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Qualitative and quantitative phytochemical analysis of methanolic extract of *Magnolia champaca* leaves#

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Abstract

Plants remain a vibrant source of diverse bioactive phytochemicals that are secondary metabolites protecting them from infections and predations. Magnolia champaca is reported to possess a multitude of phytochemicals. In the present study, the phytochemical constituents of the methanolic extract of Magnolia champaca leaves were analysed qualitatively and with gas chromatography-tandem mass spectrometry (GC-MS/MS). Fourier transform infrared (FTIR) spectroscopic analysis was performed to identify the chemical nature of the extract and to find structurally similar compounds. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, terpenes, glycosides, phenols, steroids, tannins and saponins. FTIR spectroscopic analysis revealed structurally related compounds. GC-MS/MS analysis revealed the presence of 99 diverse compounds with varied biological activities, among which 1,2,4-butanetriol, n-hexadecanoic acid, cis vaccenic acid, phytol, trans longipinocarveol and caryophyllene oxide were found predominantly. Thus, the identification and characterisation of the phytochemicals in the extract favour the development of novel therapeutic agents.

Keywords: *Magnolia champaca, GC-MS/MS, FTIR, phytochemicals, phytol, caryophyllene oxide*

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Plants remain a vibrant source of diverse bioactive phytochemicals that are secondary metabolites that protects them from infections and predations. Even though synthetic pharmaceuticals are extensively available today and quite successful at treating a wide range of disorders, several people still favour using phytomedicines because of their less critical side effects.

Magnolia champaca commonly known as champak/chembakam is an evergreen tropical tree known for their scented flowers. Traditionally the plant parts were used for their beneficial activity against nausea, haemolysis, leprosy, ulcers, gout, cough, bronchitis, malaria and dysmenorrhea (Sinha and Varma, 2017).The plant possessed diverse pharmacological activities like antioxidant (Hasan *et al.,* 2020), antimicrobial (Daphedar *et al.,* 2020), schizonticidal (Mehrotra *et al.,* 2017), spasmolytic, air-way relaxing and vasodilating potential (Saqib *et al.,* 2018).Yesmin *et al.* (2021) established the antineoplastic potential of *M. champaca* bark methanolic extract against Ehrlich ascites carcinoma (EAC) in Swiss albino mice. All these pharmacological activities are due to the diverse phytochemical constituents contained in them. Thus, the present study was conducted to screen the phytochemical constituents qualitatively and quantitatively using FTIR spectroscopy and GC-MS/MS analysis.

Materials and methods

Collection and authentication of plant material

The leaves of *M. champaca* was collected from Mannarai, Tirupur, Tamilnadu (Fig. 1). The herbarium prepared from the collected leaf was authenticated from Raw material Herbarium and Museum Department (RHMD), Council of Scientific and Industrial Research (CSIR)-National Institute of Science Communication and Policy Research (NIScPR), New Delhi.

Extraction of plant material

The extraction procedure was carried out as per the standard protocol of hot continuous extraction method using Soxhlet apparatus. The leaves of *M. champaca* was collected, cleaned off dust and debris, shade dried by spreading on the paper.The dried leaves were coarsely pulverised using an electric pulveriser. Then, it was packed in a thimble, weighed and assembled in the Soxhlet apparatus with methanol (99 per cent, v/v) at 67°C. The methanolic extracts thus obtained was concentrated in the rotary vacuum evaporator under reduced pressure and temperature (40°C) in a pear-shaped flask. The dried extracts was stored in a wide necked container at -20°C in a deep freezer until further use. The yield of the prepared extract was calculated using the formula,

$$\text{Extraction yield (per cent)} = \frac{\text{Weight of the extract}}{\text{Weight of the sample taken}} \times 100$$

Qualitative phytochemical screening

The methanolic extract was subjected to qualitative analysis for the presence of various phytochemical components namely glycosides, tannins, steroids, phenolic compounds, alkaloids, flavonoids, diterpenes, triterpenes and saponins (Harborne, 1998).

Steroids in the extract was detected using Salkowski's test. Fifty milligrams of the extract were dissolved in three millilitres of chloroform. Few drops of concentrated sulphuric acid were added through the sides of the test tube and the solution was allowed to stand. A red colour denoted the presence of steroids.

One gram of the extract was mixed with five millilitres of liquid ammonia and then extracted with an equal volume of chloroform. To this, five millilitres of dilute hydrochloric acid was added.The acid layer obtained was further utilised for the detection of alkaloids. To one millilitre of the acid extract, eight drops of Dragendorff's reagent wereadded. Development of a reddish-brown precipitate indicated the presence of alkaloids. In Mayer's test, one millilitre of the acid layer was mixed with eight drops of Mayer's reagent. A cream-coloured precipitate signified the presence of alkaloids. To one millilitre of the acid layerone

millilitre of Wagner's reagent was added. Development of reddish-brown precipitate indicated the presence of alkaloids. For Hager's test, one millilitre of the acid layer was mixed with eight drops of Hager's reagent. A yellow precipitate denoted the presence of alkaloids.

For detection of glycosides sodium hydroxide test was used. Fifty milligrams of the extract were dissolved in one millilitre of distilled water. Six drops of 10 per cent sodium hydroxide solution were added to it. A yellow colour developed denoting the presence of glycosides.

Phenols in the extract was detected using ferric chloride test, for which five milligrams of the extract was dissolved in one millilitre of water and five drops of 10 per cent ferric chloride were added to it.A bluish black colour formed which signified the presence of phenols.

For detection of tannins, ferric chloride and gelatin test were employed. Treated two milligrams of the extract with three millilitres of one per cent ferric chloride solution. A brownish green or a blue-black colouration signified the presence of tannins. Similarly, one gram of the extract was mixed with a few drops of one per cent solution of gelatin containing 10 per cent sodium chloride.A white precipitate signified the presence of tannins.

Flavonoids in the sample was detected using ferric chloride and lead acetate test. Treated two millilitres of the methanol extract (500 mg extract in 10 mL methanol) with four drops of neutral ferric chloride solution. Formation of green colour indicated the presence of flavonoids. Likewise, treated two millilitres of the alcohol extract (500 mg extract in 10 mL methanol) with three millilitres of 10 per cent lead acetate solution.A yellow precipitate signified the presence of flavonoids.

About five milligrams extract were mixed with three millilitres of five per cent copper acetate solution. Development of green colour indicated the presence of diterpenes. While for detection of triterpenes, three milligrams of extract were mixed with three millilitres of chloroform and it was shaken with three millilitres concentrated sulphuric acid.A yellow colour in the lower layer on standing denoted the presence of triterpenes.

Saponins in the sample was detected using froth/foam test. Approximately 200 mg of the extract was shaken with five millilitres of water. Persistenceof foam produced for 10 min indicated the presence of saponins.

Gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis

The phytochemical constituents from the extract were analysed using GC-MS/MS system at Central Instruments Laboratory (CIL), College of Veterinary and Animal Sciences, Mannuthy, Kerala. The components of the extract were separated using gas chromatography (Trace 1300) and the separated constituents were analysed using TSQ 8000 MS/MS.The compounds were separated on TSQ-2MS capillary column (30 m × 0.25 mm; ID 0.25 µm film).The sample was dissolved in methanol, filtered in a 0.22 µm syringe filter and then used for further analysis. The column oven temperature was programmed from an initial temperature of 80°C for two minutes then increased at a rate of 15°C/min to 150°C for one minute and finally to 250°C for five minutes which was at a rate of 10°C/min. The oven run time was 20 min. The injection temperature and ion source temperature were 290°C and 230°C, respectively. Helium was used as the carrier gas at flow rate of 1mL/min. The ionising energy used was 70 eV. All the data were obtained by collecting the full-scan mass spectra within the scan range 50-500 atomic mass units (amu). Compounds were identified using the National Institute of Standards and Technology MS Search 2.0 library (Aathira *et al.,* 2021).

Fourier transform infrared (FTIR) spectroscopic analysis

To identify the structurally similar molecular compounds present in the extract, Attenuated Total Reflectance - Fourier transform infrared (ATR-FTIR) spectrometer analysis was performed. A Perkin- Elmer Spectrum Two™ FTIR spectrometer with attenuated total reflectance (ATR) was used in the current study. Spectrum was taken from 4000 cm^{-1} to 400 cm^{-1}

range using an infra-red (IR) spectrophotometer against the blank. The sampling station was equipped with an overhead ATR accessory. The ATR diamond crystal was carefully cleaned with pure isopropanol and a small quantity of the sample was placed carefully on the diamond crystal surface to cover the ATR diamond windowto focus the laser beam. Each spectrum was recorded as absorbance under 60 N and analysed using the FLUKA library (Poonghuzhali *et al.,* 2022).

Results and discussion

Extraction of plant material

The methanolic extract of *M. champaca* was prepared using hot continuous extraction method with an extraction yield of 21.26 per cent with reference to initial dry matter of the plant material. Ruwali *et al.* (2019) reported that the extraction yield of *M. champaca* leaves using methanol was higher (19.30 per cent) compared to other solvents like ethanol, hexane and chloroform, which was in harmony with the present study.

Qualitative phytochemical screening

The qualitative phytochemical screening of the methanolic extract revealed the presence of steroids, alkaloids, flavonoids, terpenes, phenols, glycosides, tannins and saponins (Plate 1). Ruwali *et al.* (2019) reported the presence of tannins, phenols, flavonoids, sterols, glycosides, alkaloids, triterpenes and lignin in the methanolic extract of the leaves of *M. champaca*. Karthikeyan *et al.* (2016) reported the presence of saponins, tannins, terpenoids, flavonoids and proteins in the ethanolic extract of the leaves of *M. champaca.* Mohan and Krishna (2019) reported the presence of alkaloids, sterols, diterpenes, phenols, flavonoids, glycosides, saponins, carbohydrates and tannins in the methanolic extract of leaves of *M.nilagirica.* These findings are in unanimity with those of the present study.

Gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis

The chromatogram obtained on phytochemical analysis of the extract through GC-MS/MS is given in Fig.2. The analysis revealed presence of 99 compounds. Phytoconstituents with greater peak area and probability, detected on the analysis are listed in Table 1.Butane-1,2,4-triol (11.17 per cent), n-hexadecanoic acid (7.85 per cent), 3-o-methyld-glucose (7.17 per cent), cis vaccenic acid (5.5 per cent), 11-octadecenoic acid methyl ester (4.74 per cent), phytol (3.66 per cent), hexadecanoic acid methyl ester (3.65 per cent), 9-octadecynoic acid (2.85 per cent), 9,12-octadecadienoic acid (2.52 per cent), octadecanoic acid (2.05 per cent), trans-longipinocarveol (1.71 per cent), caryophyllene oxide (1.21 per cent) and acrylic acid isoamyl ester (1.04 per cent) were found to be the major phytocompounds in the extract.

Ananthi and Chitra (2013a)identified the presence of 1,2,4-butanetriol, copaene, caryophyllene oxide, (-)-spathulenol, 3-O-methyl-d-glucose, hexadecanoic acid, methyl ester, n-hexadecanoic acid, 9,12-octadecadienoic acid, methyl ester, (E,E), 9,12,15- octadecadienoic acid, methyl ester, (Z,Z,Z) and 9,12- octadecadienoic acid (Z, Z) through GC-MS analysis of the methanolic extract of flowers of *M. champaca.* Similar compounds could be detected in the present study.

Wei *et al.* (2011) reported the presence of methyl β-d-galactopyranoside, octadecatrienoic acid and phenol in the methanolic extract of *M. champaca* upon GC-MS analysis. They also reported the presence of 9,12-octadecadienoic acid, methyl ester, (E,E), butanoic acid, 2-methyl-3-oxo-, ethyl ester and oleic acid in the methanolic extract of flowers of *M. champaca.*

Fourier transform infrared (FTIR) spectroscopic analysis

Based on the absorbance spectra as displayed in Fig.3, the structurally similar compounds identified in the extract by ATR-FTIR analysis using FLUKA library are depicted in Table 2.

Fourier transform infrared (FTIR) spectroscopic analysis is a crucial method for determining functional groups of

Table 1. Gas chromatography-tandem mass spectroscopic analysis of the methanolic extract of *M. champaca*

S. No.	Compound	Retention time (Min)	Peak area (%)	Probability (%)	PubChem ID
1.	DL-Arabinose	4.37	2.4	16.47	854
2.	3-Methylformate1-butanol	5.14	1.41	36.61	8052
3.	Acrylic acid isoamyl ester	5.37	1.04	16.57	77920
4.	3-Methyl but-2-yl ester formic acid	5.42	1.66	30.07	19810797
5.	6-Acetyl β-d-mannose	6.75	1	17.44	439780
6.	2(1H) Naphthalenone,3,4,4a,5,6,7 hexahydro 1,1,4a trimethyl	7.68	0.3	25.41	535380
7.	1,2,4-Butanetriol	8.73	11.17	44.63	18302
8.	Cyclohexane, 1-ethenyl 1-methyl 2,4bis-(1-methyl ethenyl),[1S(1α,2β,4β)]	9.06	1.94	20.09	10583
9.	2,5-Octadecadiynoic acid methyl ester	9.35	0.38	35.7	42151
10.	4-O-Methylmannose	10.27	0.28	30.36	345716
11.	2,4,5,5,8a Pentamethyl 6,7,8,8a-tetrahydro 5H-chromene	10.50	0.74	14.81	605742
12.	β-copaene	10.70	0.9	5.47	87529
13.	α-D-Glucopyranoside, O-α-D-glucopyranosyl (1.fwdarw.3) β-D-fructofuranosyl	10.90	0.67	16.35	92817
14.	Bicyclo [4.4.0] dec-2-ene-4-ol, 2-methyl 9-(prop-1-en-3-ol-2-yl)	11.00	0.64	31.04	535256
15.	Caryophyllene oxide	11.66	1.21	26.48	1742210
16.	2-Butenal, 2-methyl 4(2,6,6-trimethyl 1-cyclohexen-1-yl)	12.33	1.9	10.51	102375
17.	- (-) Spathulenol	12.60	0.82	2.56	92231
18.	trans Longipinocarveol	13.28	1.71	9.85	534645
19.	β-D-Glucopyranose, 4-O-β-D-galactopyranosyl	14.31	1.25	10.27	3086428
20.	Scyllo Inositol, 1C-methyl	14.51	0.31	7.28	102511252
21.	α-d-Mannofuranoside, methyl	14.61	0.39	7.9	12897794
22.	Myo Inositol, 4C-methyl	14.67	0.46	21.77	244581
23.	3-O-Methyl d-glucose	14.89	7.17	29.18	8973
24.	7,9-Ditertbutyl 1-oxaspiro (4,5) deca 6,9-diene 2,8-dione	15.07	0.91	72	545303
25.	Hexadecanoic acidmethyl ester	15.15	3.65	69.59	8181
26.	Benzene propanoic acid, 3,5bis (1,1-dimethyl ethyl) 4-hydroxy methyl ester	15.27	1.05	96.48	14401630
27.	n-Hexadecanoic acid	15.55	7.85	51.07	985
28.	Cedrandiol, 8S,13	16.16	0.87	8.97	536384
29.	Ethyl isoallocholate	16.28	0.27	9.85	154734451
30.	Desulphosinigrin	16.36	0.99	8.43	9601716
31.	6-(1-Hydroxy methyl vinyl) 4,8a-dimethyl 3,5,6,7,8,8a hexahydro-1H -naphthalen-2-one	16.64	0.53	5.81	564373
32.	9,12-Octadecadienoic acid	16.88	2.52	15.92	3931
33.	11-Octadecenoic acid methyl ester	16.95	4.74	4.77	5364432
34.	Phytol	17.06	3.66	68	5280435
35.	Methyl stearate	17.21	1.14	54.78	8201
36.	9-Octadecynoic acid	17.31	2.85	12.95	68167
37.	Cis-Vaccenic acid	17.39	5.5	11.34	5282761
38.	Octadecanoic acid	17.61	2.05	77.57	5281
39.	Trans-Geranylgeraniol	17.94	0.34	43.62	5281365
40.	Dasycarpidan1-methanol acetate (ester)	18.59	0.02	5.99	550072
41.	9,12,15-Octadecatrienoic acid, 2- [(trimethyl silyl) oxy] 1-[[(trimethyl silyl) oxy] methyl] ethyl ester, (Z, Z,Z)	18.66	0.13	8.8	5362857
42.	5,8-Dihydroxy-4a-methyl 4,4a,4b,5,6,7,8,8a,9,10-decahydro 2(3H)-phenanthrenone	19.14	0.25	18.4	91697217
43.	Xanthinin	19.65	0.12	7.22	160533
44.	Anobin	19.85	0.49	11.39	538430
45.	Arglabin	20.01	0.5	31.77	5574924
46.	Card-20(22)-enolide, 3,5,14,19-tetrahydroxy, (3β,5β)	20.2	0.23	6.54	12308767
47.	3H-Cyclodeca[b]furan-2-one,4,9-dihydroxy-6-methyl-3,10-dimethylene-3a,4,7,8,9,10,11,11a-octahydro	20.41	0.62	35.73	6110227
48.	Eicosanoic acid	20.48	0.15	56.26	10467
49.	α-D-Mannofuranoside, farnesyl	20.76	0.02	5.37	91694681

Table 2. Fourier transform infrared (FTIR) spectroscopic analysis of methanolic extract of *M. champaca*

Absorption (cm^{-1})	Appearance	Group	Compound Class	Structurally similar compounds
3305	Strong, broad	O-H stretching	Alcohol	1-Deoxy d-mannitol, - (-) Spathulenol, Longipinocarveol trans, β-D-Mannofuranoside farnesyl
2104	Weak	C≡C stretching	Alkyne	Methyl 10,12-pentacosadiynoate
1924	Weak	C-H bending	Aromatic compound	Xanthinin, Card-20(22)-enolide, 3,5,14,19-tetrahydroxy, (3β,5β), Anobin, 5,8-Dihydroxy-4a-methyl 4,4a,4b,5,6,7,8,8a,9,10-decahydro 2(3H)-phenanthrenone
1604	Medium	C=C stretching	Conjugated alkene	E, E, Z1,3,12-Nonadecatriene 5,14-diol
	Medium	N-H bending	Amine	Alanine, 3-amino
	Medium	C=C stretching	Cyclic alkene	4-Cyclopropyl carbonyl oxytridecane, 6- Ethoxy 6-methyl 2-cyclohexenone, Benzene propanoic acid, 3,5bis (1,1-dimethyl ethyl) 4-hydroxy, methyl ester
1213	Strong	C-O stretching	Vinyl ether	Card-20(22)-enolide, 3,5,14,19-tetrahydroxy, (3β,5β)
1118	Strong	C-O stretching	Aliphatic ether	β-D-Mannofuranoside, farnesyl
	Strong	C-O stretching	Secondary alcohol	1,2,3-Butanetriol, 1,2,4-Butanetriol, Myo Inositol, 2C-methyl, Scyllo Inositol, 1C-methyl, Myo Inositol, 4C-methyl, β-D-Mannofuranoside, farnesyl
894	Strong	C=C bending	Alkene	8-Isopropenyl 1,5-dimethyl cyclodeca 1,5-diene, β-D-Mannofuranosidefarnesyl, 7-Methyl Z-tetradecen-1-ol, acetate, Ethanol, 2-(9,12-octadecadienyloxy), (Z, Z)

phytochemicals present in the plant sample, as the wavelength of light absorbed is unique to the chemical bond contained in them (Lisha *et al.,* 2017). Ananthi and Chitra (2013b) analysed the methanolic extract of the flowers of *M. champaca* using FTIR spectroscopy and reported the presence of alcohols, aromatic compounds, aliphatic ethers, phenols, ketones and conjugated ethers. Ananthi and Kalaiselvi (2016) analysed the ethanolic extract of leaves of *M. champaca* using FTIR spectroscopy and confirmed the presence of O-H stretching, C=C stretching, C-H stretching, C-N stretching, C-O stretching and C≡C bonding, which correlates with the present study.

Among the diverse phytoconstituents identified, several compounds were reported to have biological activities. Phytol (Islam *et al.,* 2018), arglabin (Adekenov, 2015), ethyl iso-allocholate (Thakur and Ahirwar, 2019), transgeranylgeraniol (Campia *et al.,* 2009), desulphosinigrin (Krishnaveni, 2015), caryophyllene oxide (Fidyt *et al.,* 2016), 1-ethenyl1-methyl2,4-bis(1-methylethenyl), [1S(1α,2β,4β)] cyclohexane (Jiang *et al.,* 2017),α-D-Glucopyranoside, O-α-D-glucopyranosyl (1.fwdarw.3) β-D-fructofuranosyl(Behera and Balaji, 2021), β-copaene (Turkez *et al.,* 2014), - (-) spathulenol (Dzul-Beh *et al.,* 2019) and dasycarpidan1-methanol acetate ester (Al-Rubaye *et al.,* 2017) were reported to have anticancer activity.

n-Hexadecanoic acid was reported to have antioxidant, hypolipidemic, pesticide and nematicide activity (Sheela and Uthayakumari, 2013). Phytol and octadecanoic acid possessed anti-inflammatory activities (Silva *et al.,* 2014). Cis vaccenic acid was reported to have hypolipidemic and antimicrobial activity (Hamazaki *et al.,* 2016). 7,9-Ditertbutyl 1-oxaspiro (4,5) deca 6,9-diene 2,8-dione was reported to have antioxidant property (Riquet *et al.,* 2016).

Conclusion

The phytoconstituents in a plant are responsible for the various activities it possesses,though the activity of many of such compounds is not yet described. The present study revealed the presence of diverse classes of phytochemicals like alkaloids, steroids, glycosides, terpenes, flavonoids, tannin and saponins in the methanolic extract of *M. champaca* leaves. The GC-MS/MS analysis of a plant extract is the initial stage in exploring the active phytochemical ingredients contained in them and this kind of research will be useful for more in-depth investigation. Further research on these phytoconstituents may confirm their important medicinal properties in the future.

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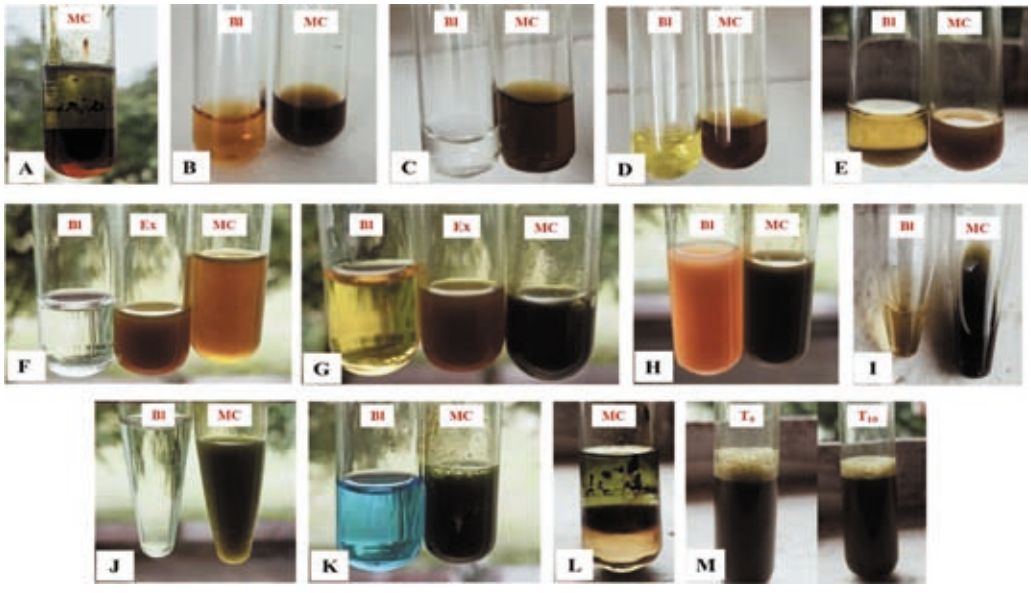
Fig. 1. Leaves of *Magnolia champaca*

Conflict of interest

The authors declare that they do not have any conflict of interest.



Bl – Blank (reagent alone); Ex – Extract alone; MC- Reagent + Extract; T_0 – Time zero; T_{10} – After 10 min

Plate 1. Phytochemical screening of methanolic extract of *Magnolia champaca.* A) Salkowski's test for steroids; B) Wagner's test C) Mayer's test; D) Hager's test; E) Dragendorff's test (B-E detection of alkaloids); F) Sodium hydroxide test for glycosides; G) Ferric chloride test for phenolic compounds; H) Ferric chloride test for tannins; I) Ferric chloride test for flavonoids; J) Lead acetate test for flavonoids; K)Copper acetate test for diterpenes; L) Salkowski's test for triterpenes; M) Foam test for saponins

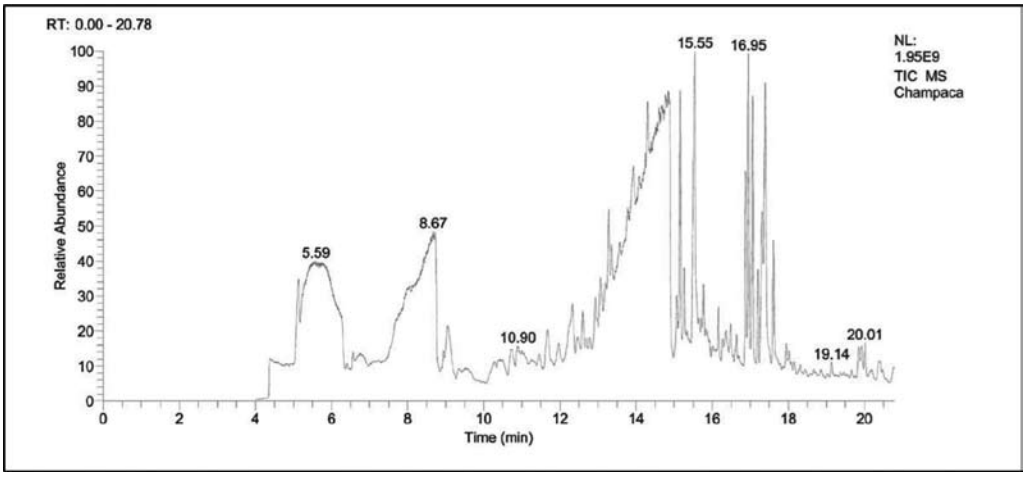


Fig. 2.Gas chromatography-tandem mass spectrometry chromatogram of methanolic extract of leaves of *Magnolia champaca* with time (min) along X axis and relative abundance along Y axis

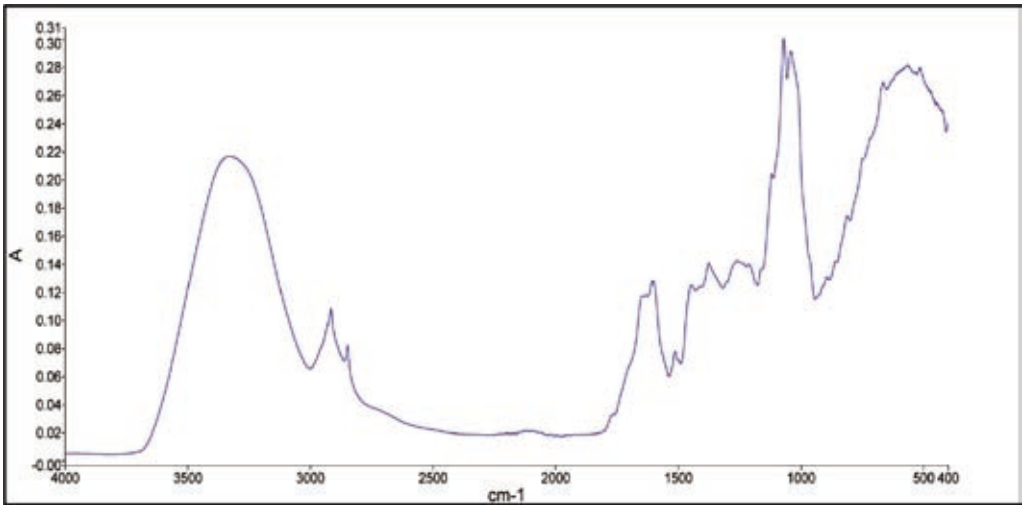


Fig. 3.FTIR spectra of methanolic extract of leaves of *Magnolia champaca* depicting the absorption peaks corresponding to chemical groups present, with wave number along X axis and absorbance along Y axis

References

Aathira, K.K., Kariyil, B.J., Dhanusha, G., Haima, J.S., Sujith, S., Shynu, M. and Nisha, A.R. 2021. Qualitative and quantitative analysis (GC-MS) of methanol extract of *Crataeva nurvala* stem bark. *J. Vet. Anim. Sci.* **52**: 135 - 141.

Adekenov, S.M. 2015. New compounds based on natural arglabin. Regio-and stereoselective synthesis. *Russ. Chem. Bull.* **64**: 743-751.

Al-Rubaye, A.F., Kaizal, A.F. and Hameed, I.H. 2017. Phytochemical screening of methanolic leaves extract of *Malva sylvestris*. *Int. J. Pharmacogn. Phytochem. Res.* **9**: 537-552.

Ananthi, T. and Chitra, M. 2013a. *In vitro* evaluation of antioxidant activity of *Michelia champaca* (L.) flowers. *Methods.* **13**:357-363.

Ananthi, T. and Chitra, M. 2013b. Analysis of flower extract of *Michelia champaca* (L.,) by FTIR spectrum. *Int. J. Phytopharm.* **4**: 68-70.

Ananthi, T. and Kalaiselvi, R. 2016. FT-IR analysis and *in vitro* anti-inflammatory

activity of leaf extract of *Michelia champaca* (L.). *World J. Pharm. Res.* **5**: 1263-1272.

Behera, P. and Balaji, S. 2021. The forgotten sugar: a review on multifarious applications of melezitose. *Carbohydr. Res.* **500**: 1-9.

Campia, I., Lussiana, C., Pescarmona, G., Ghigo, D., Bosia, A. and Riganti, C. 2009. Geranylgeraniol prevents the cytotoxic effects of mevastatin in THP-1 cells, without decreasing the beneficial effects on cholesterol synthesis. *Br. J. Pharmacol.* **158**: 1777-1786.

Daphedar, A., Ritti, M., Patil, N., Patil, S., Kakkalameli, S. and ChandrashekharappaT. T. 2020. Synthesis and characterization of silver nanoparticles from fruit extract of *Michelia champaca* L.: Their antioxidant and antibacterial activity. *Int. J. Nano Dimens.* **11**: 267-276.

Dzul-Beh, A.D.J., Garcia-Sosa, K., Uc-Cachon, A.H., Borquez, J., Loyola, L.A., Barrios-Garcia, H.B., Pena-Rodriguez, L.M. and Molina-Salinas, G.M. 2019. *In vitro* growth inhibition and bactericidal activity of spathulenol against drug-resistant clinical isolates of *Mycobacterium tuberculosis*. *Rev. Bras. Farmacogn.* **29**: 798-800.

Fidyt, K., Fiedorowicz, A., Strządała, L. and Szumny, A. 2016. β-caryophyllene and β-caryophyllene oxide—natural compounds of anticancer and analgesic properties. *Cancer Med.* **5**: 3007-3017.

Hamazaki, K., Suzuki, N., Kitamura, K.I., Hattori, A., Nagasawa, T., Itomura, M. and Hamazaki, T. 2016. Is vaccenic acid (18:1t n-7) associated with an increased incidence of hip fracture? An explanation for the calcium paradox. *Prostaglandins Leukot. Essent. Fatty Acids.* **109**: 8-12.

Harborne, J.B. 1998. *Phytochemical Methods: A guide to modern techniques of plant analysis.* (3rd Ed.) Chapman and Hall, India, 302p.

Hasan, M.M., Akter, M., Islam, M.E., Rahman, M.A.A. and Parvin, M.S. 2020. Evaluation of antioxidant and antibacterial properties of *Magnolia champaca* L. (Magnoliaceae) stem bark extract. *Bangladesh Pharm. J.* **23**: 96-102.

Islam, M.T., Ali, E.S., Uddin, S.J., Shaw, S., Islam, M.A., Ahmed, M.I., Shill, M.C., Karmakar, U.K., Yarla, N.S., Khan, I.N. and Billah, M.M. 2018. Phytol: A review of biomedical activities. *Food Chem. Toxicol.* **121**: 82-94.

Jiang, Z., Jacob, J.A., Loganathachetti, D.S., Nainangu, P. and Chen, B., 2017. β-Elemene: mechanistic studies on cancer cell interaction and its chemosensitization effect. *Front. Pharmacol.* **8**:1-7.

Karthikeyan, V., Balakrishnan, B.R., Senniappan, P., Janarthanan, L., Anandharaj, G. and Jaykar, B. 2016. Pharmacognostical, phyto-physicochemical profile of the leaves of *Michelia champaca* Linn. *Int. J. Pharm. Pharm. Res.* **7**: 331-344.

Krishnaveni, M. 2015. Docking, simulation studies of desulphosinigrin–cyclin dependent kinase 2, an anticancer drug target. *Int. J. Pharm. Sci. Rev. Res*. **30**:115-118.

Lisha, V., John, P., Usha, P.T.A., Sujith, S. and Ajith, J.G. 2017. Phytochemical screening and fourier transform infrared (FTIR) spectroscopy of *Averrhoabilimbi* fruit powd. *J. Vet. Anim. Sci.* **48**: 52-55.

Mehrotra, E., Vishwakarma, J., Tripathi, A.C., Sonar, P.K. and Saraf, S.K. 2017. Schizonticidal antimalarial sesquiterpene lactones from *Magnolia champaca* (L.) Baill. ex Pierre: microwave-assisted extraction, HPTLC fingerprinting and computational studies. *Nat. Prod. Res.* **33**: 568-572.

Mohan, M. and Krishna, M.P. 2019. Identification of phytochemical constituents of *Michelia nilagirica* leaves. *J. Phytochem. Biochem.* **3**: 1-5.

Poonghuzhali, R., Samraj, S., Nisha, A.R., Nair, S.N. and Priya, M.N. 2022. Phytopharmacological characterization of different extracts and fractions of *Cyclea peltata. J. Phytopharm*.**11**:155-158.

Riquet, A.M., Breysse, C., Dahbi, L., Loriot, C., Severin, I. and Chagnon, M.C. 2016. The consequences of physical post-treatments (microwave and electron-beam) on food/packaging interactions: A physicochemical and toxicological approach. *Food Chem.* **199**: 59-69.

Ruwali, P., Adhikari, M. and Sharma, S. 2019. Phytochemical and antioxidant properties of various extracts of *Michelia champaca* leaves. *Int. J. Pharm. Pharm. Sci.* **11**: 56-61.

Saqib, F., Mushtaq, Z., Janbaz, K.H., Imran, I., Deawnjee, S., Zia-Ul-Haq, M. and Dima, L. 2018. Pharmacological basis for the medicinal use of *Michelia champaca* in gut, airways and cardiovascular disorders. *Asian Pac. J. Trop. Med.* **11**: 292-296.

Sheela, D. and Uthayakumari, F. 2013. GC-MS analysis of bioactive constituents from coastal sand dune taxon-*Sesuvium portulacastrum* (L.). *Biosci. Discov.* **4**: 47-53.

Silva, R.O., Sousa, F.B.M., Damasceno, S.R., Carvalho, N.S., Silva, V.G., Oliveira, F.R.M., Sousa, D.P., Aragao, K.S., Barbosa, A.L., Freitas, R.M. and Medeiros, J.V.R. 2014. Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. *Fundam. Clin. Pharmacol.* **28**: 455-464.

Sinha, R. and Varma, R. 2017. Antioxidant activity in leaf extracts of *Michelia champaca* L. *J. Adv. Pharm. Educ. Res.* **7**: 86-88.

Thakur, R.S. and Ahirwar, B. 2019. A steroidal derivative from *Trigonella foenum graecum* L. that induces apoptosis *in vitro* and *in vivo*. *J. Food Drug Anal.* **27**: 231-239.

Turkez, H., Celik, K. and Togar, B. 2014. Effects of copaene, a tricyclic sesquiterpene, on human lymphocytes cells *in vitro*. *Cytotechnology.* **66**: 597-603.

Wei, L.S., Wee, W., Siong, J.Y.F. and Syamsumir, D.F. 2011. Characterization of antimicrobial, antioxidant, anticancer property and chemical composition of *Michelia champaca* seed and flower extracts. *Stamford J. Pharm. Sci.* **4**: 19-24.

Yesmin, R., Das, P.K., Belal, H., Aktar, S., Siddika, M., Asha, S.Y., Habib, F., Rakib, M. and Islam, F. 2021. Anticancer potential of *Michelia champaca* Linn. bark against Ehrlich ascites carcinoma (EAC) cells in Swiss albino mice. *J. Nat. Prod.* **11**: 85-96.

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