ORIGINAL ARTICLE

Phytochemical screening, antioxidant activity and characterization of polyherbal extract by attenuated total reflectance and gas chromatography-mass spectrometry

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Abstract

Objective: The aim of this study was to evaluate the phytochemical, antioxidant property of polyherbal extracts (Leaves of *Vernonia amygdalina*, *Ocimum tenuiflorum,* and *Murraya koenigii*) and identification, characterization of phytoconstituents present in the extract by attenuated total reflectance (ATR) and gas chromatography-mass spectrometry (GC-MS) analysis. **Materials and Methods:** Crude drug was processed and extracted with different solvent as per their polarity index. Qualitative and quantitative screening of phytochemical present in polyherbal extracts has been done as per the guidelines led by the World Health Organization. Total phenolic and total flavonoid content was done for the assessment of the antioxidant property of extracts. The antioxidant activity of extracts was evaluated by 1, 1-diphenyl-2-picrylhydrazyl assay and H_2O_2 free radical scavenging activity. The phytochemicals present in the polyherbal extract were identified and characterized by ATR and GC-MS. **Results:** The GC-MS analysis of the hydromethanolic extract of polyherbal formulation revealed the presence of many bioactive compounds with valuable biological activities. The ATR analysis indicated the presence of alcohol, alkane, alkene, alkyl halide, alkyne, amine, aromatic, carbonyl, ether, acid, aldehyde, anhydride, and ester. **Conclusion:** The phytochemical profile of the polyherbal leaf extract indicates the presence of various bioactive compounds which can be utilized further for medicinal purposes.

Key words: Antioxidant, *Murraya*, *Ocimum*, phytochemical, *Vernonia*

INTRODUCTION

Medicinal plants are the gift of nature and play a significant role in the prevention and treatment of diseases. They can be a source of chemical compounds of biological and pharmacological importance. From ancient times, they are sources of successful drugs and will continuously be important for screening of new compounds.

According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary health-care needs.[1] Various *in vitro* screening methods act as a basic tool to determine potential phytocomponents. Chemical complexity of phytoconstituents is the major barrier to determine quality parameters of plant products. As herbal preparations comprise a lot of chemical compounds, it is difficult to completely characterize all these compounds. Nowadays, the emphasis is given on qualitative/quantitative biological fingerprinting to characterize the phytocomponents.[2,3]

Vernonia amygdalina (VA) is a common shrub or small tree that grows in tropical Africa. They are well distributed also in Asia and are commonly found along drainage lines and in a natural forest or commercial plantation. It belongs to the Asteraceae family and popularly called "African bitter

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Received: 07-06-2018
Revised: 21-06-2018
Accepted: 29-06-2018

leaf" in India and Africa. The leaves are green in coloration with a characteristic odor and bitter taste.[4] The leaves of VA are used as soup condiments after washing and boiling to get rid of the bitter taste.[5] In some part of the Africa continent like Nigeria, the plant is made into tonic and drank for medicinal purposes.[6] Other popular use of VA in Africa includes traditional treatment of diseases, such as malaria, infertility, diabetes, gastrointestinal problems, and sexually transmitted diseases.[7] Ethnomedical use of VA in the treatment of ailments such as venereal diseases, gastrointestinal problems, and malaria had also been reported.[8-10] In another instance, VA had been reported in the treatment of parasite-related disease in wild chimpanzee in Tanzania.[9] VA extracts have been reported as anthelmintic, antimalarial, antitumorigenic, as well as bacteriostatic and bactericidal.[11,12]

Murraya koenigii, commonly known as curry leaf or karipatta in Indian dialects, belonging to Family Rutaceae which represents more than 150 genera and 1600 species.[13] *M. koenigii* is a highly valued plant for its characteristic aroma and medicinal value. *M. koenigii* is widely used in Indian cookery for centuries and has a versatile role to play in traditional medicine. The plant is credited with tonic and stomachic properties. Bark and roots are used as a stimulant and externally to cure eruptions and bites of poisonous animals. Green leaves are eaten raw for the cure of dysentery, diarrhea, and for checking vomiting. Leaves and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation, and itching and are useful in leukoderma and blood disorders.[14,15] Several systematic scientific studies are also being conducted regarding the efficacy of the whole plant or its parts in different extract forms for the treatment of different diseases. *M. koenigii* contains a number of chemical constituents that interact in a complex way to elicit their pharmacodynamic response. A number of active constituents responsible for the medicinal properties have been isolated and characterized. This plant has been reported to have cytotoxic, antimicrobial, antibacterial, antiulcer, positive inotropic, and cholesterol reducing activities.[16-23]

Ocimum tenuiflorum (Krishna Tulsi) Family: Lamiaceae; Labiatae, the most sacred herb commonly known as Holy Basil is well known for its huge therapeutic activities and prevention against various diseases. *O. tenuiflorum* is a well-regarded deity herb native to India, widely dispersed, cultivated over the sphere.[24] The medicinal plant is demanded its healing application not only because they are safe but also easily available, economical, and effective. The *O. tenuiflorum*, plant as a whole (leaves, stem, flower, root, and seed) had been used in traditional medicine system for its range of therapeutic activities. The therapeutic activities such as analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidemic, and antistress have been attributed to *O. tenuiflorum*.[25-27]

In the present study, we evaluated the polyherbal extract for phytochemical constituents (qualitative and quantitative) as per standard guidelines led by the WHO. The free radical scavenging activity of polyherbal extracts was screened. The analytical tools such as Fourier transform-infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS) methods were employed to characterize the phytoconstituents present in the polyherbal extract.

MATERIALS AND METHODS

Preparation of Extract

Fresh leaves of VA Dell. were collected from an eastern part of Uttar Pradesh, *O. tenuiflorum* and *M. koenigii* (VOM) were collected from the local market in the month of January 2017 and identified by Prof. N. K. Dubey, Botanist, and Taxonomist, Banaras Hindu University, Varanasi, India.

The plants were extracted using the cold maceration method. The leaves of each plant were washed, cut into small pieces, dried at room temperature and pulverized into coarse powder. 500 g of each plant material was macerated in 70% (v/v) methanol in distilled water as well as sequentially extracted with petroleum ether (PEE) and ethyl acetate (EAE) for 72 h with intermittent shaking. The extracts were then filtered using Whatman No. 1 filter papers and PEE, and EAE extracts were then concentrated in vacuum using rotary evaporator at 40°C, while hydroalcoholic extract was subjected to lyophilization and was stored in air-tight containers in refrigerator at4°C. Finally, the powdered extracts were formulated in the equal ratio for further study which was used in all biochemical assays. Ascorbic acid (1 mg/mL) was used as a reference standard while methanol was used as control in all the experiments.

Qualitative Analysis[28,29]

Extracts were further reconstituted to get a final concentration of 1 mg/ml and qualitatively tested for the presence of different phytoconstituents such as alkaloids, glycosides, amino acids, phenolic compounds, reducing sugars, flavonoids, tannins, and saponins by standard procedures.

Tests for Glycosides

Fehling's test

Equal quantities of Fehling solution A and B were added to 1 ml of the extracts, on heating formation of a brick red precipitates indicate the presence of sugar.

Borntrager's test

Few ml of dilute sulfuric acid was added to 1 ml of extract solution. The solution was boiled, filtered and the filtrate

was extracted with chloroform. The chloroform layer was treated with 1 ml of ammonia. The formation of the red color of the ammonical layer shows the presence of anthraquinone glycosides.

Test for Alkaloids

Dragendorff's test

1 ml of Dragendorff's reagent (potassium bismuth iodide solution) was added to the 1 ml of extract. An orange-red precipitate indicates the presence of alkaloids.

Mayer's test

1 ml of Mayer's reagent (potassium mercuric iodide solution) was added to 1 ml of extract. Whitish or cream colored precipitate indicates the presence of alkaloids.

Test for Tannins

Lead sub-acetate test

Basic lead acetate solution was mixed with the little quantity of test solution. Formation of white precipitates indicates the presence of tannins.

Ferric chloride test

Ferric chloride solution was added to 1 ml of the extract. Formation of a dark blue or greenish black color product shows the presence of tannins.

Test for Flavonoids

- Little quantity of extract is treated with amyl alcohol, sodium acetate, and ferric chloride. A yellow color solution formed, disappears on the addition of an acid indicates the presence of flavonoids.
- The extract is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones.

Test for Saponins

- Small quantity of alcoholic and aqueous extract was taken separately, and 20 ml of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise. Foaming indicates the presence of saponins.

Test for Carbohydrates

Molisch's test

2 ml of the extract was taken; in which 1 ml of α-naphthol solution was added, then concentrated sulfuric acid as poured through the sides of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence of carbohydrates.

Fehling's test

1 ml of the extract was taken, equal quantities of Fehling solution A and B were added, on heating formation of a brick red precipitate indicates the presence of sugar.

Test for Phenols

Ferric chloride test

1 ml of the extract was taken, ferric chloride solution was added, formation of a dark blue or greenish black color product shows the presence of phenolic compounds.

Test for Protein

Biuret test

1 ml of 40% sodium hydroxide solution and 2 drops of 1% copper sulfate solution were mixed until a blue color is produced, and then added to the 1 ml of the extract. Formation of pinkish or purple violet color indicates the presence of proteins.

Xanthoproteic test

1 ml of concentrated nitric acid was added to 1 ml of the extract. A white precipitate is formed, it is boiled and cooled. Then, 20% of sodium hydroxide or ammonia was added. Orange color indicates the presence of aromatic amino acids.

Test for Steroids and Terpenoids

Liebermann–Burchard test

1 g of the test substance was dissolved in a few drop of chloroform, 3 ml of acetic anhydride, 3 ml of glacial acetic acid was added, warmed and cooled under the tap and drops of concentrated sulfuric acid were added along the sides of the test tube. Appearance of bluish-green color shows the presence of sterols.

Test for Cardiac glycosides

Keller–Killiani test

1 g of powdered drug was extracted with 10 ml of 70% alcohol for 2 min, filtered. To the filtrate, 10 ml of water and 0.5 ml of strong solution of lead acetate were added and filtered, and the filtrate was shaken with 5 ml of chloroform. The chloroform layers were separated in a porcelain dish, and the solvent was removed by gentle evaporation. The cold residue was dissolved in 3 ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2 ml of concentrated sulfuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening withstanding.

Quantitative Phytochemical Assays

The quantitative phytochemical analysis was performed after the confirmation of the presence of phenol, flavonoid, and saponin by qualitative phytochemical tests.

Determination of Total Phenolic Content[30,31]

10 mg of each polyherbal extract was weighed accurately and dissolved in 10 ml of distilled water. To 1 ml of this solution, 0.5 ml of Folin–Ciocalteu reagent was added and vortexed. After 5 min, 1.5 ml 20% of Na_2CO_3 solution was added, and the volume was made up to 8 ml with distilled water and incubated for 90 min at room temperature. The reagent blank was also prepared in the same way except for polyherbal extract. After incubation, the absorbance against the reagent blank was determined at 765 nm. Gallic acid was used as a standard, and standard calibration curve was prepared with various dilutions (20, 40, 60, 80, and 100 µg/ml). The total phenolic content of the polyherbal extract was expressed as gallic acid equivalent (GAE) mg/g dry weight. Samples were analyzed in triplicates.

Determination of Total Flavonoid content[32]

Aluminum chloride colorimetric method was used for flavonoids determination. Polyherbal extracts (0.5 ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water and then incubated at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared using quercetin as standard.

In Vitro Antioxidant Activity

Ascorbic acid was used a reference standard while methanol was used as control in all the experiments.

Determination of 1, 1-diphenyl-2- Radical Scavenging Activity[33,34]

A stock solution of 5 mg/ml each polyherbal leaf extract and standard butylated hydroxytoluene (BHT) were prepared. Dilutions of varying concentrations (25, 50, 100, and 200 µg/ml) of polyherbal leaf extracts (hydromethanolic extract [HME]) and standard were prepared from the stock solution. To 2.5 ml solution of the extract, 1 ml of a 0.3 mM DPPH methanol solution was added and incubated at room temperature for 30 min. The same procedure was followed by standard. The optical density of different solutions was measured at 517 nm.

The capability of scavenging DPPH radical was calculated using the following equation:

DPPH Scavenged (%) = $(A_c–A_t)/A_c\times100$

Where "A_c" is the absorbance of the control reaction and "A_t" is the absorbance of the sample of the extracts. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Determination of H_2O_2 Radical Scavenging Activity[35]

4 ml of each polyherbal extract (HME) was prepared in distilled water at various concentrations and mixed with 0.6 ml of 40 mM H_2O_2 solution prepared in phosphate buffer (50 mM pH 7.4) and incubated for 20 min and its concentration was determined by measuring the absorbance at 230 nm using ultraviolet spectrophotometer against blank solution containing the phosphate buffer without H_2O_2.

The percentage of hydrogen peroxide scavenging was calculated using the given formula:

H_2O_2 radical scavenging activity = $(A_c–A_t)/A_c\times100$

Where "A_c" is the absorbance of H_2O_2 radical + methanol and "A_t" is the absorbance of H_2O_2 radical + sample extract or standard (ascorbic acid).

FTIR Spectroscopic Analysis

Attenuated total reflectance (ATR)-FTIR analysis was carried out using Bruker (Alpha Eco-ATR) spectrophotometer. Briefly, the crystal spot was cleaned with a swab of acetone and the background measurement was collected. The hydromethanolic sample of polyherbal leaf extract was then placed directly on the spot.

GC-MS Analysis

The HME was subjected to GC-MS analysis. GC-MS analysis was carried out using Shimadzu QP-2010 Plus with Thermal Desorption system (TD 20) with quadrupole detector; the injection temperature was 260°C in spilled mode. The pressure was 77.5 kPa. The column oven temperature initiated with 70°Cwith, hold time of 2.00 min the raise to 250°C at the rate of 7°C/2 min then 280°C at the rate of 10°C with, hold time of 28.00 min the ion source and interface temperature were 230°C and 270°C, respectively. The compounds were identified by comparing their spectra with the National Institute of Standards and Technology (NIST) (Wiley 8) library available in the instrument. GC-MS was done at the Advanced Instrumentation Research Facility (AIRF), JNU, New Delhi.

Sample Preparation

1 g of well-mixed sample was taken in a screw cap vial, and 10 ml of methanol was added. It was sonicated for an hour and kept for 12 h.

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) and *t*-test. Results are expressed as a mean ± standard error of the mean (n = 3). Antioxidant activity and effects of extracts in different solvents were subjected to principal component analysis.

RESULTS AND DISCUSSION

The qualitative study reported the presence of glycosides, alkaloids, tannins, saponins, flavonoids, and terpenoids in the hydromethanolic, PEE, and EAE extracts as mentioned in Table 1.

Total phenolic content was measured by Folin–Ciocalteu reagent in terms of GAE (standard curve equation: $y = 0.006x - 0.074$, $r^2 = 0.991$). The flavonoid content of the extracts was measured in terms of Quercetin equivalent (standard curve equation: $y = 0.006x + 0.031$, $r^2 = 0.996$) shown in Table 2.

DPPH Radical Scavenging Activity

The DPPH antioxidant assay is based on the reduction of ethanolic $DPPH^-$ solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH by reaction. The extract was able to reduce DPPH radical (visible deep purple color) to the yellow colored diphenylpicrylhydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant compounds present in the polyherbal extract (HME) in turns of hydrogen donating ability [Table 3].[33,34]

Table 1: Phytochemical screening of polyherbal extracts

Phytoconstituents	PEE extract	EAE	HME
Alkaloids	–	+	++
Glycosides	–	+	++
Steroids and terpenoids	+	+	++
Flavonoids	–	+	+++
Saponin	–	–	++
Tannin	–	–	+
Cardiac glycosides	–	–	+
Polyphenols	–	+	+++
Carbohydrates	–	–	–
Protein	–	–	–

HME: Hydromethanolic extract, PEE: Petroleum ether, EAE: Ethyl acetate

H_2O_2 Radical Scavenging Activity

Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells.[36] Thus, the removing of H_2O_2 is very important for antioxidant defense in cell or food systems [Figure 1].

ATR-FTIR Study

The FTIR spectrum was used to identify the functional groups present in the sample. The ATR spectrum in Figure 2 shows a broadband at 3361.79/cm assigned to O-H stretching vibrations. This may also indicate the presence of phenol and flavonoid. The sharp peak observed at 1592.40/cm (Peak 2) was attributed to aromatic C=C bending whereas peaks at 1357.90/cm and 1072.89/cm depicted strong C-H stretching in fingerprint region. The peak at 830.05/cm assigned to C-H aromatic bending vibrations which characterized the high content of eugenol. Another peak at 689.69/cm and 610.26/cm was due to a primary amine. The crude extracts subjected to FTIR analysis is used for the identification of chemical constituents present in the polyherbal extract.

GC-MS Study

GC-MS is a best technique to identify phytocompounds. The results from GC-MS analysis [Table 4] lead to the identification of number of compounds from the GC fractions of the HME of polyherbal formulation. The identification

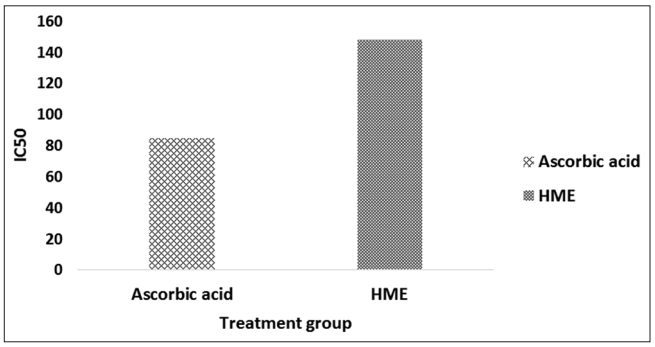

Figure 1: IC_{50} value in H_2O_2 free radical scavenging activity of polyherbal extracts (hydromethanolic extract)

Table 2: Total phenolic and flavonoid content of the extracts

Antioxidants (mg/g)	Hydromethanolic (HME)	PEE	EAE
Total phenolic content (GAE)	144.7±0.25	121.9±0.57	105.7±0.42
Total flavonoid content (QE)	437.7±0.12	378.2±0.70	392.5±0.70

QE: Quercetin equivalent, GAE: Gallic acid equivalent

Table 3: DPPH free radical scavenging activity of polyherbal extract

Concentration (µg/mL)	Percent inhibition	
	BHT	HME
25	30.65±0.012	11.52±0.03
50	39.74±0.020	18.92±0.01
100	54.30±0.030	26.44±0.04
200	62.55±0.025	31.20±0.031
IC_{50}	50.70	120.20

Value expressed as mean±SEM (*n*=3). SEM: Standard error of mean, DPPH: 1, 1-diphenyl-2-picrylhydrazyl, BHT: Butylated hydroxytoluene, HME: Hydromethanolic extract

of the phytochemical compounds was confirmed based on the peak area, retention time, and molecular formula. The mass spectra of the separated components were compared with those stored in the NIST 11 database (NIST) and Wiley 8. The name, molecular weight, and structure of the components of the test materials were ascertained. The phytochemicals identified through GC-MS analysis showed many biological activities relevant to this study are listed in Table 5. The biological activities listed are based on Dr. Duke's Phytochemical and ethnobotanical databases created by Dr. Jim Duke of the Agricultural Research Service/USDA.[37]

Table 4: Phytochemical components in polyherbal extract identified using GC-MS

Name	Retention time	%area	Molecular Wt.	Molecular formula
1,2,3-Propanetriol	5.180	3.52	92	$C_3H_8O_3$
1-Butanamine	6.578	0.26	155	$C_{10}H_{21}N$
3-ethyl-4-methyl-3-penten-2-one	6.999	0.43	126	$C_8H_{14}O$
1-butanol, 3-methyl-, acetate	7.513	10.40	130	$C_7H_{14}O_2$
1,5-anhydro-6-deoxyhexo-2,3-diulose	8.464	1.71	144	$C_6H_8O_4$
5-Methoxypyrrolidin-2-one	9.131	0.83	115	$C_5H_9NO_2$
Sec-Butyl nitrite	9.708	0.83	103	$C_4H_9NO_2$
2-hydroxycinnamic acid	9.981	0.28	164	$C_9H_8O_3$
Glycerol-1—acetate	10.446	0.48	134	$C_5H_{10}O_4$
Isoeugenol	12.675	1.09	164	$C_{10}H_{12}O_2$
3-methyl salicylaldehyde	14.326	1.33	136	$C_8H_8O_2$
2-ethylhexyl ester, decanoic acid	15.600	0.22	284	$C_{18}H_{36}O_2$
6,9,12-Octadecatrien-1-ol	16.947	0.26	264	$C_{18}H_{32}O$
1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid	17.545	3.37	192	$C_7H_{12}O_6$
1-(4,7,7-trimethylbicyclo[4.1.0] hept-3-en-3-yl) ethanone	18.023	0.29	178	$C_{12}H_{18}O$
3-O-methyl-d-glucose	18.497	0.90	194	$C_7H_{14}O_6$
Farnesyl alcohol	18.991	0.27	222	$C_{15}H_{26}O$
Methyl vanillyl ketone	19.317	0.97	180	$C_{10}H_{12}O_3$
Tetradecanoic acid	19.632	0.38	228	$C_{14}H_{28}O_2$
Benzyl benzoate	19.854	1.10	212	$C_{14}H_{12}O_2$
Farnesyl acetate 3	20.768	0.31	264	$C_{17}H_{28}O_2$
3,7,11,15-tetramethyl-2-hexadecen-1-ol	20.839	0.27	296	$C_{20}H_{40}O$
Spiro[4.4]non-3-en-2-one, 4-methyl-3-(1H-tetrazol-5-yl)-1-o	21.322	0.46	220	$C_{10}H_{12}N_4O_2$
7-Hexadecenoic acid, methyl ester, (Z)-	22.030	0.26	268	$C_{17}H_{32}O_2$
Hexadecanoic acid, methyl ester	22.112	2.51	270	$C_{17}H_{34}O_2$
N-Hexadecanoic acid	22.641	6.89	256	$C_{16}H_{32}O_2$
2,6-dimethyl-4-nitrophenol	23.008	0.52	167	$C_8H_9NO_3$
9-Octadecenoic acid	23.460	0.89	282	$C_{18}H_{34}O_2$
9,12-octadecadienoic acid, methyl ester	24.422	1.14	294	$C_{19}H_{34}O_2$
9,12,15-octadecatrienoic acid, methyl ester,	24.501	4.85	292	$C_{19}H_{32}O_2$

(contd....)

Table 4: *(continued)*

Name	Retention time	%area	Molecular Wt.	Molecular formula
Phytol	24.663	23.58	296	$C_{20}H_{40}O$
Hexadecanoic acid, 15-methyl-, methyl ester	24.860	0.52	284	$C_{18}H_{36}O_2$
9,12,15-octadecatrien-1-ol	25.044	10.89	234	$C_{16}H_{26}O$
Stearic acid	25.322	0.56	284	$C_{18}H_{36}O_2$
T-phytol	25.974	0.21	296	$C_{20}H_{40}O$
Benzyl .beta.-d-glucoside	26.347	1.03	270	$C_{13}H_{18}O_6$
3-(1-phenylethoxy) Butanoic acid	27.509	0.37	208	$C_{12}H_{16}O_3$
2-(Dimethylamino) ethyl1-adamantanecarboxylate	29.061	0.93	251	$C_{15}H_{25}NO_2$
1-Hydroxy-7-methoxy-3-methyl-9H-carbazol	29.195	0.30	227	$C_{14}H_{13}NO_2$
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	29.798	1.67	330	$C_{19}H_{38}O_4$
Dioctyl phthalate	30.123	0.69	390	$C_{24}H_{38}O_4$
Linoleic acid chloride	32.128	0.54	298	$C_{18}H_{31}ClO$
Cis, cis, cis-7,10,13-Hexadecatrienal	32.219	2.15	264	$C_{18}H_{32}O$
8-Methoxy-11-methyl-11H indolo[3,2-c] quinoline, 5-oxide	33.201	2.42	278	$C_{17}H_{14}N_2O_2$
3-Acetyl-2,5,7-trihydroxy naphthoquinone	34.044	0.36	248	$C_{12}H_8O_6$
2,3,5,6-Tetraisopropylpyrazine	36.136	6.21	248	$C_{16}H_{28}N_2$
Trimethyl (stearoyloxy) Stannane	38.229	0.53	448	$C_{21}H_{44}O_2Sn$
		100.00		

GC-MS: Gas chromatography-mass spectrometry

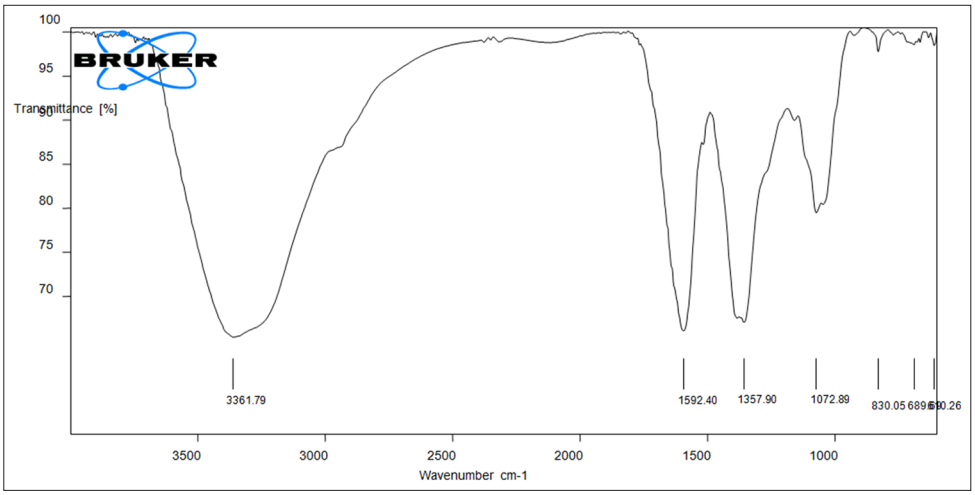

Figure 2: Attenuated total reflectance - Fourier transform-infrared spectroscopy spectra for polyherbal extract (hydromethanolic extract)

CONCLUSION

This study affirms the *in vitro* antioxidant potential of HME of the developed polyherbal extract. The result of the present study showed that the extract of polyherbal, which contains the highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity. The high scavenging property of polyherbal extract may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger. Free radicals are often generated as by-products of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases.[38] All of the extracts in this research exhibited different extent

Table 5: Biological activity of major phytocomponents identified by GC-MS in polyherbal extract

%Area	Name of compound	Nature of compound	Biological activity
23.58	Phytol	Diterpene	Antioxidant, anticancer, antidiabetic, antimicrobial, antidiuretic, anti-inflammatory, immunostimulatory
10.89	9,12,15-octadecatrien-1-ol	Fatty alcohol	Antibacterial, antiviral, drugs of urinary, nervous system disorders
10.40	1-Butanol, 3-methyl, acetate	Alcoholic compound	Antimicrobial
6.89	n-Hexadecanoic acid	Palmitic acid	Antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic, lubricant, 5- Alpha reductase inhibitor
4.85	9,12,15-Octadecatrienoic acid, methyl ester	Fatty acid ester compound	Anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary
3.52	1,2,3-Propanetriol	Phenol	Antimicrobial
3.37	1,3,4,5-Tetrahydroxy-cyclohexanecarboxylic acid	Polyphenols	Antioxidant properties

GC-MS: Gas chromatography-mass spectrometry

of antioxidant activity. Polyherbal extract showed higher potency than BHT and ascorbic acid in scavenging of DPPH and H_2O_2free radical, respectively. This may be related to the high amount of flavonoid and phenolic compounds in this plant extract. It can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in the pharmaceutical industry. These mass spectra are fingerprint of the compound which can be identified from the NIST data library. Hence, the identified phytoconstituents using GC-MS can be used as a pharmacognostical tool for the identification of active constituents. It paves the way for the development of several treatment regimens based on this extract. In addition, these active constituents may be responsible for the medicinal characteristics of the polyherbal extract.

ACKNOWLEDGMENT

We are grateful to thank for the financial support and necessary facilities granted by the Research and Development Section of IIT(BHU), Varanasi.

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Source of Support: Nil. **Conflict of Interest:** None declared.