Antibacterial and free radical scavenging activity of *Michelia champaca* Linn. flower extracts

Umadevi Parimi,[1*] Deephi Kolli[2]

[1] *Department of Chemistry, Gitam Institute of Science, Gitam University, Visakhapatnam, Andhra Pradesh, India*
[2] *Department of Chemistry, GIS, G.U, Vishakhapatnam, India*

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ABSTRACT

Introduction: *Michelia champaca* (Magnoliaceae) is a medicinal plant of paramount importance which is traditionally used against a number of diseases including inflammatory conditions. In the present study, crude hexane, ethyl acetate extracts and compounds 1-3 isolated from the extracts of *M. champaca* flower were investigated for possible antioxidant and antibacterial activity. **Method:** The DPPH radical scavenging assay was carried out by the protocol given by Nickavar et al 2006.[1] The extracts and the compounds were also investigated for antibacterial activity through disc diffusion method against four different bacterial strains viz. *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi and Shigella dysenteriae* using ampicillin as control. **Results:** In the present study the ethyl acetate extract, hexane extract exhibited strong antioxidant activity and the IC_{50} values in DPPH radical scavenging assay were found to be 160 µg/ml and 250 µg/ml, respectively while the IC_{50} values of isolated compounds 1-3 were 200 µg/ml, 220 µg/ml and 150 µg/ml, respectively. In this bioassay the extracts showed significant results compared to the individual isolated compounds. Ethyl acetate extract was more effective against all the bacterial strains followed by the hexane extract, compound-3, compound-1 and compound-2. Results of the present study suggest that *M. champaca* flower extracts and the isolated compounds possess strong antioxidant and antibacterial activity.

Keywords: *Michelia champaca*, antioxidant, antibacterial activity, catechin, ampicillin.

INTRODUCTION

Infectious diseases are a leading cause of death worldwide and antibiotic resistance has become a global concern.[2] One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents.[3] Phytomedicines derived from plants have proven as quite promising in the treatment of intractable infectious diseases[4] and the reports on the antibitotic properties of various plants are clearly documented.[5] Most of the diseases are linked to oxidative stress due to free radicals.[6] Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism.[7] The extracts of the different plant parts such as leaves, stem, root, fruits and others provided the source of natural antioxidants. 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 are the root cause of many diseases such as cancer, liver injury and cardiovascular diseases.[8] Continuous exposure to chemicals and contaminants also leads to an increase in production of free radicals that can result in irreversible oxidative damage.[9] Furthermore, due to the adverse side effects of synthetic antioxidants the search for effective and natural antioxidants has become crucial.

***Corresponding Address:**
Umadevi Parimi Associate Professor,
Department of Chemistry, Gitam Institute of Science,
Gitam University, Visakhapatnam, Andhra Pradesh, India
Email: umadevichemistry@gmail.com

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Though the antimicrobial activity and antioxidant activity of *Michelia champaca* has been reported, the studies pertaining to antimicrobial and antioxidant acitivity of individual compounds is scanty. This is the first report of comparing the antimicrobial and antioxidant activities of individual compounds with that of extracts.

Current research is now directed towards finding naturally occurring antioxidants and antibacterial agents of plant origin. In this context the flower extracts of *Michelia champaca* were screened for antibacterial and antioxidant activity. *Michelia champaca* belonging to family Magnoliaceae locally known as 'swarna champaca' or 'sampangi' is a tree with golden-yellow fragrant flowers and aggregate fruits. It is native to India and grows wild in humid tropical evergreen forests. Literature survey reveals that it possesses anti-inflammatory, antimicrobial and leishmanicidal activity.[10,11,12] And previously antidiabetic activity of flower buds of *M. champaca*[13] and antioxidant, analgesic and cytotoxic activities for methanolic extracts of *M. champaca* had been reported.[14] Literature survey also reveals that *M. champaca* flowers were effective agents for healing wounds in immune-compromised patients.[15]

MATERIALS AND METHODS

DPPH was purchased from Sigma Aldrich, India (**CAS Number 1898-66-4).** All solvents and chemicals that were used were of AR grade and were obtained from MERCK, India. The nutrient agar was obtained from Himedia (Mumbai, India).

Sample collection

The flowers of *M. champaca* were collected from Visakhapatnam. The flowers were identified by taxonomist, Dept. of Biotech, GIT, GITAM University, Visakhapatnam, and the specimen voucher is deposited in the Biotech department, GU. The flowers were washed with tap water, shade dried and finely powdered.

Extraction and isolation

Four hundred grams of finely powdered *M. champaca* flower powder was firstly extracted with 1000 ml of hexane in a soxhlet apparatus for 48 hrs. The solvent was collected and concentrated by rotary evaporator to afford 50 g of crude extract. Then the flower powder was again extracted with ethyl acetate for 48 hrs. The extracted solvent was collected and concentrated to afford 50 g of crude extract. The crude extracts were fractionated using silica gel and monitored by TLC with hexane:ethyl acetate (8:2) solvent system. The identified similar fractions of the above extracts were combined and checked by TLC. Hence from the fractionation it was identified that the two compounds from hexane and one from ethyl acetate extract have been isolated in their pure forms. The crude extracts and the three compounds were used for the current investigation.

BIOLOGICAL ASSAY

Antibacterial activity

The extracts of *Michelia champaca* flower and the isolated compounds were tested against two Gram positive and two Gram negative bacteria namely *B. subtilis, Staphylococus aureus, S. typhi and S. dysentriae.* These bacteria were obtained from MTCC, Chandigarh, India. Bacterial strains were maintained on nutrient agar slants at 4°C in Dept. of Biotech, GU. The agar disc diffusion method was employed for the determination of antimicrobial activity of the extracts. Briefly a suspension of the microorganism was spread on the solid media plates. Whatman filter paper discs (6 mm in diameter) were soaked with 10 µl of the test sample and placed on the inoculated media.[16,17] Plates were kept at 4°C for 2 hrs and then incubated at 37°C for 24 hrs. The diameter of the inhibition zones were measured in mm.

Antioxidant activity

The antioxidant activity of the test samples was estimated by the DPPH (diphenyl-2-picrylhydrazyl) radical scavenging protocol described by Nickavar et al.[1] Catechin is used as a standard. The concentration of the extracts, isolated compounds and the standard were in the range of 30 to 300 µg/ml with methanol are taken 0.3 mM solution of DPPH solution in methanol and 2 mg of catechin in 10 ml of methanol were taken. DPPH reagent alone was mixed with 2.5 ml of different concentrations of extract and standard and the mixture was shaken vigorously and incubated for 30 min in darkness at room temperature. DPPH reacts with the antioxidant in the sample and gets reduced. The colour change was observed from deep violet to light yellow. The change in absorbance was measured at 517 nm using a UV spectrophotometer (Shimadzu UV 2301). All the tests were carried out in triplicate. Radical scavenging efficacy was expressed as the inhibition percentage.

$$\text{Percentage inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} * 100$$

where $A_{control}$ = absorbance of control taken in the reaction

A_{sample} = absorbance of testing sample

RESULTS

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity.[18,19] The present study was targeted to screen the antibacterial activity and free radical scavenging activity of the different extracts of the flowers of M. champaca. The IC_{50} values calculated for scavenging DPPH free radical of hexane, ethyl acetate extracts and the three isolated compounds 1-3 were found to be 250 µg/ml, 160 µg/ml, 200 µg/ml, 220 µg/ml and 150 µg/ml, respectively. Percentage scavenging of DPPH radical was found to rise with increasing concentration of the crude extracts and the compounds (Fig. 1).

The antibacterial activity of hexane and ethyl acetate extracts and the three isolated compounds 1-3 against the different strains namely *B. subtilis, S. aureus, S. typhi, S. dysentry* is measured in diameter of zone of inhibition (in mm) and the values are given in Table 1. Ethyl acetate extract of *M. champaca* showed the highest activity against *S. aureus, B. subtilis, S. typhi and S. dysentry* (zones of inhibition: 12, 12, 14 and 8 mm) where as compound-2 showed the lowest activity against *S. aureus* and *S. dysentry* (zones of inhibition:10 and 7 mm).While the hexane extract, compound-1 and compound-3 showed

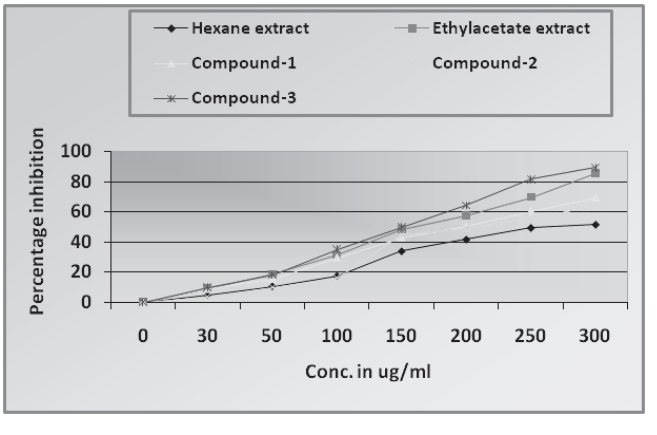
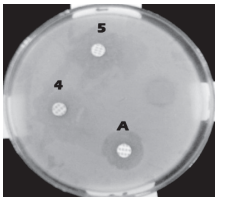


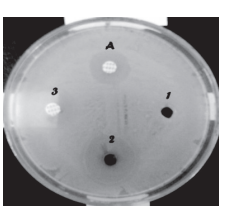
Figure 1. DPPH radical scavenging efficacy of different organic extracts of *Michelia champaca.*

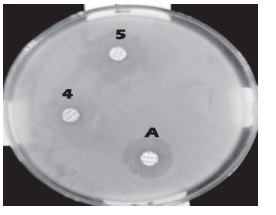
Staphylococus aureus



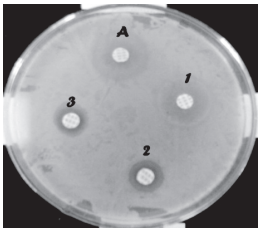
1. Hexane extract,
2. Ethyl acetate extract,
3. Compound-1,
4. Compound-2,
5. Compound-3,
6. A - Ampicilin

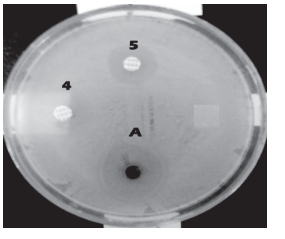
Bacillus subtilis



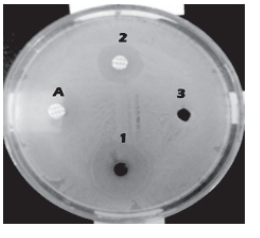


Salmonella typhi





higella dysenteriae



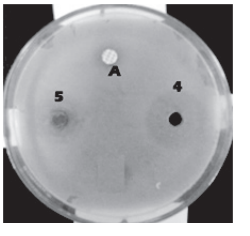


Figure 2. Zone of inhibition of the extracts and isolated compounds against the tested micro organisms.

good to moderate activity against all the used bacterial strains with different zones of inhibition.

DISCUSSION

Most of the free radical production within the body involves oxygen, and thus the free radicals are often referred to as

Table 1 Antibacterial activity of *M. champaca* flower extracts and diameter of zones of inhibition (in mm)

Name of the organism	Hexane extract	Ethyl acetate extract	Compound-1	Compound-2	Compound-3	Ampicillin (30 µg/ disc)
Gram +ve						
Staphylococcus aureus	11	12	11	10	11	18
Bacillus subtilis	10	12	8	9	12	16
Gram –ve						
Salmonella typhi	13	14	13	12	10	NE
Shigella dysentriae	8	8	8	7	7	NE

reactive or reduced oxygen species. Presence of free radicals in the body may cause cell and tissue damage. This sort of damage is known as oxidative damage.[20,21] Several mechanisms for the production of free radicals in the body have been proposed. Free radicals cause cellular damage by reacting with the phospholipid bilayer of cellular membranes. This reaction results in the production of measurable end products, primarily malondialdehyde.[22] In the present study we are reporting the isolation of three individual compounds from the flower extracts of *M. champaca*.The preliminary chemical tests showed that the compounds are phenolic in nature (positive test with ferric chloride and Folin-Ciocalteu reagent) whose structures are yet to be established. This is the first study on the comparison of antimicrobial and antioxidant properties of individual compounds with that of extracts. The DPPH-scavenging activity and the IC_{50} values clearly indicates that the individual pure components are more efficient than the extracts.The IC_{50} value of hexane extract is 250 μg/ml where as the individual compounds (compound-1 and compound-2) posses the IC_{50} values of 200 μg/ml and 220 μg/ml, respectively. The IC_{50} value for ethyl acetate and the pure compound isolated from it are 160 μg/ml and 150 μg/ml, respectively. These findings may be attributed to the fact that the individual compounds are phenolic in nature and phenols are more potent in radical scavenging activity. The antimicrobial activity of the extracts are more powerful antimicrobial agents than the pure compounds (Table 1), which infers that it is not always necessary that the drug discovery results in a single identified therapeutic agent but it can also possibly be because of the synergic action of a group of molecules that are present in the extract which are acting as better antimicrobial agents.

CONCLUSION

The present study concludes that the extracts and the isolated compounds 1-3 possess moderate to good antibacterial and free radical scavenging activity. This is the first report of the antimicrobial and antioxidant properties of the individual pure compounds isolated from *Michelia champaca* Linn. flowers. The findings justify the traditional uses of this plant in the treatment of diabetes, wounds, inflammatory conditions, worms, infestations and malarial fever. Further studies include the characterization of the isolated compounds.

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