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Correlation study among the extraction techniques, phytochemicals, and antioxidant activity of *Nepeta spicata* aerial part

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ABSTRACT

In present study, two extraction methodologies (soxhlet and ultrasonication) were employed where four solvents (methanol, acetone, chloroform, and hexane) were used to check the correlation among the extraction techniques, phytochemicals, and antioxidant activity of *Nepeta spicata* aerial part. The antioxidant potential was assesed by using DPPH free radical scavenging assay, Ferric reducing antioxidant power assay (FRAP), and total antioxidant capacity (TAC). The FTIR and GC-MS techniques were employed for the quantitative analysis of crude extracts four solvents obtained by using two extraction methodologies (soxhlet and ultrasonication). The percentage yield obtained by the soxhlet was better than the ultrasonication. Further, the results showed that the extracts obtained by polar solvents (methanol ≥ acetone ≫ chloroform > hexane) possessed a higher content of total polyphenols, total flavonoids and antioxidant activities. Overall, soxhlet extraction technique was effective and methanol have shown significant extraction. The GC-MS data of crude extracts of plant material showed the occurrence of fatty acids, steroids, long chain hydrocarbons including hexadecanoic acid, linoleic acid, phytol, hexadecanoic acid, stigmast-5-en-3-ol and stigmast-4-en-3-one. Overall, based on GC-MS study, aerial part of the *Nepeta* spicata exhibited significant among of antioxidants in polar solvents.

1. Introduction

Aromatic medicinal plants form the basis of health care throughout the world, since quite long and are still widely used due to their effectual medicinal and nutritious properties (Joshi, 2014; (Kumar et al., 2018 & 2019a). These plant based natural products contain secondary metabolites (SMs) which possess a wide range of pharmacological activities, the primary being the antioxidant activity (Kumar et al., 2019b,c&d). The latter is mainly due to the presence of large amounts of polyphenols along with other species that help in adsorption and neutralization of free radicals or decompose peroxides; hence prevent the occurrence of many diseases (Della-Greca et al., 2009; Kumar et al., 2013 & 2014). Environmental factors, genetic factors, as well as post harvest processing conditions immensely effect the composition of plants, phenolic content and the products produced from them (Albayrak et al., 2017; Aspe and Fernandez, 2011).

Aromatic medicinal plants form the basis of health care throughout the world, since quite long and are still widely used due to their effectual medicinal and nutritious properties (Joshi, 2014). These plant based natural products contain SMs which possess a wide range of pharmacological activities, the primary being the antioxidant activity. The latter is mainly due to the presence of large amounts of polyphenols along with other species that help in adsorption and neutralization of free radicals or decompose peroxides; hence prevent the occurrence of many diseases (Della-Greca et al., 2009; Kumar et al., 2019a, 2019b). Environmental factors, post harvest processing conditions as well as genetic factors greatly affect the chemical composition of plants, phenolic contents, and products produced from them. Traditional medicines have spectacular applications in the global health due to its relatively safe therapeutic results reported by World Health Organization (WHO) (Owolabi et al., 2007). Different parts of aromatic plants viz.-roots, flowers, stems, leaves as such or in the modified forms has been used as drugs, nutraceuticals, food supplements, folk medicines, food additives, flavoring agents, herbicides, fungicides, etc. (Bandh et al., 2011; Handa, 2008; Kumar et al., 2019c, 2019d; Rahman et al., 2011). Hence, there is always a constant increase in their demand. On account of this, the aromatic plants play an important role in the daily routine of especially tribal people acting as both food and medicine (Bandh et al., 2011; Batool et al., 2018; Bhati et al., 2019; Kapoor et al., 2019; Kumar et al., 2018).

The genus *Nepeta* belonged to the family Lamiaceae having almost 300 species distributed in Southern and Central Europe, North and Central America, Southwest and Central Asia and North Africa. (Bandh et al., 2011; Batool et al., 2018). The Western Himalayas are the main

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region of *Nepeta* species in India and having approximate forty one species including *spicata*. The phytochemical report of Nepeta species revealed the presence of biologically active secondary metabolites like terpenoids, steroids, glycosides, tannins, phenolic acids and flavonoids (Formisano et al., 2011). These SMs have been used as diaphoretic, febrifuge, antioxidant, anti-tussive, sedative, antiseptic, anti-asthmatic, insecticidal, antimicrobial, fungicidal and anti-viral agents, besides, being used for the healing of various problems viz. teeth troubles, kidney, ear pain, dysentery, liver infections and lots of heart problems thereby exhibiting the huge biological potential (Kumar et al., 2014; Micelia et al., 2005).

As best of our knowledge, till date there was no investigation has been carried out to study the phytochemical composition and biological potential of *Nepeta spicata*. The present work would play an essential role in highlighting the medicinal value of particular species. Keeping the above-discussion in view, the present study for the first time was aimed to explore the phytochemical composition and biological activity of *Nepeta spicata* from India.

2. Materials and methods

2.1. Chemicals and reagents

Sodium nitrite, sodium carbonate, sodium acetate, sodium hydroxide, ferrous sulphate, ferric chloride and benzoic acid were obtained from Avantor Performance Materials (RANKEM) Pvt. Ltd, Gurgaon, India. Vitamin C, sulphanilamide, rutin trihydrate, gallic acid, Folin-Ciocalteu phenol reagent, chloroform and hexane were purchased from Loba Chemie Pvt. Ltd, Mumbai, India. Sulfuric acid, hydrochloric acid, glacial acetic acid, aluminum chloride, acetonitrile (HPLC grade) and methanol were obtained from Merck, Pvt. Ltd, Mumbai, India. 2,4,6-tripyridyl-*s*-triazine (TPTZ), 2,2-Diphenyl-1-picrylhydrazyl (DPPH˙) and glacial acetic acid (HPLC grade) were purchased from Otto chemie, Pvt. Ltd, Mumbai. Sodium phosphate, sodium nitroprusside, orthophosphoric acid and ammonium molybdate were obtained from Sd fine chemicals, Pvt. Ltd, Mumbai. N-(1-naphthyl) ethylenediamine dihydrochloride was obtained from Spectrochem, Pvt. Ltd, Mumbai. All other reagents, solvents and chemicals used were of laboratory reagent grade. The millipore water used throughout the experimental work was obtained from Millipore Direct Q 3 (Millipore water kit).

2.2. Plant material

The plant was obtained from Manimahesh hills (Hadsar) in the Chamba district of Himachal Pradesh. The Hadsar (Manimhesh hills) i.e. at an elevation of more than 2000 m was the collecting point of plant in Himachal Pradesh (district Chamba). The authentication was carried out by submitting the specimen in herbarium. The Professor M.I.S Sagoo from Punjabi University (Department of Botany) was authenticated the whole plant and voucher no. is PUN58875. The dry leaves of this plant were grinded using an electrical grinder. The plant material was accumulated in sealed polyethylene packets at less than 4 °C temperature.

2.3. Extraction techniques

The dried powder of plant material of was extracted by using soxhlet and ultrasonication methods with the help of solvents of increasing polarity viz. hexane, chloroform, acetone and methanol.

2.3.1. Extraction using soxhlet method (SEM)

The dried leaves (80g) of *Nepeta spicata* were extracted by soxhlet apparatus with 300 mL of solvents and having different polarities viz. methanol, acetone, hexane and chloroform. After 12 h, when extraction was completed, the filter paper (Whatman no.1) and rotary evaporator at 40°C were used for filtration (thrice) and solvent evaporation of the extracts. The different solvents extracts of selected plant were stored in cooled (< 4°C) for further analysis i.e phytochemicals and polyphenolic analysis. The same procedure was followed for other solvent systems viz. methanol, acetone, hexane and chloroform.

2.3.2. Extraction using ultrasonication assisted extraction method (UEM)

In this method, the extraction was assisted by the use of ultrasound frequencies ranging from 20 kHz to 2000 kHz. The USA, Cole Parmer model CPX 500 (500 W, 20 kHz) was used having 3/4", 125 mm L × 19 mm diameter replaceable tip and threaded end standard Horn probe. The power of leaves (40g) of *Nepeta spicata* were extracted by solvents (200 mL) with increasing order of polarity at controlled temperature 50 °C with amplitude at 50%. The powered leaves of plant material extracted three times i.e. 25, 20 and 15 min and having 100, 60 and 40 mL solvent respectively. When extraction was completed, same steps repeated as mention above.

2.4. Phytochemical screening of extracts

The extracts of the leaves of plant *Nepeta spicata* (obtained in solvents-methanol, acetone, hexane and chloroform) were taken for phytochemical screening in order to determine the presence of phytochemicals viz. carbohydrates, glycosides, alkaloids, tannins, flavonoids, saponins, resins, diterpenoids etc. These four extracts of the plant were procured by employing two different extraction techniques viz. soxhlet extraction and ultrasonication. Specific qualitative tests for phytochemicals were performed to identify the constituents in the four different extracts of the plant.

2.5. Total polyphenolic content

The Folin-Ciocalteu reagent and gallic acid as standard used for the quantification of total polyphenolic yields of different extracts by the approach illustrated by Stoilova et al. with a few modifications. The experimental data analyzed as mg of GAE (gallic acid equivalents)/g of DPE (dehydrated plant extract). The 10 mL deionised water mixed in standard and various plant extracts (1 mL; mg/mL) followed with 1 mL Folin-Ciocalteu phenolic reagent. The whole mixture was stand up at room temperature for 5 min then adding 2 mL solution of sodium carbonate (20%). After 60 min incubation in dark, the samples absorbance (750 nm) were analyzed with the help of UV–Visible spectrophotometer (Shimadzu, 1800). All the samples were analyzed in triplicate.

2.6. Total flavonoids content (TFC)

In this method (Zhishen et al., 1999), rutin trihydrate (standard) and aluminum chloride were used for the identification of total flavonoids content of different extracts. Total flavonoid content was denoted as mg of RE (rutin trihydrate equivalents)/g of DPE (dehydrated plant extract). The deionised water (4 mL) added in standard and various extracts (1 mL; 1 mg/mL) followed by 0.3 mL of sodium nitrite solution (5% w/v). After 5 min incubation, 2 mL sodium hydroxide (1M) and 0.3 mL aluminium chloride (10%, w/v) were added in whole mixture solution. Now, 2.4 mL deionised water was deposit in to mixture solution by mean of made 10 mL volume of mixture solution. Finally, Shimadzu (1800) UV–Visible spectrophotometer absorbance (510 nm) was measured. The total flavonoid content determination of different samples was repeated triply and articulated as mg of rutin trihydrate equivalents (RE)/g by DPE.

2.7. Estimation of antioxidant potential

The DPPH radical scavenging method, Molybdenum reduction method and FRAP method were used to determine the biological potential i.e. antioxidant activities of various crude extracts of plant material.

2.7.1. Free radical scavenging activity by stable DPPH radical

Based on electron-transfer, 2,2-Diphenyl-1-picryl-hydrazyl-hydrate free radical method used by Miliauskas et al. (2004) was followed. 3 mL methanol solution of DPPH (0.004%) was added in to the standard (ascorbic acid) and different plant extracts (0.2 mL; 1 mg/mL) and whole mixture was incubated (30 min) in dark region at room temperature. Finally, the UV–Visible spectrophotometer 1800 (Shimadzu) was used for the measurement of absorbance at 517 nm. The each and every sample of crude extracts of selected plant was evaluated thrice and following equation applied for estimation of percentage inhibition (I %) of DPPH free radical. I_S and I_C are the absorbance of standard or samples and control respectively i.e. I % [DPPH free radical] = $[(I_C–I_S)/I_C] \times 100$.

2.7.2. Determination of total antioxidant capacity by Mo(VI)

The phosphomolybdenum reducing method described by the Prieto et al. (1999) was operated to determine the TAC values of the various extracts. The Ascorbic acid (standard) and each extract (0.3 mL: 1 mg/mL) were added to 3 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). At 95 °C, the test tubes mixture were incubated for 90 min and cooled at room temperature. The UV–Visible spectrophotometer, 1800 (Shimadzu) was used against blank for measuring absorbance at 695 nm. The results were carried out triplicates and denoted like mg of ascorbic acid equivalent (AAE)/1 g of DPE.

2.7.3. Determination of antioxidant capacity by FRAP method

The antioxidant potential of different extracts in FRAP (ferric ion reducing antioxidant power) was estimated by the method demonstrated by Benzie et al. Initially, FRAP solution was prepared by mixing 20 mM of $FeCl_3 \cdot 6H_2O$ solution (2.5 mL), 300 mM of acetate buffer (25 mL) and 10 mM in 40 mM HCl solution of TPTZ (2,4,6-tripyridyl-*s*-triazine, 2.5 mL). After that, 0.2 mL of standard (Ferrous sulphate) and numerous extract solutions (0.5 mg/mL) were put in FRAP solution. In dark, the samples were incubated for 30 min at room temperature. At last, the absorbance was noted with the help of Shimadzu (1800) UV–Visible spectrophotometer at 593 nm. The entire testing was approved out three times and the outcomes were stated as mg of ferrous II equivalent (Fe (II) E)/1 g of DPE.

2.8. Analytical techniques

2.8.1. FTIR analysis

The previous results shows that the Fourier Transform Infrared Spectrophotometer (FTIR) was used to identified the functional groups of dried powders of different extracts of plant material. The translucent sample discs were prepared by mixing of 10 mg KBr salt and 1 mg fine dried powder of different extracts of plant material. These pellets of powdered mixture were packed in FTIR spectroscope, Shimadzu, Japan (4 cm^{-1} resolution), having frequency range from 4000 to 400 cm^{-1}.

2.8.2. GC-MS analysis

The literature showed that the bioactive crude extracts of plant material illustrate the numerous classes of phytochemicals with the help of GC–MS technique (Udayaprakash et al., 2015). The GC-MS technique i.e. Mass spectroscopy (GC–MS, QP 2010 Ultra) attached with GC system (Shimadzu GC-2010 Plus) of different extracts with different polarity by the methods of soxhlet and ultrasonication was performed. Thought out the process, RTx-5Sil MS (Restesk USA) capillary column (30 mm × 0.25 mm × 0.25 mm) was utilized. The split ratio and flow rate of carrier gas (Helium) was 1:5 and 1 mL/min. and 280°C was the injection temperature. The temperature of the oven increased from 100°C to 250°C at the flow rate 4°C/min and maintained for 5 min. Further, temperature was raised (280°C) at the rate 5°C/min. At last, the temperature was holed at 280°C for 30 min. The electron ionization (EI) mode was used in mass spectra and having range 40–700 m/z at 70 eV. The interface and ion source temperature was upheld at 280 °C and 200°C respectively. The mass spectral data of NIST11 and Wiley8 library were used to identification of different compounds by comparing mass spectral data of both compound and library. The LOD (limits of detection) (0.002–0.14 μg/g), average recovery (85–96%) and LOQ (limits of quantification) (0.03–0.30 μg/g) values were showed the sensitivity of give method.

2.9. Statistical analysis

All the outcomes have been directed and characterized by (Statistical 7 software, MS excel and computer programmes) and (mean ± standard deviation). The results significance was resolved with the help of Duncan's multiple range tests i.e. A one way ANOVA (analysis of variance). In ANOVA, there was noticeable correlation between various assay and considered significant values of probability (p) was < 0.05.

3. Results and discussion

3.1. Percentage yield

The dried powder of leaves of *Nepeta spicata* were extracted by the two methods – soxhlet and the ultrasonication using four solvents of increasing polarities order like hexane, chloroform, acetone and methanol. The percentage yield crude extracts was ranged from 1.1 to 6.68% and depends on nature of the solvent and type of method. A comparison of the employed methodologies showed that the materials extracted from soxhlet method (SEM) were higher in yields as compared to ultrasonication technique (UEM). However, the time taken to complete the extraction in former case (12 h) was found to be higher as compared to the latter (1 h). Further, the percentage yields of the materials with different solvents were compared, it was noted that methanol extract obtained from the SEM provided the maximum yield (6.68%), whereas acetone, chloroform and hexane extracts gave 2.82%, 2.70% and 3.10% respectively. Similar, trend was observed in the case of UEM, whereas methanol, acetone, chloroform and hexane extracts provided 5.77%, 1.97%, 1.57% and 1.1% yields, respectively [Fig. 1].

The above table shows that non-polar solvent i.e. hexane in ultrasonication method gave least percentage yield of the extracted material as compared to the other solvents. From the above results, it was also clear that the soxhlet method gives better yield as compared to ultrasonication method. The percentage yield of various extracts by both the methods followed same order i.e. methanol > acetone > chloroform > hexane. It has been observed from literature that the highly polar solvents has maximum percentage yield due to the solubation of

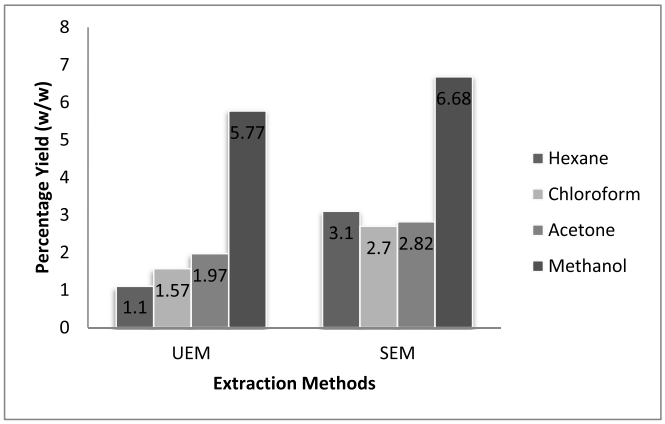

Fig. 1. The yield/percentage yield of various extracts (UEM -ultrasonic assisted extraction method and SEM -soxhlet extraction method) obtained from the powdered leaves of *N. Spicata*.

Table 1
Results of qualitative phytochemical analysis of various extracts acquired from *N. spicata*.

Phytochemicals	Methanol extract		Acetone extract		Chloroform extract		Hexane extract	
	SEM	UEM	SEM	UEM	SEM	UEM	SEM	UEM
Carbohydrates	+	+	+	+	+	+	–	–
Glycosides	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	–	–	–	–
Triterpenoids	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+
Polyphenols	+	+	+	+	–	–	–	–
Saponins	+	+	+	+	–	–	–	–
Alkaloids	+	+	–	–	–	–	–	–
Diterpenoids	+	+	+	+	+	+	–	–

UEM - ultrasonic assisted extraction method and SEM -soxhlet extraction method, + and – indicates the presence and absence of corresponding SMs in the test extract.

secondary metabolites that has maximum polar nature (Kalia et al., 2008). The literature also supported these factors in various plants like extraction of the bark of *Pinus radiate* (Aspe and Fernandez, 2011), leaves, fruit, pulp and seed of *Hippophae rhamnoides*, flower of *Opuntia ficus-indica*, whole plant of *Torilis leptophylla* (Saeed et al., 2012) and aerial parts of *Potentilla atrosanguinea* (Kalia et al., 2008) by soxhlet method has highest percentage yield.

3.2. Qualitative analysis of phytochemicals

Qualitative tests were performed in order to evaluate the chemical composition of different leaf extracts of *Nepeta spicata*. The different extracts show the occurrence of phytochemicals viz triterpenoids, carbohydrates, glycosides, resins, steroids, diterpenoids and saponins [Table 1]. The linear equations and linear regression (R^2) of different concentration of standards has been acquired by curve drawn from area under the peak in assays like TPC, TFC, TAC and FRAP [Table 3]. The quantification of various extracts, it was observed that methanol extract contains maximum number of secondary metabolites as compared to other extracts. The number of SMs in various extracts of different solvents follows the order given below: methanol extract > acetone extract > chloroform extract > hexane extract. A similar trend was observed in case of ultrasonication extracts.

All the extraction methods in different solvents have all most same results and there is no significant difference between qualitative analysis of phytochemicals. Alike results were also reported for *Nepeta nepetella's* aerial part, *N. leucophylla's* roots, *Nepeta praetervisa's* aerial parts and *Nepeta cataria's* leaves by Seladji et al. (2014), Sharma et al., (2016A), Fareed et al. (2013) and Edewor and Usman (2011), respectively.

3.3. Quantitative analysis of phytochemicals

3.3.1. TPC

Folin Ciocalteu reagent was used to evaluate the total polyphenolic content for different extracts and TPC yield of different extracts by different method lies between 1.42 and 35.71 mg of GAE/g of DPE. The results showed that the total polyphenolic content of methanol and acetone extract obtained by SEM provided highest yield, whereas higher TPC yield in hexane and chloroform extracts acquired by UEM. The extracts of plant material by SEM and UEM methods followed the decreasing order (methanol > acetone > chloroform > hexane) of TPC contents i.e. higher polarity to low polarity of different extracts [Table 2]. The polyphenolic compounds are polar in nature and their solubility maximum in polar solvents. The current results also matched with the previous results of Jayaprakasha and Patil (2007) and Saeed et al. (2012) in field of leaves of *Raphanus sativus* L. (hexane-4.97 < chloroform-22.37 < acetone-78.77 < methanol-56.69 mg catechin/g dry extracts), bark of *Terminalia arjuna* (hexane-61.73 < chloroform-73.00 < methanol-817.49 mg GAE/g), fruit of *C. media* (citron) (hexane-0.00 < acetone-29.64 < methanol-39.28 mg/g of extracts) and whole plant of *Torilis leptophylla* (hexane-54.9 < chloroform-78.0 < methanol- 121.90 mg GAE/g). In SEM, higher amount of secondary metabolites i.e. TPC contents due to presence of continuous and repetitive washing of dried power of plant material with hot solvents up to their boiling point. Therefore, higher TPC yield in polar solvent i.e. methanol extracts. *Terminalia arjuna's* bark and *Terminalia leptopphylla*'s, whole plant were also illustrate the similar trend of TPC yield. Previous research papers of aerial parts of *Galium mollugo* (Milic et al., 2013) and *Potentilla atrosanguinea* (Kalia et al., 2008) were confirmed that SEM has higher TPC value than UEM. However, in some cases UEM extracts of chloroform and hexane has more TPC values than that of SEM extracts. The high temperature and frequency of ultrasound waves may cause the higher TPC yield in some UEM extracts as compared to continuous hot solvent passed method i.e. SEM. The ultrasonic condition may be renovating the nature of SMs by degradation and oxidation. Therefore, more polar extracts (i.e. methanol and acetone) of UEM has lower yield of TPC (Vinatoru, 2001). The results shown in Table 2 have been calculated from linear equation.

$$\boldsymbol{Y} = 9.788\boldsymbol{X} + 0.0137$$

This linear equation has been obtained from the standard curve of gallic acid, where Y is the absorbance of the sample obtained from UV–visible spectrophotometer. X is the concentration of gallic acid calculated from calibration curve and listed in Table 3. From the value of X, the total polyphenol content P (mg of GAE/g of DPE) was calculated.

$$\boldsymbol{P} = \boldsymbol{X} \times \boldsymbol{V}/\boldsymbol{N}$$

Where: V = volume of extract: N = weight of plant extract in g to get the final result.

3.3.2. TFC

The flavonoids content was determined quantatively using aluminium chloride method with the help of UV-Spectrophotometer. The TFC yield of different extracts was in the range of 2.18–216.89 mg of RE/g of DPE. The acetone extract obtained by SEM and UEM showed maximum flavonoids content. The total flavonoids content in methanol extract by both the methods were comparative, whereas the chloroform and hexane extracts showed lower content of flavonoids due to less polar solvents extracts [Table 2].

The more polar crude extracts has higher amount of secondary metabolites than least polar extracts due to this reason methanol and acetone extracts has higher TFC values than that of chloroform and hexane extracts. Same types of results also uttered by whole plant of *Osbeckia parvifolia* (hexane-9.03 < ethyl acetate-9.33 < methanol-26.53 mg RE/g) (Murugan and Parimelazhagan, 2014), flowers of *Opuntia ficus* (hexane- 0.00 < dichloromethane-1.73 < methanol-

Table 2
Results of various antioxidants evaluation (DPPH, TAC and FRAP), TPC and TFC of different extracts obtained from the aerial part of *N. spicata*.

EM	SU	DPPH	FRAP	TAC	TPC	TFC
SEM	Meth	19.18 ± 0.22^{a}	26.63 ± 4.29^{a}	47.32 ± 1.20^{a}	35.71 ± 3.56^{b}	105.82 ± 01.91^{b}
	Acet	16.09 ± 0.53^{c}	33.97 ± 8.71^{e}	61.36 ± 3.40^{e}	29.94 ± 3.56^{a}	216.89 ± 0.42^{a}
	Chl	1.0 ± 0.35^{fg}	3.27 ± 0.73^{d}	18.53 ± 0.78^{c}	3.01 ± 0.56^{d}	36.24 ± 1.95^{c}
	Hex	0.21 ± 0.16^{g}	6.12 ± 2.04^{f}	11.69 ± 1.44^{c}	1.42 ± 1.11^{d}	09.62 ± 0.46^{c}
UEM	Meth	18.18 ± 0.45^{b}	26.61 ± 2.98^{b}	52.31 ± 1.51^{b}	32.13 ± 0.86^{a}	104.38 ± 1.12^{b}
	Acet	14.14 ± 0.20^{d}	19.26 ± 0.24^{d}	52.40 ± 1.52^{e}	20.02 ± 1.61^{c}	133.29 ± 0.67^{b}
	Chl	3.52 ± 0.10^{e}	2.05 ± 0.28^{e}	19.84 ± 6.70^{e}	03.11 ± 11.49^{d}	16.77 ± 0.73.18^{d}
	Hex	1.73 ± 0.22^{f}	4.14 ± 0.42^{f}	16.40 ± 1.47^{f}	02.01 ± 1.38^{d}	02.18 ± 0.58^{e}
Ascorbic acid		58.42 ± 0.16	385.3 ± 2.76	–	385.3 ± 2.76	–
Quercetin		89.52 ± 0.89	–	488.95 ± 7.95	–	–

UEM -Ultrasonic assisted extraction method and SEM -Soxhlet extraction method, EM - extraction methods, SU - solvent used, Meth - methanol, Acet - acetone, Chl - chloroform, Hex - hexane.
The results of DPPH assay were expressed as % Inhibition, FRAP as mg Fe (II) E/g DPE, TAC as mg AAE/g DPE, TPC as mg GAE/g DPE and TFC as mg RE/g DPE. The values having different superscript letters within a column were significantly different ($p < 0.05$).

Table 3
Linear equations and R^2 for different standards used to calculate the results of different assays.

Sr. No.	Name of Assay	Name of Standard and Concentration	Linear Equation	R^2
1	Total Polyphenolic Content (TPC)	Gallic Acid (0–150 mg/L)	Y = 9.788x + 0.0137	0.9993
2	Total Flavonoids Content (TFC)	Rutin Trihydrate (0–300 mg/L)	Y = 1.1643x + 0.0018	0.9984
3	Ferric Reducing Antioxidant Power (FRAP)	Ferrous Sulphate (0–300 mg/L)	Y = 4.5682x – 0.0347	0.9993
4	Total Antioxidant Capacity (TAC)	Vitamin C (0–300 mg/L)	Y = 3.5665x – 0.0503	0.9992

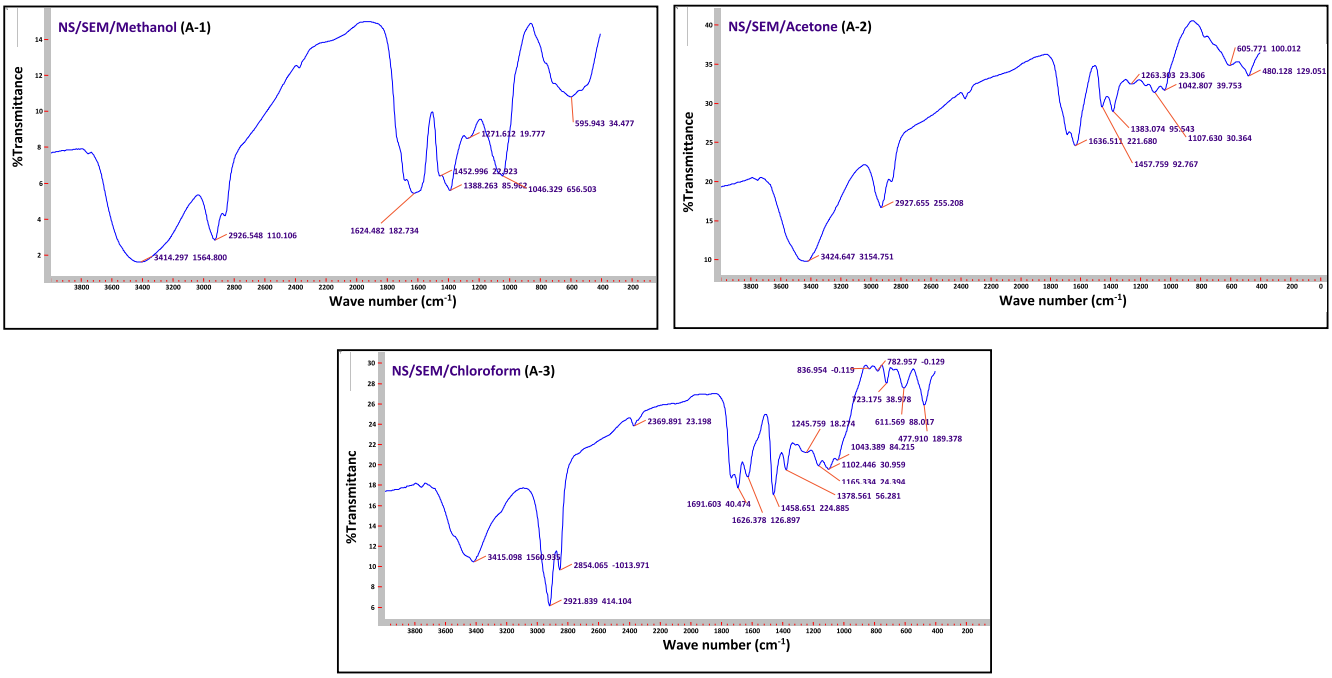

Fig. 2. FTIR-Specters of (A-1) methanol extract, (A-2) acetone extract and (A-3) chloroform extracts extract by SEM; (B-1) methanol extract, (B-2) acetone extract and (B-3) chloroform extract by UEM of different crude extracts of *Nepta spicata*.

60.81 mg RE/g) and bark of Terminalia arjuna (hexane-51.04 < chloroform 60.72 < methanol-199.12 mg quercetin Equivalent/g). In both the methods (SEM and UEM), the TFC contents of acetone extracts were more than that of methanol extracts. The literature also shows that TFC values of acetone extracts more than methanol extracts for *Bcida*'s leaf, trunk and stem (Iloki-Assanga et al., 2015) and *T. frafara* root. The results mentioned have been calculated (using same method as used for total polyphenolic content) from the linear equation derived by standard curve [Table 3] of Rutin trihydrate at different concentration.

$$\boldsymbol{Y} = 1.164\boldsymbol{X} + 0.0018$$

In this equation, Y is the absorbance of the sample obtained from UV–visible spectrophotometer. X is the concentration of Rutin trihydrate calculated from calibration curve. From the value of X, the value of total flavanoids content F (mg of RE/g of DPE mg) was calculated.

$$\boldsymbol{F} = \boldsymbol{X} \times \boldsymbol{V}/\boldsymbol{N}$$

Where: V = volume of extract: N = weight of plant extract in g to get the final result.

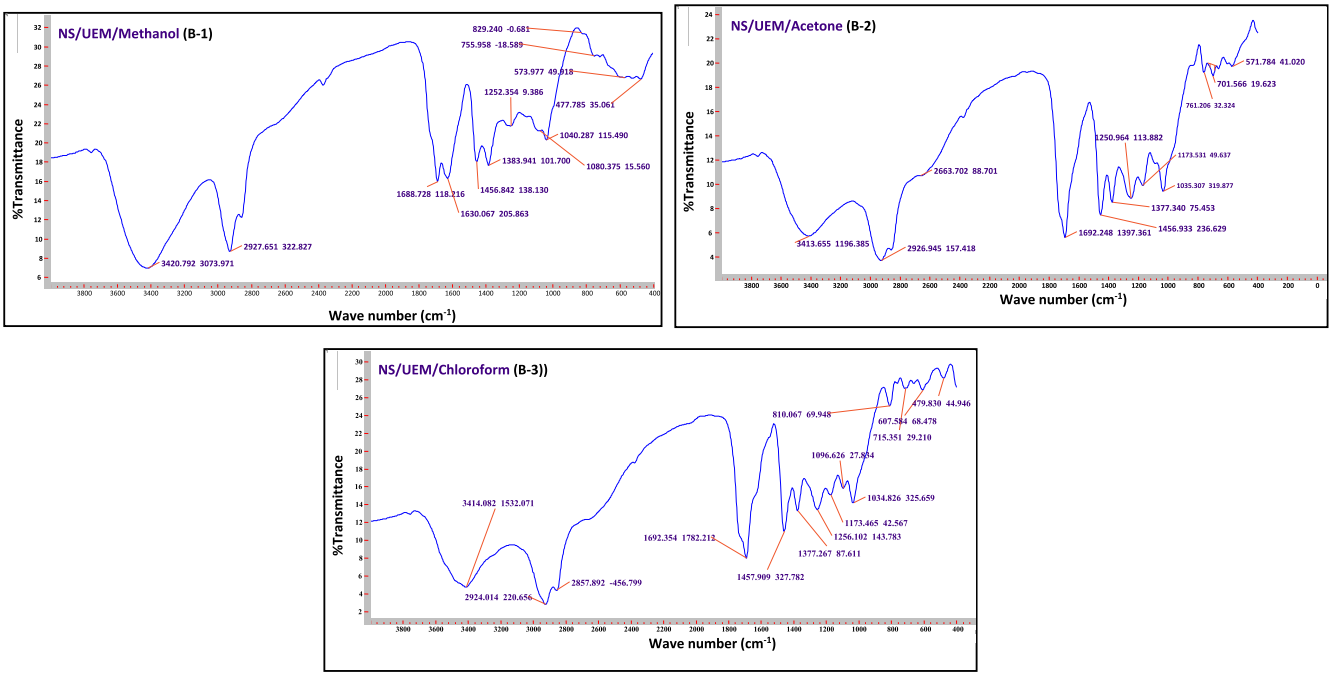

Fig. 2. (*continued*)

3.4. Antioxidant potential

The natural SMs have contained numerous antioxidants. In the plant extract, a single assay not gives the complete picture of total antioxidant potential because they shows the numerous biological reactions i.e. chain initiation prevention, continued hydrogen abstraction, decomposition of peroxides, catalysts binding in transition metal ions, radical scavenging and reductive capacity (Yildirim et al., 2000). Therefore, various antioxidant techniques used to determine the complete picture of total antioxidant potential of various crude extracts. The diverse antioxidant assays are illustrated in [Table 2].

3.4.1. DPPH radical scavenging activity

In this method, the different extracts DPPH free radical percentage of inhibition was vary from 0.21 to 19.18%. In case of UEM, the percentage inhibition for different extracts like chloroform and hexane were greater than same solvent extracts obtained from SEM method due to acoustic cavitation. The phenomenon of ultrasound waves break off the cell wall of plant cell in the plant extracts, the solvents intensely penetrated inside and diffusion of solute enhanced rapidly, called acoustic cavitation. Similar results were also given by Batool et al. (2018) chloroform (IC_{50}values(ug/mL) UEM- 56.31 > SEM - 24.91) and methanol (SEM - 12.35 > UEM - 7.68) extracts; and hexane extract of stem of *Nepeta leucophylla* shows (UEM-18.25 > SEM-16.63) by Sharma & Cannoo (2016B). The results revealed that the free radical scavenging activity of methanol and acetone extracts obtained by SEM was higher than the extracts obtained by UEM [Table 2]. The TPC and TFC showed the good correlation with DPPH activity as 0.97 and 0.93, respectively [Table-7].

So, polyphenolic and flavanoids contents are responsible for the higher value of free radical scavenging activity in polar solvent extracts as compared to less polar solvents i.e. chloroform and hexane. Alike outcomes were also showed earlier as the seed of *Origanum majorana* extracts (chloroform-84.87 < acetone-91.03 < methanol-91.89%) by Dhull, Kaur & Purewal, *H. erinacens* mushrooms extracts (hexane < chloroform < acetone) by Jiang et al. (2016), corn tassels extracts (acetone < methanol) by Mohsen & Ammar (2009) and leaves of *Ipomoea aquatic* extracts(hexane-06 < chloroform-55 < methanol-85%) by Prasad et al. (2005), respectively.

3.4.2. Total antioxidant capacity

The total antioxidant capacity of different extracts by different methods lies between 11.69 and 61.36 mg AAE/gm of DPE. It has been observed [Table 2] that using soxhlet methodology, the total antioxidant capacity of acetone extract was higher than other three extracts in increasing order as hexane, chloroform and methanol extracts. The sequence of TAC values, in both the extraction methods are acetone > methanol > chloroform > hexane i.e. SEM (61.36 mg AAE/g DPE) > UEM (52.40 mg AAE/g DPE), UEM (52.31 mg AAE/g DPE) > SEM (47.32 mg AAE/g DPE), UEM (19.84 mg AAE/g DPE) > SEM (18.53 mg AAE/g DPE) and UEM (16.40 mg AAE/g DPE) > SEM (11.69 mg AAE/g DPE), respectively. The literature also showed that TAC values of acetone extract more than methanol extracts of different rice cultures (Shel kew, Gull zag, Kaw kreed and Teli zag i.e. methanol-3.602 < acetone-3.677, methanol-0.656 < acetone-0.952, methanol-1.876 < acetone-1.956 and methanol-0.987 < acetone-1.157 mg AAE/g respectively) by Bhat & Riar and pomegranates peels i.e. at 50 and 75 (ug/mL) (methanol-1298 < acetone-1392 and methanol-1749 < acetone-1778 μmol/g of extracts) by Negi et al. (2003). However, the ultrasonication method's extracts i.e. methanol, chloroform and hexane provided better results except for acetone [Table 2]. The current results (UEM > SEM) were in concurrence with the earlier outcomes of root (Sharma & Cannoo, 2016C) and stem of *N. leucophylla* i.e. (40.8 > 33.93) mg AAE/g DPE and (8.36 > 6.68) mg AAE/g DPE for methanol and chloroform extracts respectively and the methanol extracts of whole plant of *C. aksoyi* (UAEM-136.38 > SEM-135.38 mg AAE/g extract) and *C. amaeno* (UAEM-147.52 > SEM-147.37 mg AAE/g extract) by Albayrak et al. (2017). The results were obtained using a linear equation derived from the standard curve of ascorbic acid,

$$\boldsymbol{Y} = 3.5665\boldsymbol{X} - 0.0503$$

Y = absorbance of sample; X = concentration of ascorbic acid calculated from above calibration curve. From the value of X, the value of total antioxidant activity A (mg AAE/gm of DPE) was calculated using equation:

$$\boldsymbol{A} = \boldsymbol{X} \times \boldsymbol{V}/\boldsymbol{N}$$

Where, V = volume of extract, N = weight of plant extract in g.

Table 4
Main functional groups assigned to the different vibrations present in the FTIR-Specters of (A-1) methanol extract, (A-2) acetone extract and (A-3) chloroform extracts extract by Soxhlet extraction method (SEM); (B-1) methanol extract, (B-2) acetone extract and (B-3) chloroform extract by Ultrasonic assisted extraction method (UAEM).

Sr. No.	Characteristic Absorption (cm^{-1})		Type of Bonds	Functional Group	References
	SEM (A-1, A-2, A-3)	UAEM (B-1, B-2, B-3)			
1.	3414, 3424, 3415	3420, 3413, 3414	O–H Stretching	Phenol, Polysaccharides, carbohydrates, Alkaloids, Lignins	Ishnava et al., 2012; Gouveia et al., 2012; Gonzalez-Cabrera et al. (2018); Lu et al. (2011); Schulz and Baranska (2007) and Nogales-Bueno et al. (2017)
2.	2926, 2927, 2921	2927, 2926, 2924	CH_2 Antisymmetric stretching, N–H stretching frequency	Lipids/fatty acid, protein, Alkaloids, Lignins	
3.	2855, 2856, 2854	2852, 2853, 2857	CH_2 Symmetric stretching,–O-H stretch	Lipids/fatty acid, Alkaloids, Lignins	
4.	1679, 1681, 1691,	1688, 1692, 1692	C = O carbonyl, C═O···H Stretching of carboxylic acids, N–H bend	Amino acid, Protein, hydroxyl fatty acids	
5.	1624, 1636, 1626	1630, -, –	C = C Stretching, N–H bend,	Alkenes, Terpenes, Phenolics, Pectins, Lipids/fatty acid, Protein	
6.	1452, 1457, 1458	1456, 1456, 1457	C–C aromatic, Asymmetric bend methyl(C–H), CH_2 Scissoring,	Phenolics, Polysaccharides, Terpenes	
7.	1388, 1383, 1378	1383, 1377, 1377	C–C Alkanes, symmetric bend methyl (C–H), COO- Symmetric Stretching/CHC bending	Polysaccharides, Pectins, Terpenes, carboxylate ions.	
8.	1271, 1263, 1245	1252, 1250, 1256	In plane O–H Bending, N═O stretching, Out of phase C–C–O stretching	Nitro compound, Phenol	
9.	-, 1107, 1165	-, 1173, 1113	C–O stretches, C–C Stretching, C–O–C stretching	Polysaccharides, aliphatic amines, Phenol, Alkaloids	
10.	1046, 1042, 1043	1080, 1035, 1034	Alcohol C–O Stretching, C–C stretching, C–N ring	Phenols, Polysaccharides	
11.	595, 605, 611	573, 571, 607	C–Cl stretching	Haloalkanes	
12.	-, -, 723	755, 701, 715	primary and secondary amines, CH_2 Rocking Vibration	protein, Lipids/fatty acid, Alkaloids, Phenolics	

3.4.3. The ferric reducing antioxidant power assay

The FRAP values of different extracts as obtained from in this technique fluctuated from 2.05 to 33.97 mg of $FeSO_4$ E/g dw and SEM and UEM has following order acetone (33.97 mg of $FeSO_4$ E/g of dw) > methanol(26.63 mg of $FeSO_4$ E/g of dw) > hexane(6.12 mg of $FeSO_4$ E/g of dw) > chloroform(3.27 mg of $FeSO_4$ E/g of dw) and methanol (26.61 mg of $FeSO_4$ E/g of dw) > acetone (19.26 mg of $FeSO_4$ E/g of dw) > hexane(4.14 mg of $FeSO_4$ E/g of dw) > chloroform (2.05 mg of $FeSO_4$ E/g of dw), respectively. Also, from the results it was obvious that the FRAP values of all the extracts acquired by SEM was more in amount than that obtained by UEM as displayed in Table 2. The SEM gives the best results of FRAP as compared to the UEM also shown by *Nepeta leucophylla* areal part (Sharma & Cannoo, 2016A), root (Sharma & Cannoo, 2016C) and stem (Sharma & Cannoo, 2016B) and *Dorcycinum Pentapyllum* subsp. Haussne chtii herb areal part (Uysal et al., 2017). In some cases, FRAP results followed the trend: methanol > acetone > chloroform. The trend tracked by stem and leaf of *Plectranthus stocksii* Hook: methanol-124.76 > acetone-102.05 > chloroform-76.99 and methanol-154.17 > acetone-98.75 > chloroform-58.32 respectively (Muniyandi et al., 2017); fresh leaves of *B. vahlii*: methanol-3257.8(m mol Fe(II)/g extracts) > acetone-3028.9(m mol Fe(II)/g extracts) > chloroform-734.4(m mol Fe(II)/g extracts). In some cases, FRAP results followed the order acetone > methanol extracts i.e. bark of *Terminalia arjuna* (acetone-212.5 > methanol-35.50 μM) by Kumar et al. (2013), leaves of *Bidenspilosa* (acetone-2431.93 > methanol-561.68 μmol Fe(II)/g) and *Chenopodium album* (acetone-263.96 > methanol-57.47 u mol Fe(II)/g) by Adedapo, Jimoh & Afolayan.

The linear equation obtained by standard curve [Table 3] of $FeSO_4$ used to get the results is as follows:

$$\boldsymbol{Y} = 4.5682\boldsymbol{X} - 0.0347$$

Y = absorbance of sample; X = concentration of ferrous sulphate calculated from above calibration curve. From the value of X, the value of ferric reducing antioxidant power R (mg of $FeSO_4$ E/g dw) was calculated using equation:

$$\boldsymbol{R} = \boldsymbol{X} \times \boldsymbol{V}/\boldsymbol{N}$$

Where, V = volume of extract, N = weight of plant extract in g.

Since, the antioxidant activity of any extract is due to the occurrence of polyphenols, flavonoids, alkanoids, etc. in it (Holasova et al.,

Table 5
List of main constituents recognized by GC-MS study for the extracts attained by SEM and UEM.

Name of Extract	RT	Name of compound (IUPAC)	MF	MW	Peak Area (%)
Methanol (SEM)	3.281	Benzene, 1,4-dichloro-	$C_6H_4Cl_2$	146	2.12
	23.397	Neophytadiene	$C_{20}H_{38}$	278	3.19
	23.534	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	4.59
	25.717	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	27.07
	26.650	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.98
	29.841	Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294	5.99
	29.979	9,12,15-Octadecatrienoic acid, methyl ester Phytol	$C_{19}H_{32}O_2$	292	7.94
	30.287	7-Tetradecenal	$C_{20}H_{40}O$	296	12.40
	30.910	Methyl 18-methylnonadecanoate	$C_{14}H_{26}O$	210	5.19
	35.346	UI	$C_{21}H_{42}O_2$	326	2.44
	35.905		–	–	2.61
Acetone (SEM)	23.407	2-Hexadecen1-ol,3,7,11,15 Tetramethyl-,[R-[R*,R*-(E)]]- UI	$C_{20}H_{40}O$	296	10.43
	23.573	Hexadecanoic acid	–	–	4.14
	26.724	Phytol	$C_{16}H_{32}O_2$	256	27.07
	30.293	9,12-Octadecadienoic acid (Z,Z)-	$C_{20}H_{40}O$	296	3.73
	30.811	7-Tetradecenal, (Z)-	$C_{18}H_{32}O_2$	280	5.58
	30.971	Octadecanoic acid	$C_{14}H_{26}O$ $C_{18}H_{36}O_2$	210	15.64
	31.543	Stachan-16-one	$C_{20}H_{32}O$	284	5.04
	34.037	Dehydro abietol db5-3391	C_{20} H_{30} O	288	2.29
	35.909			286	4.23
Chloroform (SEM)	10.858	Long chain hydrocarbons	–	–	3.21
	14.152	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	5.51
	16.638	Long chain hydrocarbons	–	–	4.24
	22.267	Long chain hydrocarbons	–	–	4.21
	23.421	Long chain hydrocarbons	–	–	2.74
	47.788	Long chain hydrocarbons	–	–	3.53
	48.367	Long chain hydrocarbons	–	–	2.82
	48.933	Long chain hydrocarbons	–	–	6.81
	51.059	Long chain hydrocarbons	–	–	2.41
	52.032	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412	5.40
	53.511	Long chain hydrocarbons	–	–	15.86
	55.584	Long chain hydrocarbons	–	–	2.76
	56.397	Long chain hydrocarbons	–	–	2.24
	59.596	Long chain hydrocarbons	–	–	5.08
	59.916	Long chain hydrocarbons	–	–	8.62
Hexane (SEM)	23.550	6,10,14-trimethylpentadecan-2-one	$C_{18}H_{36}O$	268	2.18
	26.818	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.96
	30.310	Phytol	$C_{20}H_{40}O$	296	5.48
	31.135	7-Tetradecenal, (Z)	$C_{14}H_{26}O$	210	10.12
	44.052	Long chain hydrocarbons	–	–	2.01
	48.955	Long chain hydrocarbons	–	–	3.96
	50.478	Long chain hydrocarbons	–	–	2.37
	53.560	Long chain hydrocarbons	–	–	6.24
	55.584	Long chain hydrocarbons	–	–	2.76
	55.638	Long chain hydrocarbons	–	–	2.24
	56.440	UI	–	–	2.63
	56.397	Long chain hydrocarbons	–	–	2.36
	59.691	Stigmast-5-en-3-ol, (3.beta.)-	$C_{29}H_{50}O$	414	12.23
	59.980	Long chain hydrocarbons	–	–	3.69
	60.924	beta.-Amyrin	$C_{30}H_{50}O$	426	2.63
	61.455	UI	–	–	4.06
	62.802	UI	–	–	5.42
	64.719	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412	2.48
Methanol (UEM)	12.990	2-Isopropyl-5-Methycyclohexyl	$C_{12}H_{20}N_2O_2$	224	16.16
	23.411	2,6,10-Trimethyl,14-Ethylene-14-pentadecene 9H-Carbazole,9-Ethyl-$9Ethylcarbazole	$C_{20}H_{38}$	278	4.15
	23.720	Hexadecanoic acid	$C_{14}H_{13}N$	195	6.44
	26.703	2-hexadecen-1-ol	$C_{16}H_{32}O_2$	256	15.62
	30.299	7-Tetradecenal	$C_{20}H_{40}O$	296	2.03
	30.959	Stearic acid	$C_{14}H_{26}O$	210	5.03
	31.540	Stigmast-4-en-3-one	$C_{18}H_{36}O2$	284	2.58
	41.435	Long chain hydrocarbons	$C_{29}H_{48}O$	412	24.10
	53.485	Stigmast-5-en-3-ol, (3.beta.,24s)-	–	–	3.15
	59.565		$C_{29}H_{50}O$	414	9.78

(*continued on next page*)

Table 5 (*continued*)

Name of Extract	RT	Name of compound (IUPAC)	MF	MW	Peak Area (%)
Acetone (UEM)	14.140	Phenol, 2,4-bis (1,1-dimethylethyl)-)-	$C_{14}H_{22}O$	206	6.69
	17.955	(1S-(1alpha,2beta,5alpha))-2-Methyl-5-(1-methylvinyl)cyclohexan -1-ol 1H-3A,7-Methazulene-6-ol	$C_{10}H_{18}O$	154	5.38
	19.526	2,6,10-trimethyl,14-ethylene −14-pentadecene	$C_{15}H_{26}O$	222	4.98
	23.412	Hexadecanoic acid 9,12-Octadecadienoic acid (Z,Z)-	$C_{20}H_{38}$	278	9.20
	26.674	Long chain hydrocarbon	$C_{16}H_{32}O_2$	256	21.67
	30.937	Long chain hydrocarbon	$C_{18}H_{32}O_2$	280	17.20
	48.921		–	–	4.91
	53.497		–	–	7.72
Chloroform (UEM)	10.858	Long chain hydrocarbon	–	–	2.36
	14.156	Phenol,2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	5.59
	16.640	Long chain hydrocarbon	–	–	3.82
	22.273	Long chain hydrocarbon	–	–	3.81
	23.429	Long chain hydrocarbon	–	–	4.11
	26.719	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.72
	27.493	n-Tetracosanol-1	$C_{29}H_{50}O$	354	3.41
	32.290	Long chain hydrocarbon	–	–	2.65
	48.936	Long chain hydrocarbon	–	–	3.98
	53.523	Hexatriacontane	$C_{36}H_{74}$	506	9.30
	59.612	UI	–	–	13.74
	59.932	Long chain hydrocarbon	–	–	5.98
	61.420	UI	–	–	2.36
	61.545	UI	–	–	2.37
	64.673	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412	3.35

UEM - Ultrasonic assisted extraction method and SEM - Soxhlet extraction method RT-retention time, MF- molecular formula, MW- molecular weight, UI-unidentified.

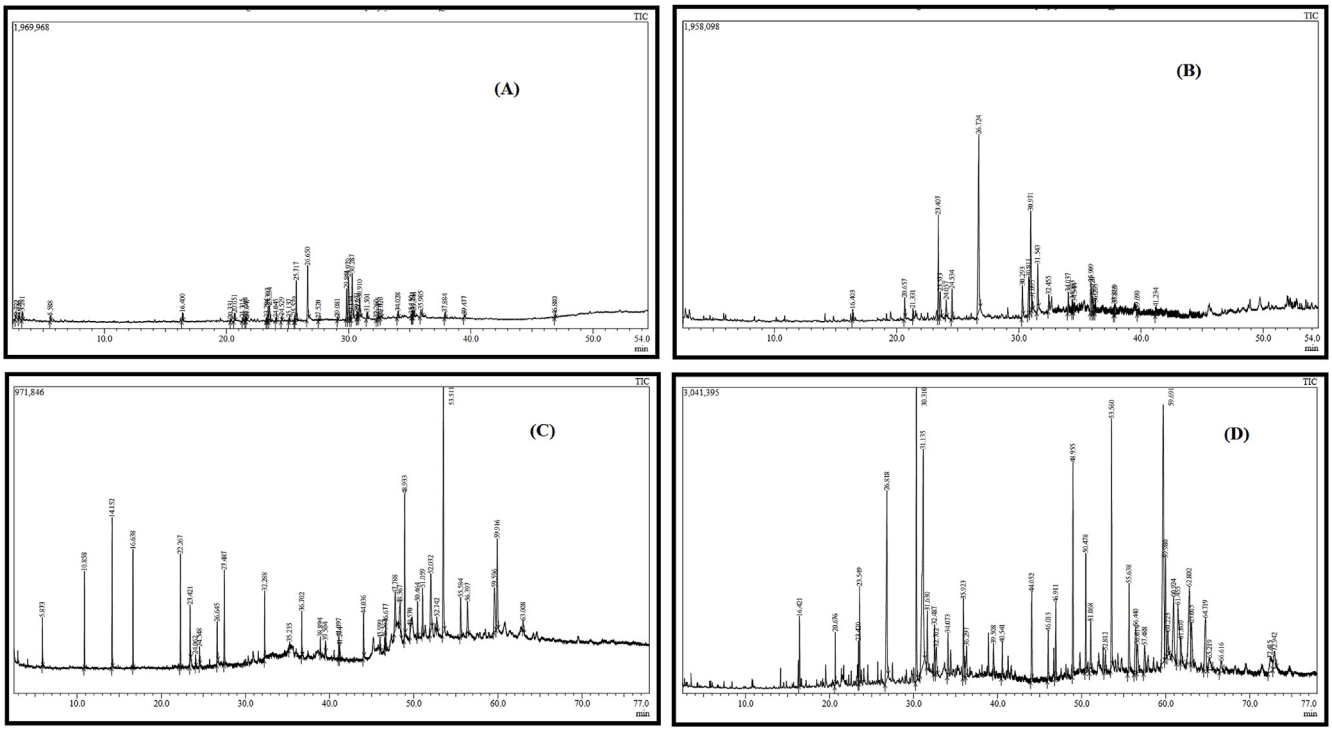

Fig. 3. GC-MS chromatogram of (A) methanol extract, (B) acetone extract, (C) chloroform extracts and (D) hexane extract by SEM; (E) methanol extract, (F) acetone extract, (G) chloroform extract by UEM.

2002). From the above tables, it is clear that the polar extracts possess higher concentration of TPC and TFC; therefore these extracts show maximum antioxidant activity. However, TPC and TFC in hexane extract are quite less that bears direct proportionality with its anti-oxidant potential.

3.5. Analytical techniques

The analytical techniques i.e. FTIR (Fourier-transform infrared spectroscopy) and GC-MS (Gas chromatography-mass spectrometry) of different crude extracts of areal part of *Nepeta spicata* revealed the existence of long chain hydrocarbons, steroids, fatty acids etc.

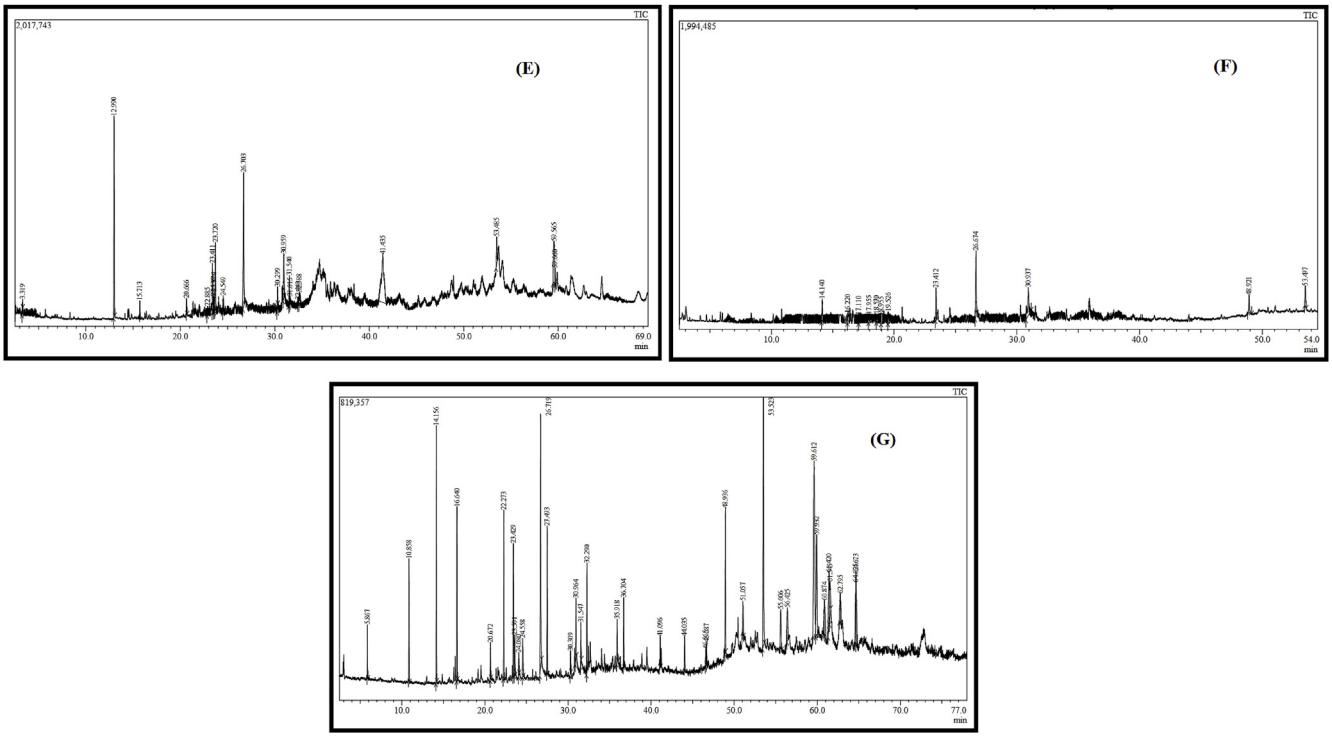

Fig. 3. (*continued*)

3.5.1. FTIR analysis

IR of various crude extracts of *Nepeta spicata* plant obtained from SEM and UEM using different solvents viz. methanol, acetone and chloroform was recorded using a KBr pellet of different extracts. Since, the extracts were crude containing a mixture of compounds, hence, the peaks have been assigned arbitrarily to the vibrational frequency of different functional group present in the structures obtained from GC-MS study (Fig. 2).

3.6.1. IR spectra of different extracts (A-1, A-2, A-3, B-1, B-2 & B-3) obtained by SEM and UEM. In the Infrared spectra (Fig. 2 and Table 4; A-1, A-2, A-3, B-1, B-2 & B-3), peaks at 3414, 3424, 3415, 3420, 3413, 3414 cm^{-1} corresponds to the O–H stretching frequencies (Ishnava et al., 2012; Gouveia et al., 2012; Gonzalez-Cabrera et al., 2018; Lu et al., 2011; Schulz and Baranska, 2007 and Nogales-Bueno et al., 2017) where as at 2926, 2927, 2921, 2927, 2926, 2924 cm^{-1} corresponds to CH_2 antisymmetric and N–H stretching vibrations and at 2855, 2856, 2854, 2852, 2853, 2857 cm^{-1} corresponds to CH_2 symmetric and C–H stretching (aldehyde) represents the presence of phenol, polysaccharides, carbohydrates, lipids, protein, alkaloids and lignins (Gouveia et al., 2012; Gonzalez-Cabrera et al., 2018; Lu et al., 2011; Schulz and Baranska, 2007; Nogales-Bueno et al., 2017). The Amino acid, protein, hydroxyl fatty acid, alkenes, terpenes, pectins observed by the peak at 1679, 1681, 1691, 1688, 1692, 1692 cm^{-1} indicates the presence of carbonyl (C═O) streching, carboxylic acid (C═O ….H) stretching and amine (N–H) bending frequencies and at 1624, 1636,1626,1630 cm^{-1} indicates the presence of alkene (C═C) stretching and (N–H) bending frequencies (Ishnava et al., 2012; Lu et al., 2011; Schulz and Baranska, 2007 and Nogales-Bueno et al., 2017). It was observed that absorption bands at 1452, 1457, 1458, 1456, 1456, 1457 cm^{-1} corresponds to the aromatic C–C stretching, methyl C–H asymmetric bending and CH_2 scissoring bending specified the phenolics, polysaccharides and terpenes whereas at 1388, 1383, 1378, 1383, 1377, 1377 cm^{-1} corresponds to alkanes C–C stretching, methyl C–H symmetrical bending, COO- symmetric stretching and CHC bending vibrations specified the polysaccharides, pectins, terpenes and caboxylate ions (Schulz and Baranska, 2007; Nogales-Bueno et al., 2017). The phenols and nitro compounds peaks observed at 1271, 1263, 1245, 1252, 1250 and 1256 cm^{-1} signifies the in plane O–H bending, N═O stretching and out of phase C–C–O stretching vibrational frequencies (Ishnava et al., 2012). The IR spectrum of all extracts showed the presence of haloalkanes (C–Cl stretching) at 595, 605, 611, 573, 571,607 cm^{-1} (Ishnava et al., 2012) and phenols and polysachharides (alcohol C–O stretching, C–C stretching and C–N stretching) at 1046, 1042, 1043, 1080, 1035, 1034 cm^{-1}. The study of infrared spectra (IR) exposed the existence of diverse class of primary and secondary metabolites i.e. carbohydrates, protein, fatty acid, amino acid, lipids, polysaccharides, alkaloids, phenol, lignins, terpenes etc. as shown in Table 4.

3.5.2. GC-MS analysis

GC-MS was employed to determine the chemical composition of the constituent compounds. The various studies (GC-MS) of numerous extracts viz. methanol, acetone, chloroform and hexane of *Nepeta spicata* by SEM contained 31, 18, 29 and 31 chemical constituents, respectively. Whereas, the methanol, acetone and chloroform extracts obtained by UEM contained 15, 8 and 31 chemical constituents respectively. The main constituents (> 2.0%) were identified from the data of various extracts and have been reported in the [Table 5] and [Fig. 3].

The GC-MS data of hexane extracts as given in the above table reveals the presence of non-polar and long chain hydrocarbons. The latter showed the presence of Stigmast-5-en-3-ol, (3.beta.) which has significant antioxidant potential and is used in food and beverage (including additives, contaminants) and pharmaceuticals. Further, the analysis GC-MS shows that the polar extracts (methanol, acetone, chloroform) contained numerous steroids, phenolic etc which impart them a better antioxidant activity as compared to hexane. The major compounds detected (> 12%) by GC-MS technique obtained by SEM and UEM of different extracts are listed in [Table 6] and [Fig. 4]. The major compounds are Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$); Phytol ($C_{20}H_{40}O$); Hexadecanoic acid ($C_{16}H_{32}O_2$); Hexatriacontane ($C_{36}H_{74}$); Stigmast-5-en-3-ol,(3.beta.) ($C_{29}H_{30}O$); Stigmast-4-en-3-one ($C_{29}H_{48}O$); 9,12-Octadecadienoic acid(Z,Z) ($C_{18}H_{32}O_2$) with similarity index 95, 97, 90, 96, 85, 78, 84 and peak area 27.07, 12.40, 27.07,

Table 6
List of biologically active major compounds detected for the various extracts obtained by SEM and UEM by GC-MS analysis.

Sr. No.	Name of Major compounds (IUPAC)	RT (min)	SI	MF	MW	Peak area (%)	Compound nature	Biological activities
1.	Hexadecanoic acid, methyl ester	25.717	95	$C_{17}H_{34}O_2$	270	27.07	Fatty acid methyl esters	Antioxidant, Flavor, Hypocholesterolemic Pesticide, 5-Alpha reductase inhibitor
2.	Phytol	30.287	97	$C_{20}H_{40}O$	296	12.40	Acyclic diterpene alcohol	Antimicrobial, Anticancer, Cancer preventive, Diuretic Antiinflammatory, Antifungal against *S. typhi*, resistant gonorrhea, joint dislocation, headache, hernia, stimulant and antimalarial
3.	Hexadecanoic acid	26.724	90	$C_{16}H_{32}O_2$	256	27.07	Saturated fatty acid (Palmitic acid)	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor Antipsychotic
4.	Hexatriacontane	53.511	96	$C_{36}H_{74}$	506	15.86	Higher Alkane	Antifungal against fungal spores germination, Antioxidant, Antitumour, Antibacterial
5.	Stigmast-5-en-3-ol, (3.beta.)	59.691	85	$C_{29}H_{50}O$	414	12.23	Phytosterols	Anticancer, anti hypercholesterolemic, antiinflammatory, antibacterial, antifungal, and anti-hyperlipoproteinemic activities inhibits tumor promotion, anti HIV reverse transcriptase Antimicrobial Antiasthma, Anti-arthritic, Antiproliferative and Anti-diabetic
6.	Stigmast-4-en-3-one	41.435	78	$C_{29}H_{48}O$	412	24.10	Steroid	Antioxidant, hypoglycemic and thyroid inhibiting properties, precursor of progesterone, antimicrobial, anticancer, antiarthritic, antiasthama, anti inflammatory, diuretic
7.	9,12-Octadecadienoic acid (Z,Z)	30.937	84	$C_{18}H_{32}O_2$	280	17.20	Essential fatty acid (Linoleic acid)	Antiinflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic, 5-Alpha reductase inhibitor, Antiandrogenic, Anticoronary

RT-retention time, SI- similarity index, MF- molecular formula, MW- molecular weight.

15.86, 12.23, 24.10, 17.20%, respectively and these major compounds has various biological activities like antioxidant, antimicrobial, anticancer, antimalarial, antiandrogenic, antipsychotic, antihyperchlolemic, anti-inflammatory etc listed in [Table 6] (Kumar et al., 2019e; Krishnamoorthy and Subramaniam, 2014).

Kim and Chung (2009) reported that n-Hexadenoic acid and Phytol has been found in dried boxthorn fruit of *Lycium chinensis* and stigmast-4-en-3-one, 9, 12 - Octadecadienoic acid (Z,Z)-methyl ester, n-Hexadenoic acid and Hexadecanoic acid, methyl ester has been found in heartwood of *A. adianthifolia* and stem bark of *P. angolensis*, respectively. The methanolic extracts of leaf, stem and tuber parts of *Solena amplexicaulis* (Lam.) showed the presence of n-Hexadecanoic acid, Phytol and Hexadecanoic acid, methyl ester by Krishnamoorthy and Subramaniam, 2014. The ethyl acetate (32 compounds) and methanol (26 compounds) roots extracts of *C. ciliaris* has been show the presence of Hexadecanoic acid (5.54%) and Stigmast-5-en-3-ol, (3.beta.) (8.50%), respectively. The methanol extract of aerial part of *Nepeta leucophylla* by different extraction methods (SEM, UEM and maceration method) has been show the presence of Hexadecanoic acid-methyl ester, n-Hexadecanoic acid, Linolenic acid, Stigmast-5-en-3-ol, (3.beta.)- etc (Sharma & Cannoo, 2016A). The different extracts of leaves of *P. microphyllum* shows the presence of 9, 12 - Octadecadienoic acid (Z,Z)-methyl ester (hexane-nil, DCM-2.08%, chloroform-2.12% and ethyl acetate-nil), Stigmast-5-en-3-ol, (3.beta.)-(hexane-5.85%, DCM-5.61%, chloroform-5.08% and ethyl acetate-6.48%) and Stigmast-4-en-3-one (hexane-8.72%, DCM-6.75%, chloroform-5.68% and ethyl acetate-8.53%) by Bastos et al.

So, the bioactive compounds (classes of primary and secondary metabolites) like steroids, higher alkanes, terpenes, fatty acids along with polyphenolics and flavonoids in crude extract dependable for the antioxidant potential.

3.6. Correlation

The range of correlation coefficients (r) of various extracts of aerial part of *Nepeta spicata* by different extraction methods are 0.93–1 as specified in Table 7 for TFC, TPC, FRAP, TAC and DPPH scavenging. The results of total polyphenols, total flavonoids and different extraction methods showed the excellent correlation (i.e. r value). The r values of correlation of TAC results with TPC, TFC, DPPH and FRAP were equal to 0.98, 0.98, 0.95 and 0.98, respectively; of FRAP results with TPC, TFC, DPPH were equal to 0.99, 0.99 and 0.97, respectively; of DPPH results with TPC and TFC were equal to 0.97 and 0.93, respectively. The correlation values exposed the fact that there are direct relationships between total flavonoids and phenolics contents with different antioxidant activities. At last, DPPH scavenging activity, TAC, FRAP, TFC and TPC showed good correlations with itself. Therefore, results prove that the higher values of antioxidant assays are due to the occurrence of poly-phenolics and flavanoids contents.

4. Conclusion

It has been concluded that the extracts of the leaves of plant *Nepeta spicata* obtained from SEM produced better yield than UEM. Further, the yield of compounds was higher in the polar solvent viz. methanol and acetone probably due to the presence of higher concentration of polar constituents. The evaluation of total polyhenolic content revealed that the methanol extract obtained from UEM possessed higher polyphenolic content than other crude extracts. Furthermore, it has been concluded that the total flavonoids content in acetone extract obtained by SEM was higher than other extracts. The acetone extract procured by SEM gives higher antioxidant potential by Mo reducing assay and FRAP assay whereas, methanol extract furnished better free radical scavenging activity in DPPH. In general, more polar solvents exhibited higher values of TPC and TFC which possibly was responsible for their efficient antioxidant activity. The conclusions have been enumerated as follows:

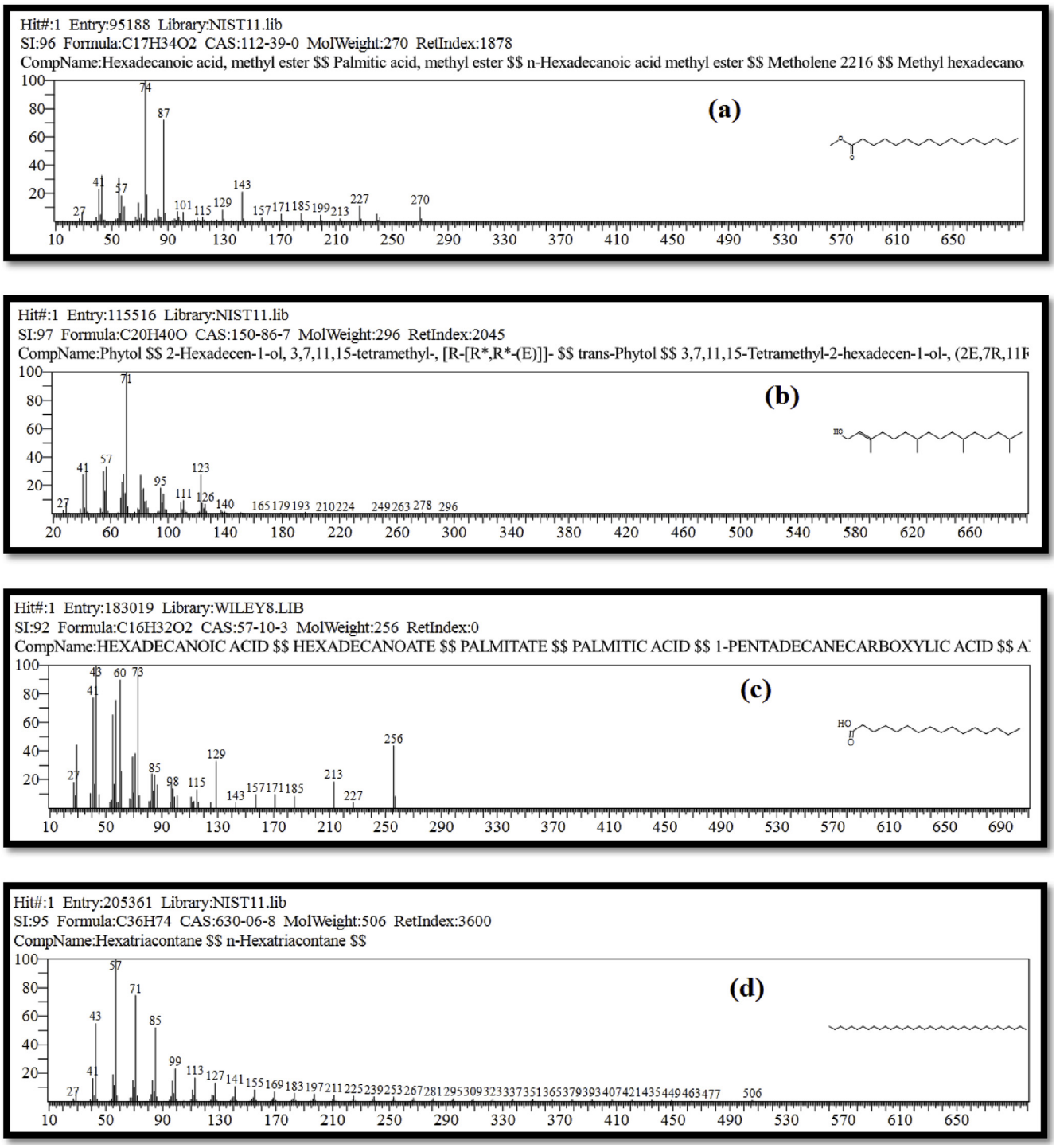

Fig. 4. The Structures and Mass Spectra of Major Compounds in crude extracts detected by GC-MS i.e. (a) Hexadecanoic acid, methyl ester, (b) Phytol, (c) Hexadecanoic acid, (d) Hexatriacontane, (e) Stigmast-5-en-3-ol, (3.beta.), (f) Stigmast-4-en-3-one and (g) 9,12-Octadecadienoic acid (Z,Z)- methyl ester.

The plant *Nepeta spicata* exhibited significant antioxidant potential.

- The polar solvents used for extraction in both employed techniques i.e SEM and UEM exhibited higher total polyphenolic contents and total flavonoids contents and subsequently showed better antioxidant activity than other extracts.
- Despite longer extraction time, SEM provided better yields than UEM.
- The FTIR and GC-MS analysis of various extracts illustrated the existence of antioxidant activities due to the presence of bioactive compounds i.e. higher alkanes, steroids, polyphenols, fatty acids, terpenes and flavonoids.
- The different crude extracts of leaves of *Nepeta spicata* could be used in pharmaceutical and agricultural industries regarding their antioxidant power. The future scope of this work will be isolation of new noble bioactive compounds and their derivatization.

Conflicts of interest

We certify that there are no conflicts of interest.

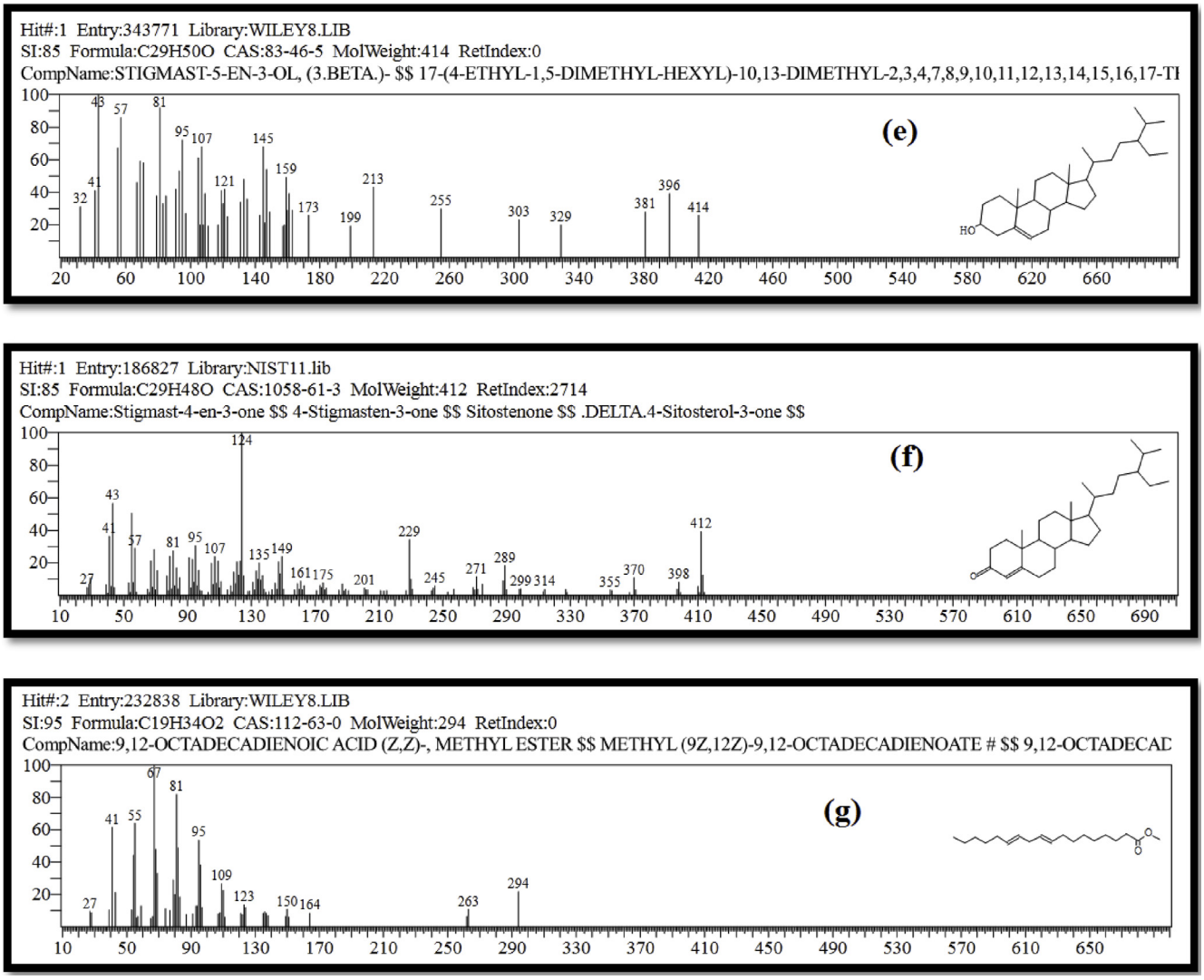

Fig. 4. (*continued*)

Table 7
Correlation among the different assay viz. TPC, TFC, DPPH scavenging, FRAP and TAC.

	TPC	TFC	DPPH	FRAP	TAC
TPC	1.00				
TFC	0.97	1.00			
DPPH	0.97	0.93	1.00		
FRAP	0.99	0.99	0.97	1.00	
TAC	0.98	0.98	0.95	0.98	1.00

TPC – total polyphenolic contents, TFC – total flavanoids contents, TAC – total antioxidant capacity, FRAP – ferric reducing antioxidant power, DPPH – 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101275.

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