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GC-MS and FTIR guided phytochemical profiling and antioxidant activity evaluation of *Mitragyna parvifolia* (Roxb.) Korth. bark extracts obtained via ultrasound-assisted extraction

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ABSTRACT

Background: Plants have secondary metabolites, which can act as antioxidants to neutralize free radicals preventing oxidative stress related health issues. *Mitragyna parvifolia* (Roxb.) Korth. an ethnomedicinal medicinal plant of the Rubiaceae family that has extensive pharmacological properties. **Objective:** To study the effect of different solvents on extraction yield, phytochemical content and antioxidant potential of *M. parvifolia* bark and to profile the major phytochemicals in the most bioactive extract. **Materials and Methods:** Bark was extracted using methanol, ethanol, aqueous and hydroethanol via the ultrasound-assisted extraction method. Total phenolic content (TPC) and flavonoid content (TFC) of extracts are determined by folin-ciocalteau and aluminium chloride method. Antioxidant activity was evaluated using DPPH and Nitric oxide scavenging (NOSA) assays. The metabolite profiling of the bioactive rich extract was performed using Fourier transform infrared spectrometry (FTIR) and Gas chromatography-mass spectrometry (GC-MS). **Results:** Among the different solvent extracts of *M. parvifolia* bark, hydroethanolic extract (HEE) yielded maximum extraction efficiency (11.6 %) with the presence of phenols, alkaloids, flavonoids, saponins, terpenoids, glycosides, and steroids. HEE exhibited greater levels of TPC (120.78 ± 1.165 mg GAE/g) and TFC (76.89 ± 1.424 mg QE/g). In terms of antioxidant activity, HEE showed strongest DPPH and NOSA radical scavenging potential compared to other extracts with IC_{50} values of 114.27 ± 1.021 ug/mL and 123.47 ± 3.801ug/mL, respectively. The FTIR analysis confirmed the presence of alkanes, alkenes carboxylic and hydroxy groups indicating alcohols, phenols, terpenoids, alkaloids. GC-MS profiling of the HEE revealed the presence of 12 major bioactive phytochemicals, mainly 9-octadecenoic acid, methyl ester (60.67%), Hexadecanoic acid, methyl ester (10.41%), Squalene (1.96 %) and Pterin-6-carboxylic acid (1.25 %). **Conclusion:** The findings suggest that hydroethanol is the most effective solvent for extracting bioactive phytochemicals from *M. parvifolia* bark, supporting its traditional therapeutic relevance and potential as natural antioxidant source.

Keywords: *M. parvifolia*, Hydroethanol, Phytochemicals, Phytochemistry, Medicinal plant, Radical scavenging.

INTRODUCTION

During normal cellular metabolism, free radicals (FR) such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), are generated as metabolic by-products and play a vital role in cell signalling, immune response, and enzymatic regulation at low level [1]. However, when the human body is exposed to exogenous sources such as environmental toxins, UV radiation, cigarette smoking, and alcohol consumption, along with endogenous sources, leads to excess production of FR leading to oxidative stress. Oxidative stress and inflammation are interlinked, as an excess of FR can activate inflammatory pathways such as NF-κB, leading to the release of cytokines (TNF-α and IL-6), while immune cells activated by inflammation generates more FR [2]. This loop contributes to the pathogenesis of chronic inflammations such as cancer, diabetes, rheumatoid arthritis, accelerated aging, cardiovascular, and neurodegenerative diseases [3-5].

Under adverse conditions, plants synthesize wide range of secondary metabolites (phytochemicals) for survival, such as phenolic compounds, flavonoids, terpenoids, glycosides, carotenoids, and alkaloids. These bioactive compounds show antioxidant properties by inhibiting or neutralizing FR through different mechanisms such as radical scavenging, chelating or quenching [6]. It is estimated that approximately 80% of the world population still relies on herbal medicines as primary health care due to its effectiveness, accessibility, and lower side-effects [7]. Therefore, the evaluation of antioxidant potential can be an effective approach for screening of medicinal plants with anti-inflammatory potential.

Mitragyna parvifolia (Roxb.) Korth., commonly known as 'Kadamba' or 'kaim' (Figure 1). is an endangered tree belonging to the Rubiceace family [8]. It grows medium to large tree reaching up to 27 meters in height, is widely distributed throughout the tropical and subtropical regions of Africa, India, China, Bangladesh, Myanmar, Sri Lanka and Southeast Asia [9]. *M. parvifolia*, has been integral to traditional medicine among tribal and rural communities of Indian Subcontinent. The leaf juice is consumed for jaundice and burning sensations, while leaf paste to treat pimples, ulcers, wounds and skin infections. The bark and roots are used as a decoction for fever, colic, cough, cold, stomach and gynaecological disorders, or as a paste for muscular pain, wound healing, lice infestation, snake bites, leprosy and oral-health problems. The fruits are consumed for eye disorders, rheumatic conditions and also used as galactagogues and lactodepurants [9-13]. The phytochemistry of *M. parvifolia* have revealed the presence of a wide range of secondary metabolites including flavonoids, phenolics, terpenoids, saponins, with alkaloids as the predominant constituents [9]. Plant reported to contain oxindolic (pteropodine, isopteropodine, speciphylline, uncarine F, isomitraphylline, mitraphylline 16,17-dihydro-17β-hydroxy isomitraphylline, 16,17dihydro-17β-hydroxy mitraphylline) and indolic (tetrahydroalstonine, alkyamigine) type of alkaloids, along with pyroligneous acids, ketones and aldehydes [14,15]. The pharmacological studies of *M. parvifolia* leaves and barks extracts reported to show anticonvulsant, antimicrobial, anthelmintic, analgesic, anti-inflammatory, antinociceptive, and antioxidant properties [16-19]

Extraction is a critical primary step in recovering of phytochemicals from plant material as it influences extraction efficiency and the chemical profile of the extract [20]. In the present study, ultrasound-assisted extraction (UAE), a modern green extraction technique, was employed. UAE works on the principle of non-thermal acoustic cavitation phenomenon, enhancing cell wall disruption and improved solvent penetration, enabling efficient extraction of bioactive phytochemicals. In contrast to traditional methods such as Soxhlet extraction, UAE offers reduced solvents, extraction time, energy and eliminates the degradation of thermosensitive [21,22]. Second, the choice of the solvent for extraction strongly influences yield, phytochemical composition and biological activity of the extracts [23]. Despite ethnomedicinal relevance, *M. parvifolia* bark has not been systematically studied for the effect of solvent on their phytochemistry and antioxidant potential. This study investigates the influence of different solvents (methanol, ethanol, aqueous, and hydroethanol) on extraction yield, total phenolic and flavonoid content and *in vitro* antioxidant activities of *M. parvifolia* bark extract obtained using UAE. Further, the most bioactive extract was characterized using FTIR and GC-MS analyses to profile major phytoconstituents. These findings aim to validate the traditional use of *M. parvifolia* as a natural antioxidant source and providing a basis of future pharmacological investigations.

MATERIAL AND METHODS

Plant Material Collection and Authentication

The Raw bark of *M. parvifolia* was collected in June 2023 from Somaiya Vidyavihar campus, Mumbai (Maharashtra, India) (Figure 1). The specimen was authenticated at the Blatter Herbarium, St. Xavier's College, Mumbai and was identified as *M. parvifolia* (Roxb.) Korth. belonging to the family Rubiaceae. The specimen matched with the Blatter Herbarium specimen no. 433 of *H. Santapau*. Collected bark was washed under running tap, shade-dried, and pulverized into fine powder using a domestic electric grinder for further experiments.

Phytochemical Extraction Procedure

In this study, the UAE was employed based on the method suggested by Seyrekoğlu *et al*. [26] with minor modification. Briefly, 10 g of powdered *M. parvifolia* bark were extracted separately with 100 mL of methanol, ethanol, aqueous and hydroethanol (70:30, v/v). Extraction was carried out in an ultrasonic bath (ATS-3, Athena technology, India; 33 kHz, 100 W) for 45 min. The resulting mixture were filtered through Whatman No.1 and the filtrates were concentrated under reduced pressure using a rotary evaporator. The dried crude methanolic (ME), ethanolic (EE), Aqueous (AE) and hydroethanolic (HEE) bark extracts of *M. parvifolia* were weighed and stored at 4 °C for further analysis.

Extraction yield (%) was calculated using the following equation (1):

$$\text{Extraction Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{weight of initial plant material}} \times 100 \quad (1)$$

Qualitative phytochemical Screening

The Qualitative phytochemical screening for the presence of major secondary metabolites classes such as alkaloids, phenols, flavonoids, saponins, tannins, steroids, glycosides and terpenoids was assessed in all extracts of *M. parvifolia* bark using standard procedures [27].

Quantitative determination of Phytochemicals

Total Phenolic Content (TPC)

The Total phenolic content (TPC) was determined using the Folin-Ciocalteu (FC) method described by Singleton and Rosi [26], with minor modifications. Briefly, 0.5 mL of extract (1mg/mL) prepared in methanol was mixed with 2.5 mL of 10% FC reagent and 2 mL of 3.5 % sodium carbonate solution. The reaction mixture was incubated in the dark at room temperature for 60 min, and then the absorbance was measured at 765 nm using a UV-visible spectrophotometer (Jasco V-630 double-beam). TPC was calculated using a gallic acid calibration curve (10-100 μg/mL) and the results was expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined using the aluminium chloride colorimetric method described by Phuyal *et al*. [27] with slight modification. In detail, 0.5 mL of each extract (1 mg/mL) prepared in methanol was mixed well with 0.150 mL of 5% sodium nitrite and 0.150 mL of 2% aluminium chloride solution. After 10 min incubation, 1 mL of 1 M sodium hydroxide was added, and the total volume was adjusted to 5 mL with distilled water. The mixture was incubated in the dark for 30 min, and absorbance was recorded at 510 nm. The TFC was calculated using a quercetin calibration curve (10-120 µg/mL) and the results was expressed as milligrams of quercetin equivalent per gram of dry extract (mg QE/g).

In vitro antioxidant activity

DPPH Radical Scavenging Assay

The free radical scavenging capacity of *M. parvifolia* bark extracts was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method suggested by Blois [28]. Briefly, 2.9 mL of 0.2 mM DPPH solution prepared in methanol was mixed with 0.1 mL of extracts or standard (12.5 - 400 µg/mL). After 30 min of incubation in the dark at

RT, absorbance was recorded at 517 nm. The percentage scavenging of DPPH was calculated using the following formula:

$$\text{DPPH Radical Scavenging Activity (\%)} = \left[\frac{(A_0 - A_S)}{A_0}\right] \times 100 \quad (2)$$

Where A_S and A_0 is the absorbance of the DPPH solution with and without extract respectively.

Nitric Oxide Scavenging assay

The nitric oxide (NO) scavenging activity was evaluated according to the method described by Marcocci *et al*. [29] with slight modifications. Briefly, 0.5 mL of plant extract (12.5–100 µg/mL) was added to a mixture containing 2 mL of 10 mM sodium nitroprusside and 0.5 mL of phosphate-buffered saline and incubated at room temperature for 120 min. Then, 2 mL of the reaction mixture was treated with 2 mL of the freshly prepared Griess reagent [1:1 ratio of 1% sulphanilamide in 2.5% phosphoric acid and 0.1 % N-(1-naphthyl) ethylenediamine dihydrochloride (NED)] and incubated for 30 min. The absorbance of the resulting pink chromophore was recorded at 546 nm. Ascorbic acid was used as reference standard. The percentage scavenging of NO was calculated using equation (2). The antioxidant potency of each extract was compared based on their half-maximal inhibitory concentration (IC_{50}), the extract concentration required to scavenge 50% of the free radicals. The IC_{50} values were estimated from a nonlinear dose-response curve of scavenging percentages against different extract concentrations.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

Functional groups present in the HEE bark extract of *M. parvifolia* was identified using FTIR spectrophotometer (Shimadzu IRSpirit) equipped with an ATR accessory. Spectrum was recorded over the range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. The recorded FTIR peaks were interpreted using previous literatures to identify characteristic vibrational frequencies of key functional groups.

Phytochemical profiling by Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of the HEE extract of *M. parvifolia* was conducted using an Agilent 7890B GC system connected with a 5977A MSD, equipped with a DB-5MS Ultra Inert fused capillary column (30 m×250 µm ID × 0.25 µm film thickness). Helium gas (purity of 99.99%) was used as carrier gas at a constant flow rate of 1.2 mL/min. A 1 µL sample was injected in spitless mode. The injector and ion source temperatures were maintained at 230 °C. The oven program was initially held at 60 °C for 2 min, then ramped to 150 °C at 10 °C/min and then stabilized to 280 °C at same rate and held for 5 min [30]. The total run time was 20 min, with a 3 min solvent delay. The ionization energy was set at 70 eV and mass spectra were recorded in the m/z range of 35 to 600 amu. The chromatogram, retention times and fragmentation patterns were collected using a MassHunter software and compound identification was done using National Institute Standard and Technology (NIST version 2.3) spectral library.

Statistical Analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$. IC_{50} values were calculated from nonlinear dose response curves using GraphPad Prism (version 9.1.0). extracts.

RESULTS

Extraction yield

Highest yield was observed in the HEE (11.64%), followed by ME (7.9%), EE (6.12%), and least in AE extracts (4.9%) respectively.

Qualitative phytochemical screening

Different bark extracts of *M. parvifolia* were qualitatively screened for phytochemical classes and the observations depicted in Table 1. HEE and ME showed the presence of all major classes of phytochemicals including alkaloids, phenols, flavonoids, saponins, glycosides, tannins, steroids and terpenoids while the alkaloids and glycosides were absent in EE extract. The AE extract showed only phenolics, flavonoids, saponins and tannins.

Quantitative determination of Phytochemicals:

The total phenolic and flavonoid content of *M. parvifolia* bark extracts are summarized in Table 2. Results indicates that the HEE comprises higher level of phenolic content (120.78 ± 1.165 mg GAE/g) and flavonoid content (76.89 ± 1.424 mg QE/g) followed by ME, EE and least in AE extract.

***In vitro* antioxidant activities**

DPPH radical scavenging activity:

The DPPH radical scavenging activity of *M. parvifolia bark* extracts is shown in Figure 2(A). Scavenging profiles of all extracts and ascorbic acid was found to be concentration dependent manner. At concentration 400 ug/mL, HEE showed the maximum radical scavenging activity (91.01±1.235 %) and least in AE extract (57.93 ± 1.495%) compared to ascorbic acid (95.25±0.108%). The extract with lower IC_{50} values indicates higher activity (Figure 3). The HEE showed strongest activity with least IC_{50} value (114.27±1.021 ug/mL) compared to standard ascorbic acid (85.57±1.421 ug/mL)

Nitric oxide scavenging activity (NOSA)

The Scavenging activity of all extracts and ascorbic acid was found to in concentration dependent manner and is depicted in Figure 2 (B). At maximum concentration 400 ug/mL, maximum activity showed by HEE (79.78 ± 0.672%) and least in AE (59.19 ± 0.627%) respectively. The HEE bark extract of *M parvifolia* showed similar trend with lower IC_{50} value (123.47 ± 3.801 ug/mL) compared to standard ascorbic acid (109.53±3.009 ug/mL). The HEE of *M. parvifolia* bark showed higher antioxidant potential correlated with its rich phenolic and flavonoid content, was selected for further phytochemical characterization by FTIR and GC-MS analysis.

FTIR fingerprinting

The FTIR spectrum of the HEE extract of *M. parvifolia* bark over the frequency range of 4000 - 400 cm^{-1} depicted the distinct absorption peaks corresponding to major functional groups (Figure 4). A characteristic broad band at 3286.46 cm^{-1} corresponds to -OH stretching vibrations, indicating the presence of phenolics and aliphatic hydroxyl groups. Stretching vibrations of C-H group was detected at 2925.12 and 2854.86 cm^{-1}, indicating methylene and aromatic methoxy groups. A peak at 1691.98 cm^{-1} represents the stretching vibrations of C≡C groups, at 1442.48 cm^{-1} reflects the bending vibrations of amines and amides, indicating amino acids or proteins. The absorption peak at 1366.49 cm^{-1} indicates the presence of nitro compounds and at 1279.02 cm^{-1} corresponds esters and ethers. C-O stretching at 1039.56 cm^{-1} indicates the presence of primary alcohols. Furthermore, the peak at 874.67 and 767.13 cm^{-1} indicates the presence of alkenes, alkynes and alkyl halides [31-33].

Table 1: Qualitative phytochemical screening of Different solvent extracts of *M. parvifolia* bark

Phytochemical Class	ME	EE	AE	HEE
Alkaloids	+	-	-	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Glycosides	+	-	-	+
Tannins	+	+	+	+
Steroids	+	+	-	+
Terpenoids	+	+	-	+
Where signs indicate, '-': absence, '±': moderate, '+': presence				

Table 2: Total phenolic content and total flavonoid content of Different solvent extracts of *M. parvifolia* bark

Extract	TPC (mg GAE/g)	TFC (mg QE/g)
ME	102.48±1.561[b]	67.4±2.371[b]
EE	84.08±3.279[c]	41.9±2.544[c]
AE	65.49±1.306[d]	27.42±2.552[d]
HEE	120.78±1.165[a]	76.89±1.424[a]
Data are presented as means ± standard deviation of three replicates. ME: methanolic; EE: ethanolic extract; AE: Aqueous extract; HEE: hydroethanolic bark extracts. Statistical significance was determined at $p<0.05$ and indicated with different superscripts		

Table 3: List of identified major compounds of hydroethanolic (HEE) bark extract *of M. parvifolia*

S. No	RT (min)	Area %	Identified phytoconstituents	MF	MW
1	3.433	0.95	N-dl-Alanylglycine	$C_5H_{10}N_2O_3$	146.07
2	4.771	1.12	3-Octen-2-one, 3-butyl-	$C_{12}H_{22}O$	182.17
3	5.648	1.26	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	207.04
4	7.333	2.56	Methyl 6,8-octadecadiynoate	$C_{19}H_{30}O_2$	290.22
5	10.93	10.41	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.25
6	13.04	60.67	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.27
7	13.30	5.58	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	298.29
8	13.56	1.22	Cholestan-3-ol, 2-methylene-, (3β,5α)-	$C_{28}H_{48}O$	400.37
9	13.96	0.66	n-Propyl 9,12-hexadecadienoate	$C_{19}H_{34}O_2$	294.25
10	15.07	2.08	Cis-13-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324.30
11	16.112	1.08	Methyl 21-methlydocosanoate	$C_{24}H_{48}0_2$	368.36
12	17.32	1.96	Squalene	$C_{30}H_{50}$	410.01

Figure 1: *M. parvifolia* (Roxb.) Korth. (A) Tree (B) Bark

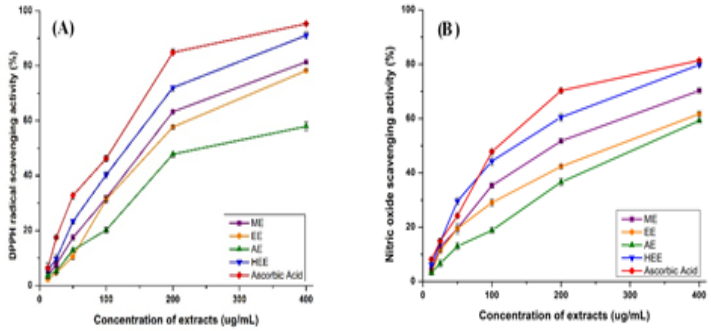
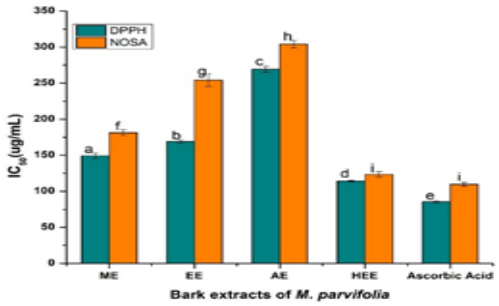


Figure 2: (A) DPPH radical scavenging activity (B) Nitric Oxide scavenging activity of different solvent extracts of *M. parvifolia* bark



ME: Methanolic extract; EE: ethanolic extract; AE: Aqueous extract; HEE: hydroethanolic extract. Statistical significance was determined at $p<0.05$ and indicated with different superscripts

Figure 3: Half maximal inhibitory concentration of *M. parvifolia* bark (IC_{50} ug/mL)

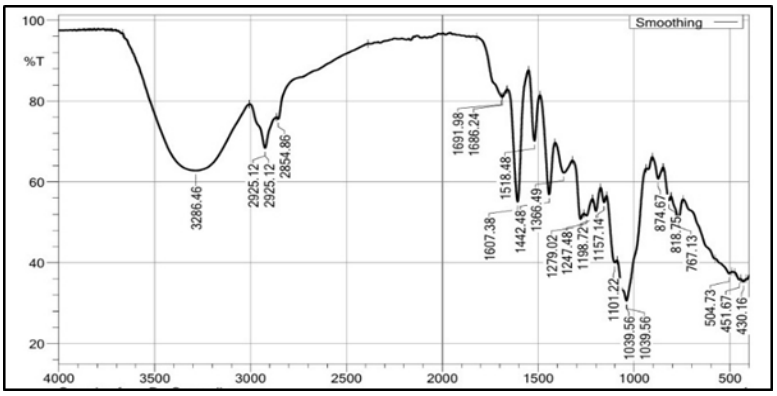


Figure 4: Infrared spectrum of the hydroethanolic (HEE) bark extract of *M. parvifolia*

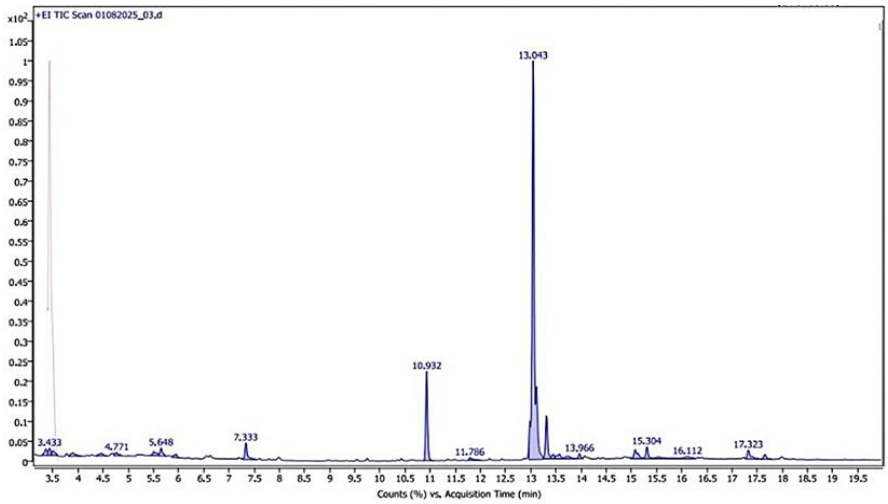


Figure 5: GC-MS chromatogram of hydroethanolic bark extract of *M. parvifolia*

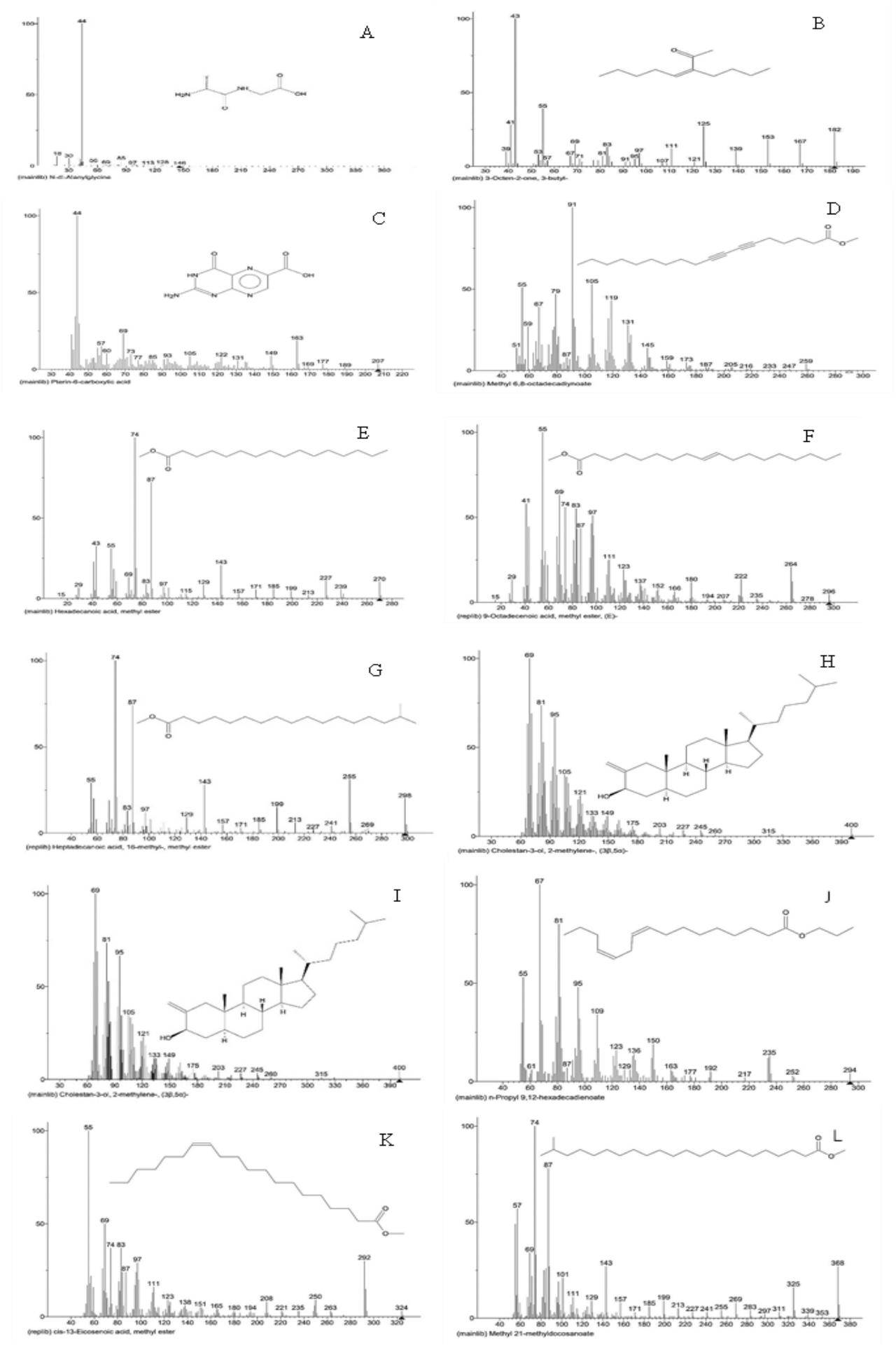


Figure 6: Mass spectra of identified compounds

Gas Chromatography-Mass Spectrometer analysis

The GC-MS chromatogram of HEE of *M. parvifolia* bark detected a total 28 peaks (Figure 5). Among them, 12 major bioactive compounds were identified based on their retention time, molecular weight, molecular formula, peak area and mass strum. The chromatogram showed dominant abundance of 9-Octadecenoic acid, methyl ester (60.67%), Hexadecanoic acid, methyl ester (10.41%) and heptadecanoic acid, 16-methyl-, methyl ester (5.58%), The other identified observations are summarized in Table 3 and mass spectrum of identified compounds are shown in Figure 6.

DISCUSSION

In this study, the impact of solvents polarity on extraction of Yield, phytochemical contents and antioxidant properties was investigated. Efficiency of solvent in terms of yield showed the order, HEE > ME > EE > AE. The higher yield observed in the HEE may be attributed to the synergistic polarity of ethanol and water, which facilitates efficient solubilization of both polar and moderately non-polar phytoconstituents [34]. The phytochemical screening indicated the presence phytochemicals including alkaloids, phenols, flavonoids, saponins, glycosides, tannins, and terpenoids in HEE bark extract of *M. parvifolia*. The observation agrees with previous reports that stem of *M. parvifolia* comprises of flavonoids, phenolics, saponins, and tannins [35]. This suggests that *M. parvifolia* bark possess significant bioactive properties, as these phytochemicals are known to exhibit antimicrobial, antifungal, antioxidant, and anti-inflammatory potentials [36].

Phenolics and flavonoids are known for their antioxidant properties in medicinal plants due to their structural ability to scavenge, neutralize, inhibit lipid peroxidation preventing oxidative damage [29]. TPC and TFC profiles of *M. parvifolia* bark showed order HEE > ME > EE > AE. These results are consistent with previous reports where hydroalcoholic solvent enhanced the extraction efficiency of phenolics and flavonoids. Another study conducted by Traore *et al*. reported that ultrasound method efficiently extracted the total polyphenols and flavonoids from *Cynometra ananta* stem bark with improved antioxidant activity [37]. In the present study, the antioxidant potential assessed through DPPH and NOSA assays. Different assays are used to check the capacity of phytochemicals in scavenging multiple radicals. DPPH is a purple-coloured stable free radical that accepts an electron or hydrogen to become yellow coloured stable diamagnetic molecule. The ability of the extracts to donate hydrogen to DPPH radicals was determined by decrease in absorbance at 517 nm [28]. Similarly, NO, a very unstable species converts in to nitrates and nitrites under aerobic conditions, leads to tissue damage and activation of pro-inflammatory mediators associated with inflammation [38]. NO is generated spontaneously by sodium nitroprusside under physiological pH and converts to nitrites. These nitrites react with Griess reagent and undergo diazo coupling reaction to form a pink azo dye. A decrease in absorbance at 546 nm indicates the extract's ability to prevent the nitrite formation [39]. Results indicated that the HEE showed the consistently strongest DPPH and NO scavenging activity with lower IC_{50} values with order HEE > ME > EE >AE for both assays. However, maximum activity was observed for DPPH than NO. These variation in results is due to different phytochemicals present in extract act differently in scavenging radicals [39]. Kaushik *et al*. reported leaves of *M. parvifolia* showed maximum activity at 500 ug/mL, suggesting the bark *M. parvifolia* may exhibit more potency correlated with rich phytochemicals composition [40]. The variation in results can be due to the geographical variation of medicinal plants, the nature of the antioxidant assays, and the season of the sample collection.

The FTIR analysis of bioactive rich HEE extract of *M. parvifolia* bark confirmed the presence of functional groups indicating the presence phytochemicals such hydroxyl, carbonyl, methylene groups suggesting the presence of terpenoids, phenols, tannins, and flavonoids, responsible for its antioxidant activities. The existence of diverse bioactive compounds in the HEE bark extract *M. parvifolia* was confirmed by GC-MS analysis. The 12 major identified compounds are reported to possess several biological activities. Among them, 9-Octadecenoic acid, methyl ester reported to show antioxidant, anticancer, and anti-inflammatory activities [41]. Hexadecenoic acid also called palmitic acid reported for antioxidant, antimicrobial properties [42]. Pterin-6-carboxylic acid reported anti-psychotic, mood stabilizer and anti-parasite properties [43]. Squalene was known to have antibacterial, antioxidant, cancer preventive and immunostimulant properties [44]. Other compounds identified (Table 4) also reported to show several bioactive properties. There is no previous report published the GC-MS profiling of hydroethanolic bark extract of *M. parvifolia*. The observations of the current investigation highlights that the antioxidant activity of *M. parvifolia* bark may be due to the presence of diverse class of phytochemicals including phenols, fatty acid esters and flavonoids.

CONCLUSION

In the present work, it was found that *M. parvifolia* bark possess a promising source of antioxidants. Among the various solvents used for extraction, HEE exhibited the highest phenolic and flavonoid contents and potent antioxidant activity. UAE proved to be an effective green technique for extracting bioactive phytochemicals. Furthermore, FTIR and GC-MS analysis confirmed and identified the presence of several compounds in the extract of *M. parvifolia* bark responsible for its activity. These findings validate the traditional use of *M. parvifolia* and support as a good source of antioxidants. Further isolation of bioactive components and in vivo studies are needed to explore its full pharmacological potential.

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Conflict of interest

The authors declared no conflict of interest.

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