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Original Article

LC-MS and HPTLC Profile of Crude Ethanol Extract of Plant *Michelia champaca* Linn.

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ABSTRACT

Aim: The plant *Michelia champaca* L. plant is a very rich source of phytoconstituents with medicinal properties. The aim of the study is to analyze the chemical constituents present in the crude extract responsible for various pharmacological actions. **Materials and Methods:** The aerial parts of the plant Extracted with ethanol and subjected to LC-MS and HPTLC analysis by using Water Q-TOF micro coupled with 2795 HPLC and CAMAG TLC SCANNER -3. **Results:** LC-MS analysis showed the various peaks with low and high molecular weights indicating the presence of various phytochemicals. The compounds identified in the ethanol extract include parthenolides, quercetin, stigmasterol, sitosterol, and liriodenine and some unknown compounds by comparison with the SDBS database. HPTLC of the ethanol extract showed the R_f value of five compounds, were found to be more prominent. 0.15, 0.3, 0.45, 0.6, 0.75. identified by comparison with HPTLC - derived database. **Conclusion:** The compounds identified by LC-MS and HPTLC of the ethanol extract of the aerial plant *Michelia champaca* Linn. may be responsible for its various pharmacological activities, and hence this plant can be used in the treatment of various ailments.

Keywords: Chromatography, Extract, Plant, Spectroscopy.

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INTRODUCTION

The plant kingdom is an extraordinary reservoir; around 400,000-500,000 novel molecules are found in plant species.[1] Plants contain secondary metabolites that are responsible for their therapeutic properties.[2] The largest class of natural compounds consists of steroids, alkaloids, flavonoids, and their glycosides.[3] For quality control of new compounds, there is a need to develop methodologies for the analysis of flavonoids and steroids in medicinal plants.[4]

Michelia champaca Linn. (Magnoliaceae) is a higher plant or evergreen tree commonly referred to as champa. It is a native of the southern parts of India and is cultivated in gardens and temples in various parts of the country.[5] *Michelia champaca* L. is reported to have a significant wound healing, anti-microbial, anti-diabetic, anti-tumor, anti-inflammatory, antioxidant, anti-infective properties, anti-hyperglycemic activity, diuretic activities, and anti-fertility activity in leaves and stem bark.[6]

It is reported that leaves of *Michelia champaca* Linn. contain parthenolide, stem bark contains michampanolide, 8-acetoxy parthenolide, Magno grandiose, costunolide, dihydro parthenolide, -sitosterol, liriodenine, ushinsunine, magnoflorine, and micheliolide from root bark. The flowers of *M. champaca* contain flavonoids, quercetin, and flavonoid.[7]

The aims of the LC-MS and HPTLC study of the ethanol aerial extract of *Michelia champaca* Linn is to investigate the phytochemical constituents responsible for its pharmacological properties.

MATERIALS AND METHODS

Collection of the Plant

The aerial parts (flowers, leaves, and branches) of *Michelia champaca* Linn. were collected from the herbal garden, Panjab University, Chandigarh. A voucher sample of the plant is provided in letter No. RBIPH/17/169. Dated May 23, 2017, it was submitted to the Raw Material Herbarium and Museum, Delhi (RHMD), and the plant was identified and authenticated by Dr. Sunita Garg, emeritus scientist, CSIR-NISCAIR, with reference number NISCAIR/RHMD/Consult/2017/3078-27 for future reference.

Extraction

The aerial parts (flower, leaves, and branch) were shade-dried for two weeks and coarsely powdered. The plant material is macerated with petroleum ether (60-80%). Filtrate discarded.

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The marc was subjected to continuous hot soxhlet extraction with Ethanol (70-80%). Then the extract was filtered and evaporated under reduced pressure, and the yield of the extract was found to be 5.3%. Then it was stored in the refrigerator until further use.

Phytochemical Screening

The ethanol extract was subjected for the qualitative preliminary phytochemical screening for the investigation of phytoconstituents (alkaloid, terpenoids, Glycosides, flavonoids, phenols, tannins)

Liquid Chromatography-Mass Spectrometry (LC-MS) studies

The chemical constituents of the ethanol extract were determined using LC-MS. For the LC-MS analysis Waters micromass Q-Tof micro instrument used. Q-TOF mass spectrometer interfaced with waters HPLC 2795 was fitted with an ESI source. From m/z 100 to 1200 m/z Full-scan mode was performed with a source temperature of 140°C. A total flow rate of 0.1 mL/min was used for the Solvents. The solvent was eluted by isocratic elution. The positive ion mode was acquired in MS spectra. The temperature of the drying gas (N2) was 350°C at a gas flow rate of 6 mL/min and a Nebulizing pressure (N2) of 25 psi. About 0.5 g of sample extracts were diluted with solvents and filtered with a 0.22-m nylon filter prior to analysis. A 5-liter volume of the extracts was injected into the analytical column for analysis. The mass fragmentations were identified by using the spectrum database for organic compounds in the SDBS application.[8]

HPTLC Analysis

The Ethanol aerial extract of *Michelia champaca* Linn. subjected for HPTLC analysis. A small quantity of extract was dissolved in ethanol and sample was applied in precoated plate with the help of Linomat IV applicator. The HPTLC fingerprint scanned at 254 and 366 nm.The rf values of the estimated compounds were compared with marker compounds by using HPTLC derived database used for the analysis of phytconstituents.

Chromatographic conditions

Following are the chromatographic conditions required to get an effective resolutions by selected mobile phase: Toluene: Ethylacetate (EA): Glacial Acetic Acid (GAA): (5:2:0.1).

Stationary phase: HPTLC precoated, silica gel G 60 F254 (Merck, Germany).

Size: 10x10 cm.

Developing chamber: Twin trough glass chamber Mode of application.

Band Band size: 5 mm.

Separation technique: Ascending.

Temperature: 20±5°C.

Saturation time: 30 min scanning.

Wavelength: 254 nm / 366 nm.[9]

RESULTS

Photochemical Screening

The phytochemical screening of ethanol extracts of *Michelia champaca* Linn. aerial parts revealed the presence of steroids, flavonoids, terpenoids, and tannins. Therefore, LC-MS analysis of the extract and various fractions was performed for the identification of phytoconstituents.

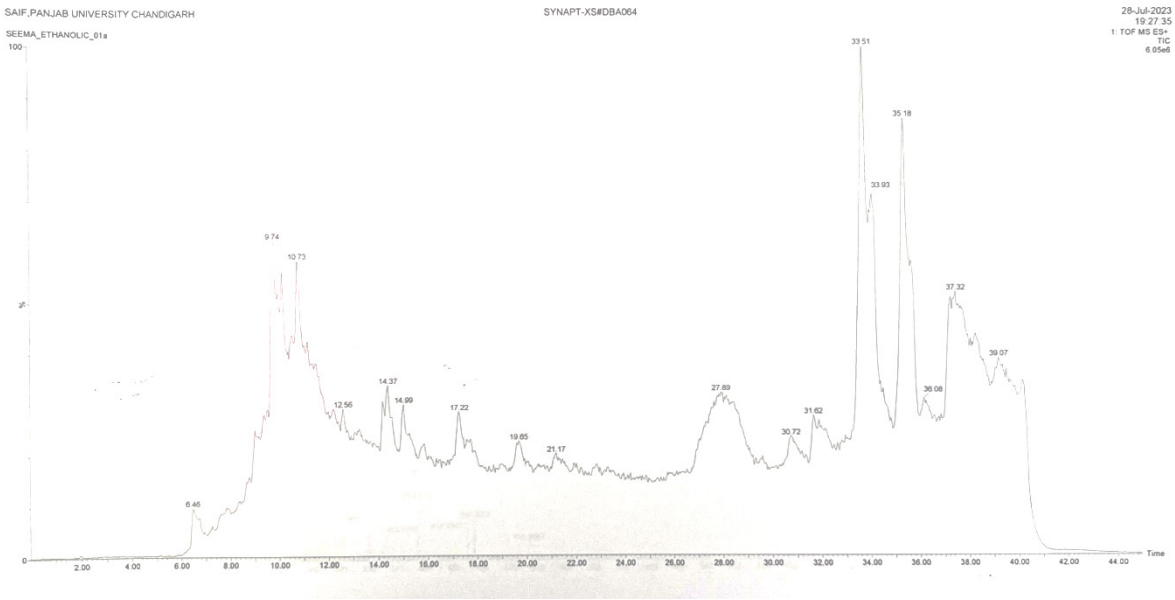


Figure 1: LC-MS Chromatogram of Ethanolic extract of *M. champaca* Linn.

Liquid Chromatography and mass spectroscopy studies

The LC-MS chromatogram of ethanol extract of *Michelia champaca* Linn. is shown in Figure 1 and Mass spectra of detected compound are shown in Figures 2-4. At different retention time there were different peaks. LC-MS revealed the compound at a particular retention time, and fragmentation patterns revealed the relative abundance of a particular compound. The compounds elucidated based on their molecular weight, peak area, retention time. The highest peak at the particular retention time showed the presence of compound Quercetine, sitosterol, stigmasterol, parthenolide and Liriodenin, as shown in Table 1. The compound Quercetine is plant flavonoid has anti-inflammatory and antioxidant property. Stigmasterol and sitosterol are phytosterol have steroidal property has anti-cancer, anti-fertility, ant-lipidemic action. Parthenolide is sesquiterpene lactone useful in fever, headache and arthritis. Liriodenin is a alkaloid has anti-microbial, anti-cancer property.[10]

Table 1: Compounds elucidated in EAEMC by LC-MS.

Peak	Retention Time	Compound Formula	Name of the compound	M.W
1	6.5	$C_{15}H_{20}O_3$	Parthenolide	248.317
2	9.7	$C_{14}H_7O_4N$	Liriodenine	275.26
3	10.78	$C_9H_5BrF_6$	1-bromomethyl-3,5-bis(trifluoromethyl) benzene	306
4	12.56	$C_8H_9IO_2$	1-acetamidopyridinium iodide	264
8	19.64	$C_8Cl_2N_2O_2$	2,3-dichloro-5,6-dicyano-p-benzoquinone	227
10	27.89	$C_{28}H_{38}O_{19}$	D-(+)-sucrose octaacetate	678.5
12	31.85	$C_{24}H_{16}N_6O_6$	4,4'-(3,3'-dinitro-4,4'-biphenylylenebisazo) diphenol	484
13	33.51	$C_{25}H_{24}N_2O_6S_2$	5-ethyl-5-phenyl-1,3-ditosyl-2,4-imidazolidinedione	512
15	35.17	$C_{37}H_{64}O_2$	cholesteryl decanoate	540
16	36.07	$C_{29}H_{50}O$	B-sitosterol	414
17	37.32	$C_{29}H_{48}O$	Stigmasterol	412.69
18	39.07	$C_{15}H_{10}O_7$	Quercetine	301

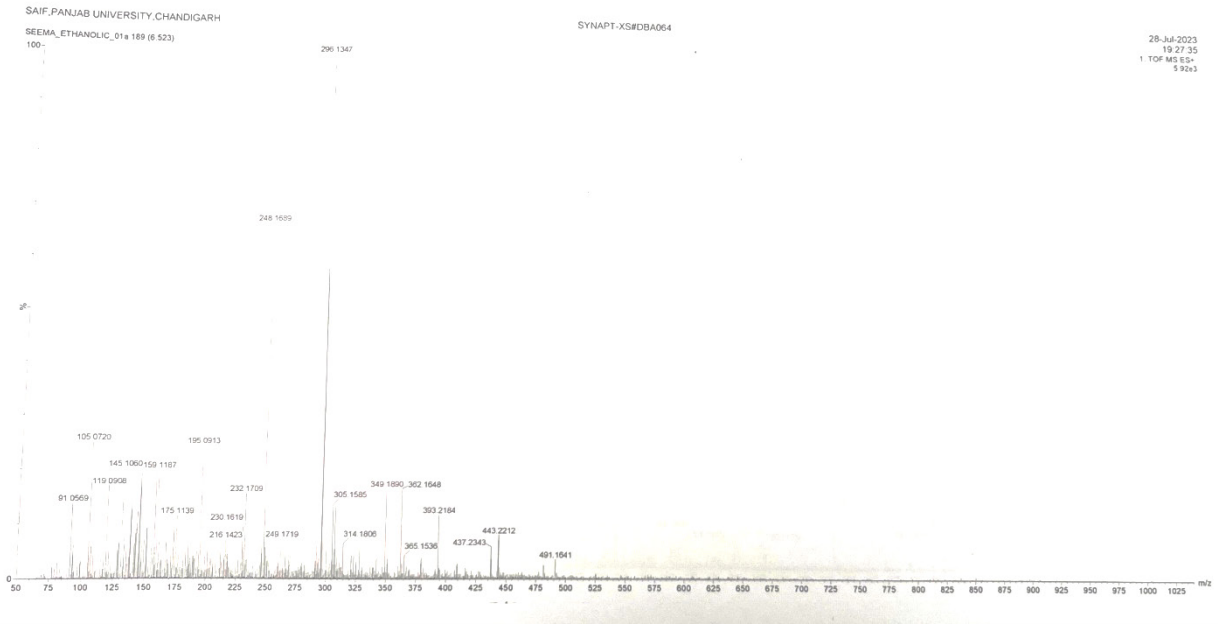


Figure 2: Mass spectrum of *M. champaca* Linn. at retention time 6.

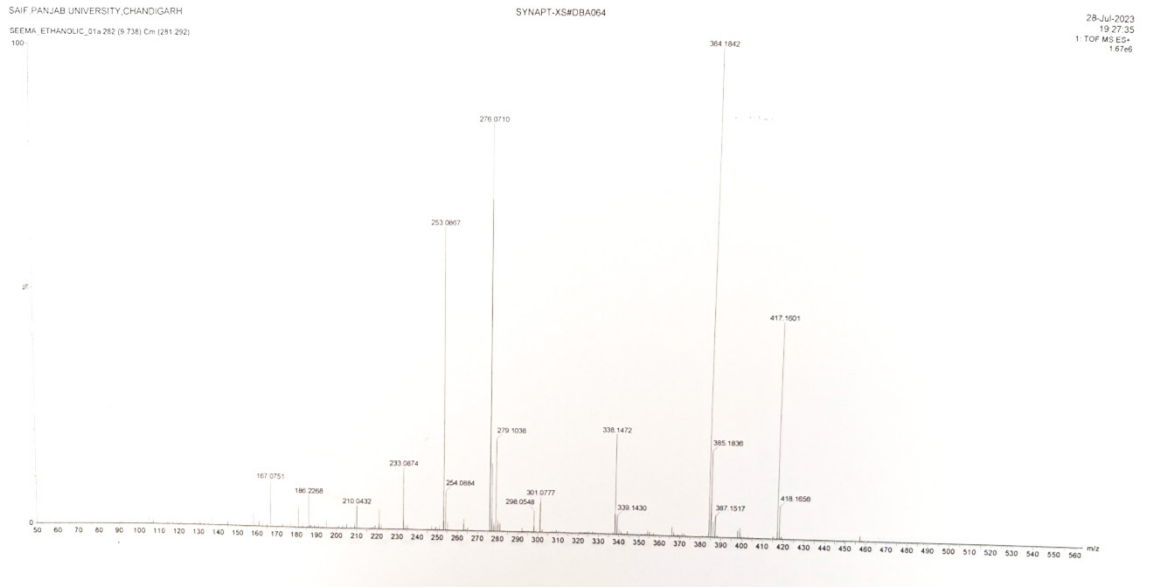


Figure 3: Mass spectrum of *M. champaca* Linn. at retention time 9.73.

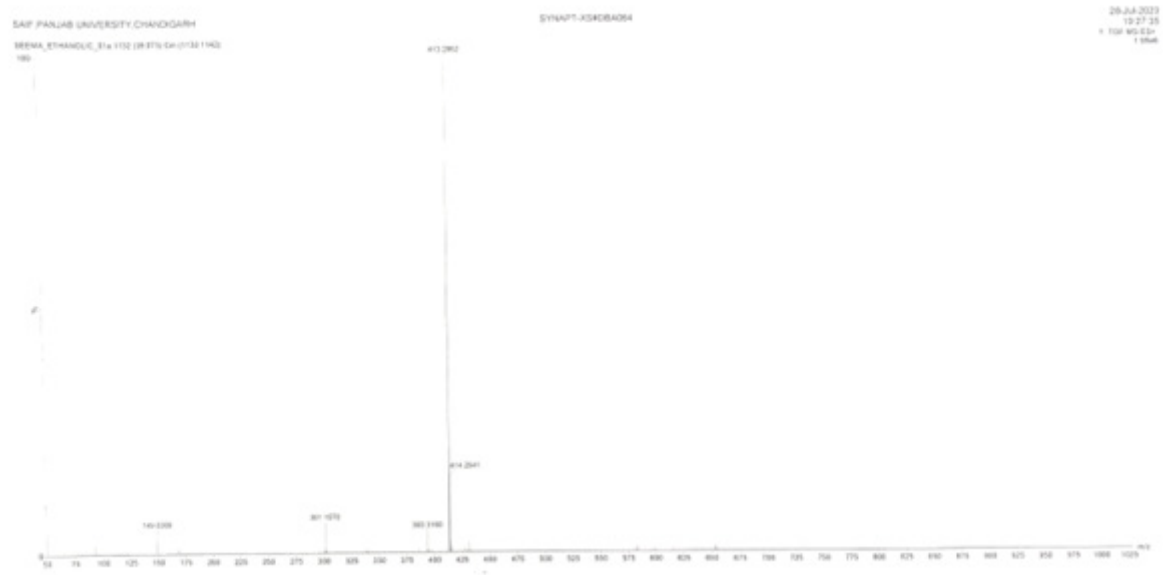


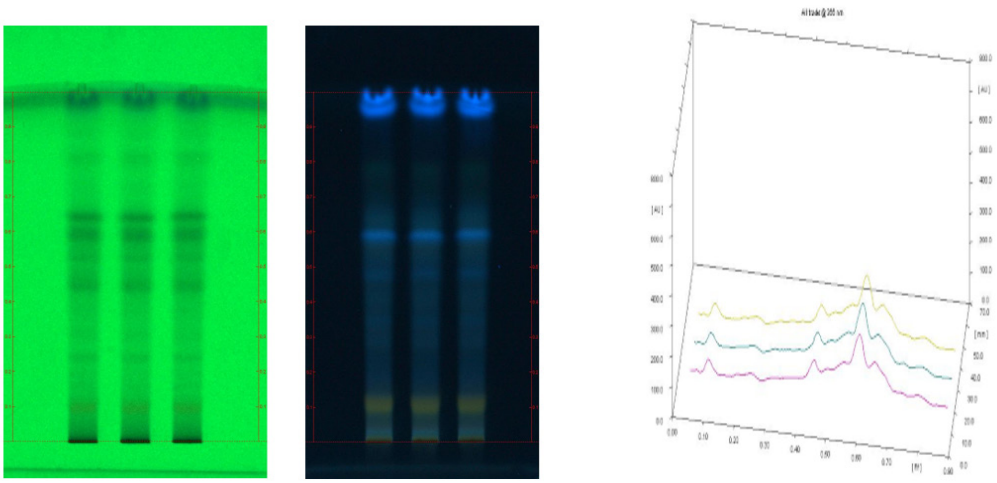
Figure 4: Mass spectrum of *M. champaca* Linn. at retention time 39.07.

HPTLC Analysis (High performance thin Layer chromatography)

The chromatogram shown in Figures 1-3 indicates that sample of Ethanol aerial extract of *Michelia champaca* Linn. was clearly separated without any tailed and diffuseness. It is evident from Figure 2, that 5 spots were visualized from the HPTLC of *M. champaca* at 399nm. The compound identified include Parthenolide, stigmasterol, sitosterol, Quercetin, Liriodenine showing R_f value 0.15, 0.3, 0.45, 0.6, 0.75 compared with Medline data base.

DISCUSSION AND CONCLUSION

The *Michelia champaca* Linn. is a traditional medicine plant used in the treatments of various ailments. The plant was extracted with ethanol and subjected for biological activity in our other study. The biological active extract explored for the active constituents responsible for its activity. The extract was subjected for preliminary phytochemical screening for the preliminary estimation of constituents. LC-MS of ethanol extract showed the various peaks at different retention time. The mass of the identified compounds compared with marker compounds using



(I) (II) (III)

I: HPTLC Florescence image of Ethanol extract of *M. champaca* 254 nm. **II:** HPTLC Florescence image of Ethanol extract of *M. champaca* 366 nm. **III.** HPTLC Chromatogram at 366 nm.

SDBS database. The elucidated compounds include parthenolide, Quercetine, Liriodenine, Stigmasterol and sitosterol and some unknown compounds. The HPTLC analysis confirmed presence of these compounds as per their R_f value compared with HPTLC-Derived database. These compounds belong to the category of alkaloids, flavonoids and steroids, Literature confirmed their presences in plant *M. champca* L. Hence the plant *M. champaca* should be further explored for the development of standard Herbal medicine.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

All the authors contributed to designing the manuscript, conceptualized, literature review, final draft, and contributed to data acquisition and data analysis for manuscript preparation, manuscript editing and revision at every stage.

SUMMARY

The plant *M. champaca*, known as yellow champa is used as traditional medicine. The ethanol extract of aerial parts of plant subjected for LC-MS and HPTLC analysis for the elucidation of phytoconstituents.

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