:**

The methanolic, Ethanolic and aqueous extracts of the flowers shown antimicrobial activities on *S. aureus* ATCC 25922 and *B. subtilis* ATCC 6633, which are Gram-positive bacteria (Table 5). These extracts show detectable antimicrobial activity on *E. coli* ATCC 25923 and P. aeruginosa ATCC 27853(expect methanolic ext), which are Gram-negative bacteria and also antimicrobial activity against a C. albicans ATCC 60192 fungus. These results could suggest that some of the

studied extracts are effective against the Gram-positive bacteria, Gram-negative bacteria (expect methanolic ext) and fungi. The reason for this could be the subject of further studies

Conclusion:

The study clearly indicates that the extract possesses antioxidant and antimicrobial activity. Thus, this investigation is the first report on the comparative analysis of the antimicrobial and antioxidant properties of various extract of *Michelia champaca Linn* flowers. These findings justify the traditional uses of this plant. Flavonoid present in the flowers is responsible for antioxidant and antimicrobial activity. Further research is necessary for elucidating other the active principles.

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Tab: 1 %scavenging activity of different extract in DDPH assay method.

SI NO	Concentration µg/ml	%scavenging activity						
		Pet. Ether	Ben.	Chloro.	Methanol	Ethanol	Aqueous	Std Gallic acid
1	50	3.18	6.02	5.68	54.74	42.02	44.02	54.71
2	100	3.2	6.38	5.89	67.76	54.70	54.69	67.29
3	150	3.25	6.72	6.04	89.02	65.08	64.98	88.67
4	200	3.8	7.12	6.54	92.56	88.12	87.08	91.40
5	250	3.96	7.23	6.86	94.42	91.24	92.16	93.27
6	500	4.02	7.42	7.10	96.58	93.88	93.72	95.56

Tab: 2 EC_{50} values (µg/ml) of different extract in DDPH assay method.

SI no	Extract	EC_{50} µg/ml
1	Blank	-
2	Methanolic ext	117.15
3	Ethanolic ext	130.48
4	Aqueous ext	130.14
5	Standard(gallic acid)	118.26

Tab: 3 Reducing powers activity of various extract

SI NO	Concentration mg	Absorbance in 700nm						
		Pet. Ether	Ben.	Chloro.	Methanol	Ethanol	Aqueous	Std Gallic acid
1	5	0.077	0.368	0.324	2.32	2.14	2.04	2.24
2	10	0.082	0.524	0.472	2.465	2.28	2.19	2.382
3	15	0.097	0.786	0.628	2.654	2.32	2.41	2.523
4	20	0.102	0.912	0.921	2.824	2.52	2.58	2.635

Tab: 4 Total flavonoid (g/100 g crude extract) determined by Aluminum nitrate colorimetric method

SI No	Extracts	Total flavonoid content (g/100 g)
1	Methanolic ext	20.4± 0.22
2	Ethanolic ext	12.2 ± 0.12
3	Aqueous ext	14.4± 0.22

Tab: 5 The zone of Inhibition for Methanol, Ethanol and Aqueous *Michelia champaca Linn* flowers

SI No	Name of Extract	Dose Loaded µg/disc	Zone of Inhibition in mm						
			*S. aureus**	*B. subtilis**	*E. coli**	P. aeruginosa*	S.typi*	S.para typi*	C. albicans*
1	Methanol	500	13±0.22	13±0.42	12±0.64	13±0.62	16±0.4	16±0.12	11±0.42
2	Ethanol	500	12±0.12	11±0.24	10±0.48	11±0.24	12±0.3	13±0.26	10±0.24
3	Aqueous	500	12±0.82	11±0.12	10±0.32	11±0.22	13±0.1	13±0.56	10±0.32
4	ciprofloxacin	100	17±0.132	18±0.12	18±0.13	18±0.12	17±0.2	18±0.2	-
5	ketoconazole	100	-	-	-	-	-	-	14±0.13

*Data represents an average of 3 determinations.

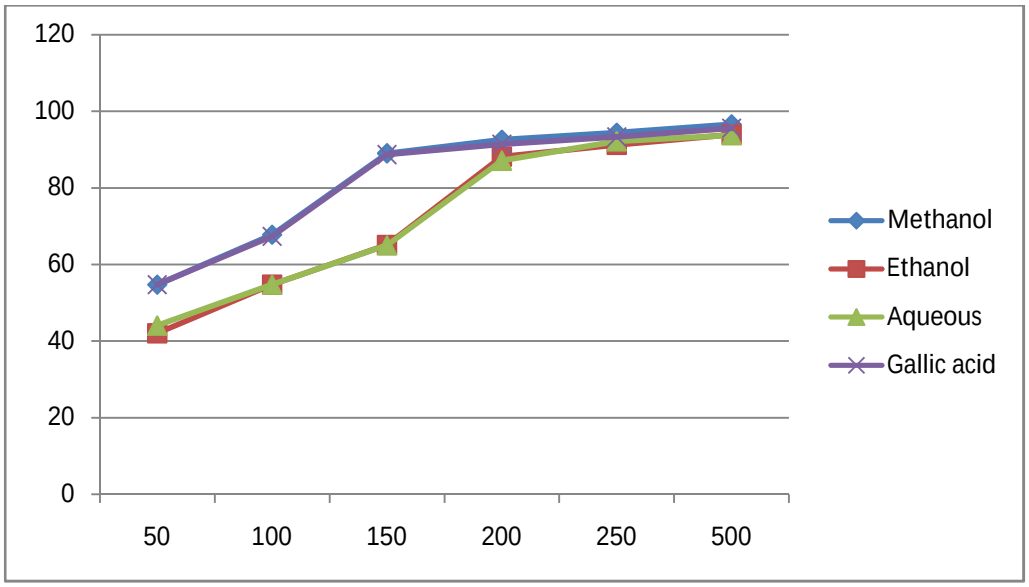

Figure 1 Free radical-scavenging capacities of the extract and standard (gallic acid) measured in DPPH METHOD

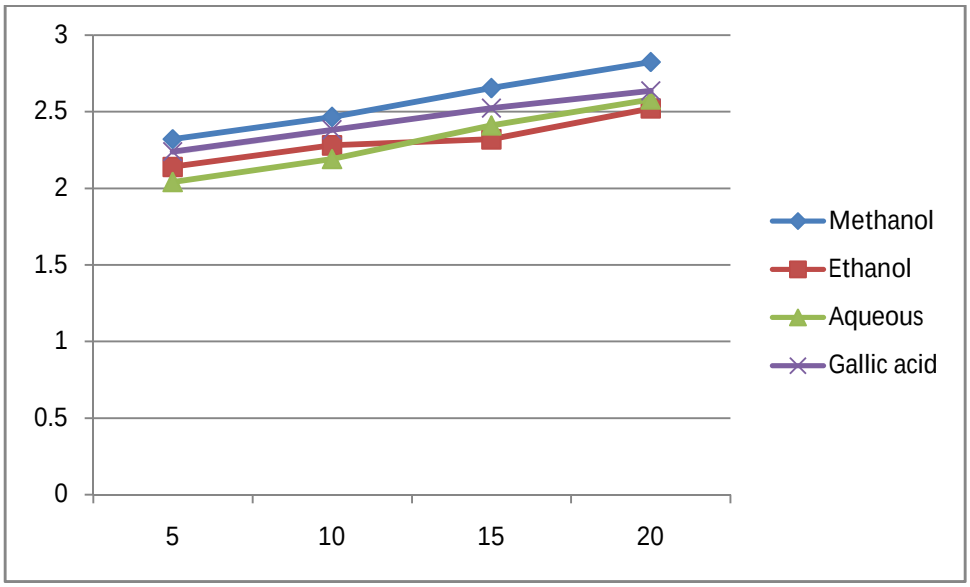

Figure 2 Reducing power of the extract and standard (gallic acid) measured absorbance at 700 nm.