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# *In vitro* antimicrobial activity and phytochemical screening of leaf and stem extracts of *Michelia champaca* Linn

Manhas, N. and *Dahiya, P.

*Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida-201303, Uttar Pradesh, India*

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**Abstract**

*Michelia champaca* leaf and stem extracts have been evaluated for phytochemical constituents, *in vitro* antimicrobial efficacy and TLC bioautography assay. Qualitative phytochemical analysis of various solvents and water extracts demonstrated the presence of tannins, glycosides, steroids, flavonoids, anthraquinones, coumarins and lactones, phlobatanins, and reducing sugars. Polysaccharides was not observed in both the extracts and saponins was absent in all the leaf extracts tested. The *in vitro* antimicrobial activity of various solvents and water extracts was further assessed against nine bacteria and two fungi respectively. Hexane and chloroform extracts were found to be more potent being capable of exerting significant inhibitory activities against majority of the isolates such as *Pseudomonas* sp., *Salmonella* sp., *Staphylococcus aureus* 1 and 2 and *Aspergillus niger*. The highest *in vitro* inhibitory activity was observed for *S. aureus* 1 with wide zone of inhibition diameter (31 mm) followed by *Aspergillus niger* (29 mm). Thin layer bioautography assay of hexane leaf extract demonstrated a single large well-defined growth inhibition zone against *S. aureus* 1 observed at *Rf* value of 0.67.



## Introduction

Antimicrobial chemotherapy is one of the constant challenges, particularly in view of the rapid evolutionary changes and wide variety of pathogens encountered. The continuous emergence of Gram-positive and Gram-negative MDR bacteria drastically reduces the efficacy of our antibiotic armory and, consequently, increases the frequency of therapeutic failure (Rice, 2006). World Health Organization (WHO) is keenly interested in the development and utilization of medicinal plant resources in the traditional system of medicine in the developing countries so as to extend the health care to maximum number of population in these countries (Goud *et al*., 2005). It is therefore necessary and urgent to fight against emerging and re-emerging infectious diseases. Several investigators evaluated the bioactivity of medicinal plant extracts and their constituents against the serious infectious organisms around the globe. Thus, screening of compounds obtained from plants for their pharmacological assay has indeed been the vast source of innumerable therapeutic agents.

*Michelia champaca* Linn. known as champaca is a large evergreen tree belonging to the Magnoliaceae family. It is known for its fragrant flowers, and its timber is used in woodworking. The tree is native to the Indo-malaya ecozone consisting of South Asia, Southeast Asia-Indochina, and southern China. It occurs in forests from 300 meters to 1200 meters above sea level. The leaves are simple, alternate, petiole 1 to 3 cm long, elliptic, lanceolate, spiral, and reticulate. Flowers are solitary, yellow, dull-yellow when fresh, orange when old and fragrant (Gamble, 1921). *M. champaca* is widely used in both Ayurveda and Siddha medicine. All parts of this plant provide various benefits. Juice of the leaves of *Michelia champaca* is given with honey in cases of colic. The volatile oil obtained from the flower is useful in cephalalgia, opthalmia and gout (Mehta *et al*., 2010) whereas, the bark is used as a stimulant, expectorant, astringent and febrifugal properties (Varier, 2003).

In the present investigation, *Michelia champaca* was selected, as one of the medicinally important plant. There are few scientific reports on the phytochemical analysis and antimicrobial properties of leaves and stem of this plant. The present study relates to phytochemical screening, *in vitro* antimicrobial activity and TLC bioautography assay of various extracts of *Michelia champaca*.

## Materials and Methods

### *Plant material and extracts preparation*

The leaves and stem of *M. champaca* were

*Corresponding author.
Email: *pdahiya@amity.edu, praveen_sang@yahoo.com*

broken down into small pieces then after they were oven dried at 50ºC, thoroughly ground and extracted successively with n-hexane, chloroform, methanol, ethanol and water. The extracts obtained were concentrated at reduced pressure to dryness using a Soxhlet evaporator. After complete solvent evaporation, extracts were dissolved in 10% DMSO to a final concentration of 20 mg/ml. The concentrated extracts are then stored at 4°C in labeled sterile screw-capped bottles till further analysis.

*Preliminary Phytochemical screening*

The extracts were evaluated for the presence of different phytochemicals to ascertain the presence of metabolites such as saponins, tannins, steroids, phlobatanins, anthraquinones, cardiac glycosides, alkaloids, reducing sugars and flavonoids by using wet reactions (Yun *et al*., 2011; Ghamba *et al*., 2012).

*Microbial cultures*

The microbial cultures included clinical isolates of *Salmonella* sp., *Staphylococcus aureus, Escherichia coli, Klebsiella* sp., *Pseudomonas* sp. and *Acinetobacter* sp. Standard strains *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used for quality control. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and sub-cultured on to nutrient broth for 24 h prior to testing. Two fungal isolates studied includes *Aspergillus* sp. and *Aspergillus niger.* The cultures were maintained on potato dextrose agar at 4°C. These microbial isolates served as test pathogens for antimicrobial activity assay.

*Antibacterial activity assay*

The agar well diffusion method was employed with slight modifications (Dahiya and Sharmistha, 2011) to determine the antibacterial activities for various solvent and aqueous extracts of *M. champaca*. About 25 ml of nutrient agar and potato dextrose agar was poured into each petri plate. Once the agar solidified, the microbial cultures were inoculated on the surface of the plates ($1 \times 10^8$ CFU/ml). Subsequently, the surface of the agar was punched with a 6 mm diameter wells. Each well was filled with 50 µl of each plant extract. The concentration of the extracts employed was 20 mg/ml. Control wells containing the same volume of hexane, chloroform, methanol, ethanol and distilled water served as negative controls while standard antibiotic discs of imepenem (10 µg) and vancomycin (30 µg) were used as the positive controls. The plates were observed for zones of growth inhibition after 24 h of incubation at 37°C. The diameter of zone of clearance was measured in millimeters. All the tests were performed in triplicate and the antimicrobial activity was expressed as the mean of inhibition.

*TLC bioautography assay*

Hexane extracts of leaf extract of *M. champaca* showing significant antimicrobial potential against *S. aureus* 1 as determined by agar well diffusion method was investigated using thin layer chromatography (TLC) bioautographic agar-overlay method (Purkayastha and Dahiya, 2012). About 10 µl of extract was chromatographed on pre-coated aluminium silica gel G 25 plates with toluene: ethyl acetate (93:7) as mobile phase. TLC bioautography was carried out using the selected strain of bacteria. The developed TLC plates were thinly overlaid with molten nutrient agar inoculated with an overnight culture of bacteria. The plates were incubated in a dark and humid chamber overnight at 37°C. Subsequently, the bioautogram was sprayed with an aqueous solution of 2, 3, 5 triphenyl tetrazolium chloride and further incubated for at 37°C for 4 h. Microbial growth inhibition appeared as clear zones against a pink background. The *Rf* values of the spots showing inhibition were determined.

## Results and Discussion

*Phytochemical screening*

Preliminary phytochemical screening of the various extracts revealed that *M. champaca* leaves and stem extracts contain most of the phytochemicals tested [Table 1] The phytoconstituents are tannins, glycosides, steroids, flavonoids, anthraquinones, coumarins and lactones, phlobatanins, and reducing sugars. However, some phytoconstituents were absent in some extracts. It was observed that polysaccharides were absent in both leaf and stem extracts, whereas, saponins was absent in all the leaf extracts. In a similar study, the ethanolic leaf extracts of *M. champaca* contained alkaloids, glycosides, and flavonoids, the aqueous extract contained flavonoids, the chloroform extract contained alkaloids, glycosides, amino acids and sterols (Geetha *et al*., 2011). Mullaicharam and Surendra kumar, (2011) reported the presence of alkaloids, flavonoids, glycosides, tannins and sterols in the leaf alcoholic extract, Whereas the leaf aqueous extract was devoid of alkaloids, carbohydrates, glycosides, and sterols. Alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pains, developed resistance against disease and endurance against stress. On contrary, Dhanlaxmi *et al*. (2012) observed the presence of phytoconstitutent saponins

Table 1. Phytochemical analysis of various extracts of *M. champaca*

| Phytoconstituents | *Leaf extract* | | | | | *Stem extract* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | H | C | M | E | Aq | H | C | M | E | Aq |
| Reducing sugar | - | - | + | + | + | - | - | - | + | + |
| Flavonoids | - | - | + | + | + | - | + | + | + | + |
| Steroids | - | - | - | - | + | + | + | + | + | + |
| Tannins | + | + | - | - | - | + | + | - | - | - |
| Phlobatanins | + | + | - | - | - | + | - | - | - | - |
| Saponin | - | - | - | - | - | + | + | - | - | - |
| Cardiac glycosides | - | + | + | + | - | + | + | + | + | + |
| Anthraquinones | + | + | - | - | - | + | + | - | - | - |
| Polysaccharides | - | - | - | - | - | - | - | - | - | - |
| Coumarins and Lactones | + | + | + | - | - | + | + | + | - | - |

H: hexane extract, C: chloroform extract, M: methanol extract, E: ethanol extract,
Aq: aqueous extract, +: present, -: not present

in the ethanolic leaf extract.

The methanolic flower extract of *Michelia champaca* was found to contain phenolics, flavonoids, tannins, alkaloids, and carbohydrate which exhibit antioxidant and free radical scavenging activities (Ananthi and Chitra, 2013). This suggests that the plant extracts are a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. The presence of polyphenols in the flowers of *Michelia champaca* provides the basis for its wide uses of the therapeutic potential. As reported by Jarald *et al.* (2008), flower buds extraction of petroleum ether was found to contain fats and alkaloids, acetone extraction contained only tannins, the chloroform extract contained sterols and alkaloids, ethanol extract contained carbohydrates, flavonoids, alkaloids and tannins and the aqueous extract contained carbohydrates, flavonoids, alkaloids and saponins.

*Antimicrobial activity assay*

The antimicrobial activity of *M. champaca* leaf and stem extracts against nine bacterial and two fungal clinical isolates by agar well diffusion method is as shown in Table 2. The results revealed that the antibacterial activity of *M. champaca* leaf and stem extracts on the agar plates varied for the different solvent and aqueous extracts tested. The extracts showed significant to moderate antimicrobial activity toward few tested strains except *E. coli, Klebsiella* sp. and *Acinetobacter* sp. Hexane and chloroform extracts were found to be more potent being capable of exerting significant inhibitory activities against majority of the isolates such as *Pseudomonas* sp., *Salmonella* sp., *S. aureus* 1 and 2 and *Aspergillus niger*. Highest inhibitory activity was observed for *S. aureus* 1 with wide zone of inhibition (31 mm) followed by *Aspergillus niger* (29 mm) and *S. aureus* 2 (20 mm). The growth of *Salmonella* sp, *S. aureus* 1 and 2 were only inhibited by the hexane and chloroform extract of *M. champaca* stem extract. All the stem extracts showed poor antifungal activity and did not inhibited the growth of fungal strains tested. The control plate did not exhibit inhibition on the tested bacteria where as standard antibiotics Imepenem and Vancomycin produced significantly larger inhibition zones against Gram-negative and Gram-positive bacteria respectively.

Aqueous extracts exhibited no inhibitory effect against the assayed bacterial and fungal isolates. *M.champaca* stem extracts only inhibited the growth of *Salmonella* sp. and *S.aureus* 1 and 2. Whereas, Khan *et al*. (2002) reported that the methanol extracts of leaves, stem and root barks of Michelia champaca and the different obtained fractions exhibited a broad spectrum of antibacterial activity. This observation confirmed the evidence from a previous study which reported that alcohol is a better solvent for extraction of antimicrobial substances from medicinal plants than water (Rojas *et al.*, 2006).

The methanolic, ethanolic and aqueous extracts of the flowers of *M. Champaca* showed antimicrobial activities against Gram-negative and Gram-negative bacteria tested (Kumar *et al.*, 2011) including *S. aureus* ATCC 25922, *Bacillus subtilis* ATCC 6633. The extracts show detectable antimicrobial activity against *E. coli* ATCC 25923, *Pseudomonas*

Table 2. Antibacterial activity of *M. champaca* leaf and stem extracts by agar well diffusion assay

| Test Microorganism | Zone of Inhibition (in mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *M. champaca* leaf extract | | | | | *M. champaca stem* extract | | | | |
| | H | C | M | E | Aq | H | C | M | E | Aq |
| *Acinetobacter sp.* | 7 | - | - | - | - | - | - | - | - | - |
| *E.coli 1* | 6 | 7 | - | - | - | - | - | - | - | - |
| *E.coli 2* | 5 | - | - | - | - | - | - | - | - | - |
| *Pseudomonas sp.* | 15 | 9 | 7 | - | 9 | - | - | - | - | - |
| *Klebsiella sp.1* | - | 5 | - | - | - | 5 | - | - | - | - |
| *Klebsiella sp.2* | - | - | - | - | - | - | - | - | - | - |
| *Salmonella sp.* | 9 | 11 | - | - | - | 10 | 9 | - | - | - |
| *S. aureus 1* | 31 | 19 | 12 | - | - | 11 | 7 | 5 | - | - |
| *S. aureus 2* | 20 | - | - | - | - | 15 | 6 | - | - | - |
| *Aspergillus sp.* | 8 | 9 | - | NT | NT | - | - | - | NT | NT |
| *Aspergillus niger* | 29 | 28 | - | NT | NT | - | - | - | NT | NT |

Zone of inhibition is the mean of three readings, H: hexane extract, C: chloroform extract, M: methanol extract, E: ethanol extract, Aq: aqueous extract, -: no inhibition, NT: not tested

*aeruginosa* ATCC 27853 and *Candida albicans* ATCC 60192 fungus.

*TLC bioautography assay*

Bioautographic assay are used to screen for antimicrobial activity by separating components onto the surface of chromatographic plates followed by overlaying the TLC plate with molten bacterial agar. Hexane extract of M. champaca leaf extract revealed a significant antibacterial activity against *S. aureus* 1as characterized by both TLC-bioautography and agar well diffusion methods. Bioautography showed presence of three active compounds at different Rf values. It includes one big spot and two smaller spots (data not shown).

One large inhibitory zone with *Rf* value 0.67 was observed against the growth of *S. aureus* 1 on the TLC plates B and C as white spot on pink background when sprayed with aqueous solution of 2, 3, 5 triphenyl tetrazolium chloride. The observed inhibition was may be due to one or more active compounds which may be overlapping possibly due to the solvent system used for screening. No zone of clearance was observed for other two spots on reference chromatogram. This could be attributed to evaporation of the active components, photo-oxidation or insufficient amount of the active component (Masoko and Eloff, 2005).

## Conclusion

The present study involves the investigation of antimicrobial potential of various solvent and aqueous extracts of leaves and stem of *Michelia champaca* against clinical isolates. Phytoconstituents were also found to be present in both the extracts tested leading to the antimicrobial potential. The extracts are the potential candidates to be developed into next generation of antimicrobials to combat bacterial and fungal infections. Further study is needed to check the efficacy of crude extract in herbal medicine that can serve as a base for the development of novel potent drugs and phytomedicines.

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## References

Ananthi, T. and Chitra, M. 2013. *In vitro* evaluation of antioxidant activity of *Michelia champaca* (L.) flowers. American Journal of Advanced Drug Delivery 1(5): 734-742.

Dahiya, P. and Purkayastha, S. 2011. Phytochemical screening and antimicrobial potentials of *Alangium salvifolium* and *Piper longum* against multi-drug

resistant bacteria from clinical isolates. International Journal of Pharmacy and Pharmaceutical Sciences 3(5): 462-465.

Dhanalakshmi, M., Jayasree, T. and Sharmila, S. 2012. Anti-arthritis activity leaves of *Michelia champaca* L. by complete freund's adjuvant. International Journal of Pharmacy and Technology 4 (3): 4607-4615.

Geetha, K.N., Jeyaprakash, K. and Nagaraja, Y.P. 2011. A preliminary pharmacognostical study on leaves and flowers of *Michelia champaca* L. Magnoliaceae. Journal of Applied and Natural Science 3(2): 228-231.

Gamble, J.S. 1921. Flora of the presidency of madras. 1st ed. London: Adlard and son Ltd.

Ghamba, P.E., Agbo, E.B., Umar, A.F., Bukbuk, D.N. and Goje, L.J. 2012. In vitro antibacterial activity of crude ethanol, acetone and aqueous *Garcinia kola* seed extracts on selected clinical isolates. African Journal of Biotechnology 11(6):1478-1483.

Goud, P.S.P., Murthy, K.S.R., Pillaiah, T. and Babu, G.V.A.K. 2005. Screening for antibacterial and antifungal activity of some medicinal plants of Nallamalais, Andhra pradesh, Indian Journal of Economic and Taxonomical Botany 29(3): 704-708.

Jarald, E.E., Joshi, S. B. and Jain, D.C. 2008. Antidiabetic activity of flower buds of *Michelia champaca* Linn. Indian Journal of Pharmacology 40: 256-260.

Kumar, R. V., Kumar, S., Shashidhara, S., Anitha, S. and Manjula, M. 2011. Antioxidant and Antimicrobial activities of various extracts of *Michelia champaca* Linn flowers. World Applied Sciences Journal 12(4): 413-418.

Khan, M.R., Kihara, M. and Omoloso, A.D. 2002. Antimicrobial activity of *Michelia champaca.* Fitoterapia 73(7-8):744-748.

Ling-Yun, C., Feng-Xiang, S. and Xia, G. 2011. Preliminary phytochemical analysis of *Acanthopanan trifoliatus* (L.) Merr. Journal of Medicinal Plants Research 5(17): 4059-4064.

Masoko, P. and Eloff, J.N. 2005. The diversity of antifungal compounds of six South African *Terminalia* species (Combretaceae) determined by bioautography. African Journal of Biotechnology 4: 1425-1431.

Mehta, K., Chauhan, D., Kumar, S., Nair, A. and Gupta, S. 2010. Morphological changes and Antihyperglycemic effect of *M. champaca* leaves extract on Beta-cell in alloxan induced diabetic rats. Recent Research in Science and Technology 3(1): 81-87.

Mullaicharam, A.M. and Surendra kumar, M. 2011. Effect of *Michelia champaca* Linn on pylorous ligated rats. Journal of Applied Pharmaceutical Science 1(2): 60-64.

Purkayastha, S. and Dahiya, P. 2012. Phytochemical analysis and antibacterial efficacy of Babchi oil *(Psoralea corylifolia)* against multi-drug resistant clinical isolates. International Conference on Bioscience, Biochemistry and Bioinformatics 31: 64-68.

Rice, L.B. 2006. Unmet medical needs in antibacterial therapy. Biochemical Pharmacology 71: 991-995.

Rojas, J.J. Ochoa, V.J. Ocampo, S.A. and Munoz, J.F. 2006. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. BMC Complementary and Alternative Medicine. 6: 2-7.

Varier, P.S. 2003. Indian medicinal plants. 1st ed. Chennai: Orient Longman Pvt. Ltd.