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EVALUATION OF BIOLOGICAL ACTIVITY AND PHARMACOLOGICAL ATTRIBUTES OF *PARIPLOCA APHYLLA*: POTENTIAL SOURCE OF FUNCTIONAL FOOD AND NUTRACEUTICALS

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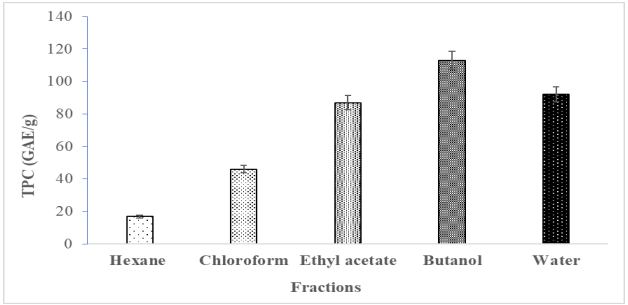
Functional foods and nutraceuticals are gaining popularity as they are being developed and commercialized with the aim of reducing the risk of various diseases and their treatment. In this study, antiradical activity guided fractionation of *Pariploca aphylla* (*P. aphylla*) crude extract was conducted. A crude methanolic extract of *P. aphylla* was prepared and then fractionated using different solvents, including hexane, chloroform, ethyl acetate, butanol, and water. This approach allowed for the isolation and characterization of different fractions of *P. aphylla* with potential antioxidant activity. These fractions were tested for the determination of total phenolic content, antiradical (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$) potential and HPLC quantification of phenolic acids. Among all the tested fractions, butanol fraction exhibited highest phenolic content and significant antiradical potential. Butanol faction was further sub-fractionated using a silica gel-loaded column and 18 sub-fractions were obtained. Antiradical potential of all 18 sub-fractions was determined and sub-fraction 12 was found having highest radical scavenging potential. Structures of compounds in sub-fraction 12 were identified and quantified using GC-MS. Among all the identified compounds, 7-Trimethylsilyloxytridecane ($C_{16}H_{36}OSi$) and 1,2-*bis*(trimethylsiloxy)ethane ($C_8H_{22}O_2Si_2$) were found in highest ratio. All of the compounds can be declared having high anti-radical potential that can be extracted or synthesized for possible exploitation as functional food or nutraceuticals.



INTRODUCTION

The process of bioactivity-guided fractionation is crucial in the isolation of a bioactive compounds of interest. However, this approach may lead to the elimination of several compounds that possess promising biological activities. In certain cases, a single compound may not exhibit the same level of biological activity as a group of compounds, due to pharmacokinetic influences or synergism.[1–5] For instance, the saponins fractions extracted from ginseng were found to be more potent than the isolated saponins compounds. Moreover, while isolating one compound of interest using bioactivity-guided methods, many other compounds with varying activities are often

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overlooked. For example, *Catharanthus roseus* plant, which was initially recognized due to anti-diabetic potential but was later discovered due to high anti-tumor agents.[6–10]

For many years, researchers have concentrated on extracting bioactive components from crude extracts. However, the isolation process has its limitations, including the consumption of large solvents volume, the need for state-of-the-art instrumentation, and lesser yields or the loss of targeted compounds, particularly which were present in lesser amount. These limitations have caused a decline in researchers' interest in isolation over the last decade.[11] Additionally, various studies have revealed that compounds in group form showed higher activity compared to isolated compounds. Considering these factors, the isolation of fractions rich in bioactive components from crude extracts is now mostly selected over isolation, and enhanced activities reported for these bioactive compound rich fractions in several cases compared with raw extracts or isolated constituents.[6,12,13]

P. aphylla is a member of the *Periploca genus* (*Asclepiadaceae*). Antitumor constituents were found in the root and bark of this plant, which are useful in the treatment of congestive heart failure.[14,15] The essential oil of *P. aphylla* has been reported to possess biological activities, including antimicrobial, insecticidal, and antioxidant properties. *Periploca laevigata*, another species of this family, is used in herbal formulations for the treatment of diabetes and headaches.[16] *P. aphylla* is commonly known as "Bata" or "Barara", and the plant produces a milky fluid that is used to treat swellings and tumors. Extracts of this plant can also be used to treat swollen joints, coughs, flu, and other diseases such as ulcers, skin infections, and constipation. Two new components that were lupane derivatives isolated from *P. aphylla* stems. Various constituents separated from *P. aphylla* have exhibited inhibitory effects against α-glucosidase type VI.[17] Researchers are currently investigating and reporting[18,19] different phytochemicals and biological attributes of *P. aphylla*. However, a comprehensive study is still needed to bridge existing gaps in knowledge.

In order to exploit botanical materials as nutraceutical or functional food product, it is necessary to find out their most potent fraction with strong antioxidant activity. According to the best of our literature survey, no research work has been reported summarizing antioxidant potential of *P. aphylla* fractions followed by HPLC and GC-MS analysis. Current study was designed to carry out antioxidant activity guided fractionation of *P. aphylla* crude extract. Fraction showing highest antioxidant activity was further processed for sub-fractionation and sub-fraction with strong antioxidant potential was then analysed using GC-MS. The structure of compounds with the highest antioxidant potential has been identified and reported. These compounds can be extracted for exploitation as functional food or nutraceuticals.

MATERIAL AND METHODS

Preparation of *P. aphylla* crude extract and solvent fractions

The plant material, *P. aphylla*, was collected from peripheral areas of Kallar Kahar, District Chakwal, Pakistan. The plant material was washed thoroughly with tap water and dried under shade. Once dried, it was ground into a powder. To prepare a crude extract, 500 g of the powdered plant material was extracted with methanol using an orbital shaker. The solvent was then recovered using a rotary evaporator, and the resulting crude extract was suspended in distilled water (500 mL) in a separating funnel. A second solvent, n-hexane (500 mL), was added to the mixture. The separating funnel was shaken to ensure good separation of the two solvent layers, and the first hexane fraction was collected. The residual aqueous layer was then mixed with chloroform (500 mL) to obtain the second chloroform fraction. This process was repeated three times for each solvent, including ethyl acetate (EtOAc) and butanol (ButOH) fractions. Finally, an aqueous (HOH) fraction was also collected separately. All the fractions (n-hexane, chloroform, EtOAc, ButOH, and HOH) were concentrated, dried, and stored at –80°C for further analysis.[20]

Determination of antioxidant potential

The antioxidant potential of the different solvent fractions (n-hexane, chloroform, ethyl acetate, butanol, and aqueous) was determined by measuring the total phenolic content (TPC) using a spectrophotometric assay with Folin-Ciocalteu (FC) reagent. A fraction of each extract was mixed with freshly prepared and diluted FC reagent (1:10, 100 µL) and then sodium carbonate solution (7.5%, 2 mL) was added. Deionized water was added to the resulting solution to make a total

volume of 7 mL, and the mixture was placed in the dark for 2 h at room temperature. The absorbance was then recorded at 765 nm, and the amount of TPC was calculated as gallic acid equivalents (mg/g) using a standard curve of gallic acid.

The antiradical potential of the fractions (n-hexane, chloroform, ethyl acetate, butanol, and aqueous) was evaluated using DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays. For the DPPH$^{\bullet}$ assay, a stock solution of DPPH$^{\bullet}$ was prepared by dissolving 0.4 mg of 2,2-diphenyl-1-picryl-hydrazyl-hydrate in methanol and adjusting its absorbance to 0.7 at 515 nm by diluting the solution. The reaction mixture was prepared by mixing 2 mL of diluted fraction and 0.5 mL of the DPPH$^{\bullet}$ solution, and the resulting mixture was allowed to react in the dark at room temperature for 45 min. After incubation, the absorbance was recorded at 515 nm against a methanol blank.[21,22]

The radical cation (ABTS$^{\bullet+}$) scavenging assay was conducted to assess the radical scavenging potential of the samples in the current study. First, a 4 mM aqueous solution of ABTS$^{\bullet+}$ radicals was prepared by oxidizing 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) with MnO_2. The resulting ABTS$^{\bullet+}$ solution was then diluted with phosphate buffer solution (pH = 7.4) to achieve an absorbance of 0.9 at 734 nm. The sample fraction was further diluted with 5 mM phosphate buffered saline (PBS, pH = 7.4) and added to 7 mL of the ABTS$^{\bullet+}$ solution. The mixture was then allowed to mix for 30 min at room temperature, and the absorbance was measured using PBS as a blank. The percent inhibition of the samples was calculated using the formula;

$$Inhibition\ (\%) = [\frac{Ac - As}{Ac}] \times 100$$

where A_c is the absorbance of the control (ABTS$^{\bullet+}$ solution without extract) and A_s is the absorbance of the sample.[21,23]

HPLC Quantification of phenolic acids in *P. aphylla* fractions

Preparation of samples

To identify and quantify phenolic compounds in different solvent fractions of *P. aphylla*, an HPLC (High Performance Liquid Chromatography) method was performed. 25 mg of each fraction were dissolved in 5 mL of 6M HCl, followed by the addition of 10 mL of methanol. The resulting solution was then incubated at 90°C for 2 h and filtered using a 0.2 µm Millipore membrane filter before being injected into the HPLC system. This step ensured that the samples were prepared in a consistent and standardized manner for accurate analysis of phenolic compounds using HPLC.[24]

HPLC analysis

The HPLC method was used to quantify phenolic acids in fractions obtained from different solvent systems, including n-hexane, chloroform, ethyl acetate (EtOAc), butanol (ButOH), and water. The samples were injected into an HPLC instrument equipped with a UV-VIS detector, using an Agilent auto sampler. Phenolic components of each fraction were separated on an Agilent C_{18} column (20 RBAX Eclipse, XDB: 5 µm; 4.6 × 150 mm, Agilent USA) using an isocratic mobile phase of acetonitrile and 0.05% phosphoric acid solution (20:3:77, v/v/v) at 1 mL/min a flow rate and the mobile phase was passed through a 0.2 µm millipore membrane filter before use, and sonication was performed to degas the solvent mixture. The separated components were identified at 280 nm, which is a commonly used wavelength for detecting phenolic compounds in HPLC analysis.[25]

Procedure for sub-fractionation

The antioxidant potential of all the fractions was evaluated in this study, and sub-fractionation was carried out specifically on the Butanol (ButOH) fraction, which exhibited the highest antioxidant activity. A glass column was uniformly packed with 100 grams of silica gel with a mesh size of 200, and the ButOH fraction was loaded onto the silica gel. To prevent sample disturbance while adding solvent, 5 g of silica gel were added on top of the sample. For gradient elution, a mixture of solvents (butanol, ethanol, and methanol) was used, and the sub-fractions obtained were analyzed by thin layer chromatography (TLC). Sub-fractions that exhibited similar TLC profiles were pooled together. The concentration of the pooled sub-fractions was performed using a rotary evaporator.[26–28]

Estimation of bioactive compounds by GC-MS

Agilent 5975 MSD, and the instrument was operated using Chemstation GC-MS software.[6] A DB-5 MS Agilent column with dimensions of

30 m × 0.25 mm × 0.25 µm, composed of 5% diphenyl – 95% dimethylpolysiloxane, was used. Helium (99.999%) was used as the carrier gas with a flow rate of 1 mL/min. The mass spectra were developed and ionization was achieved at 70 eV using electron impact mode. To avoid solvent overloading, ionization was kept off for the first 1 min. The level of ionization storage was set up to 35 m/z with a maximum ionization time of 25,000 µs. The quadrupole triple axis MS detector was maintained at a temperature of 250°C, and the mass spectrum obtained was used for the identification of bioactive compounds using the NIST and Wiley database library.

For the derivatization of the sub-fraction samples, 25 mg of sub-fraction was added to a sampling vial containing 1 mL of methanol, followed by the addition of an internal standard (5 mg of 3-hydroxybenzoic acid). Derivatization was performed using 300 µL of pyridine, 50 µL of trimethylchlorosilane (TMCS), and 150 µL of N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). After thorough mixing, the sample vial was placed in an oven at 80 °C for 1 hour to complete the derivatization process. The samples were allowed to cool and then centrifuged. The supernatant liquid (0.2 µL) was injected using a split-less injection system into the GC-MS instrument with a mass selection range of 90–550 m/z. The obtained results were in the form of trimethylsilyl esters.[29,30]

RESULTS AND DISCUSSION

Total phenolic content (TPC)

Figure 1 displays the total phenolic content (TPC) of crude extracts from *P. aphylla* plant in various solvent fractions. The results indicate that the butanol fraction exhibited the highest level of total phenolics, followed by the water fraction, and the lowest amount in the hexane fraction. Statistical analysis revealed significant differences ($p << 0.05$) in the TPC among all the fractions. These findings are in accordance with previous studies that have reported the ability of high polarity solvents such as butanol and water to solubilize a significant portion of phenolic components in crude extracts. Similar results were also observed during the fractionation process with water and methanol showing comparable potential. These findings are consistent with the findings reported by Lee *et al.*[31] for the butanol fraction of olive oil, confirming the affinity of polar solvents for phenolic compounds.

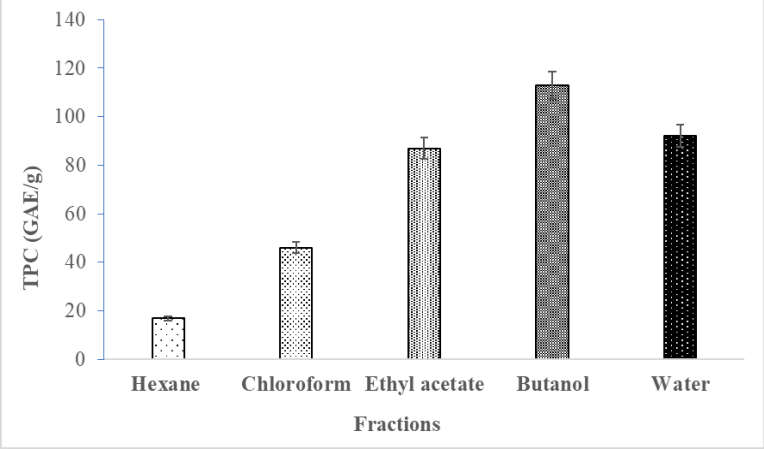

Fig. 1 – Total phenolic content in *P. aphylla* solvent fractions.

DPPH radical scavenging activity

This assay was performed by measuring the discoloration of DPPH free radical upon reaction with hydrogen donating species, such as antioxidants present in plant extracts. In this study, different fractions of *P. aphylla* were investigated for their antiradical potential using the DPPH assay. Figure 2 displays the percentage inhibition of DPPH radical by various fractions, including hexane, chloroform, ethyl acetate, butanol, and water ($p << 0.05$). The results indicate that the butanol fraction exhibited the strongest antiradical potential, followed by the ethyl

acetate fraction. On the other hand, the hexane fraction showed the lowest DPPH radical scavenging potential. These findings confirm that phenolic components can be extracted from a crude extract suspended in water using polar solvents, as evidenced by the results obtained in this study.

Butanol and ethyl acetate have been commonly used as solvents to recover polar antioxidant compounds due to their ability to solubilize phenolic components effectively. Furthermore, the DPPH• assay is known for its ability to evaluate antioxidant potential in aqueous media. Therefore, the improved performance of the DPPH• assay in aqueous media in this study may be attributed to the higher antiradical potential of the butanol, ethyl acetate, and water fractions. These results are consistent with previous studies, indicating similar findings.[31]

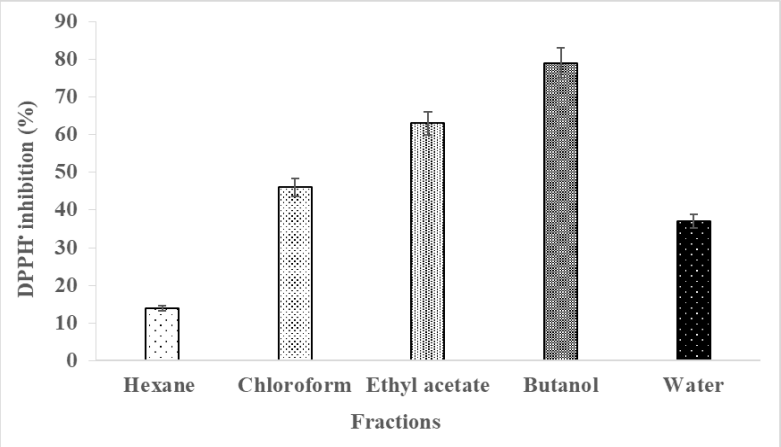

Fig. 2 – DPPH• %age inhibition potential of *P. aphylla* solvent fractions.

ABTS radical cation scavenging activity

The $ABTS^{\bullet+}$ scavenging method is similar to the $DPPH^{\bullet}$ method in terms of the working mechanism for the evaluation of antioxidant activity. However, the $ABTS^{\bullet+}$ method is considered more reliable due to the solubility of the $ABTS^{\bullet+}$ reagent in both aqueous and organic solvents and its rapid reaction with both lipophilic and hydrophilic antioxidant species, compared to $DPPH^{\bullet}$. In this study, the fractions of *P. aphylla* were evaluated for their antiradical potential using the $ABTS^{\bullet+}$ assay, as shown in Fig. 3, where the radical scavenging activity is presented as percentage inhibition. No statistically significant difference was found among the various solvent fractions ($p >> 0.05$). The methanolic fraction exhibited the highest radical scavenging activity, followed by the butanol fraction. Therefore, the butanol fraction can be considered to contain a significant amount of antiradical agents and may be further subjected to sub-fractionation or isolation for further characterization.

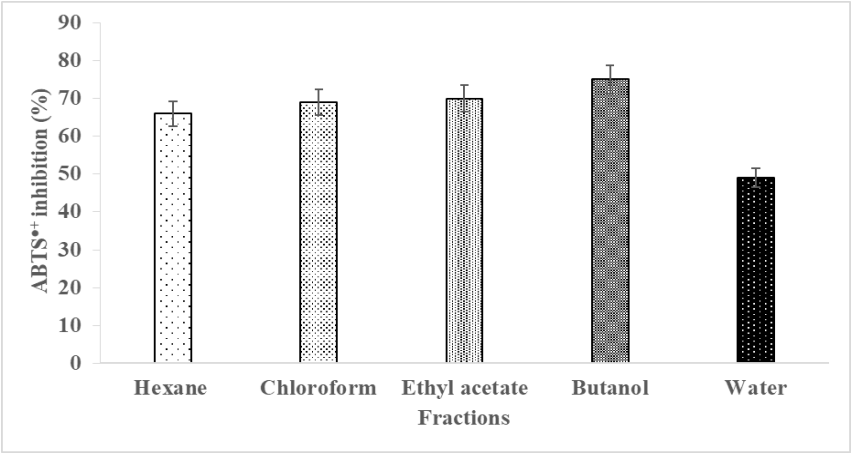

Fig. 3. $ABTS^{\bullet+}$ %age inhibition potential of P. *aphylla* solvent fractions.

HPLC quantification of phenolic acids from *P. aphylla* solvent fractions

Phenolic compounds are the most significant kind of exogenous antioxidants and widely recognized free radicals scavenger, due to their ability to donate hydrogen and electrons.[32] Numerous degenerative and age-related illnesses have been observed to occur less often if phenolic compounds are taken in regularly through food. The majority of these phenolic compounds are acidic in nature and may be separated into two main groups: those made from benzoic acid derivatives and those made from cinnamic acid derivatives. Fruits, vegetables, and other herbs contain phenolic acids of both groups, with varying amounts of hydroxylation. Some well-known hydroxybenzoic acids from the first group are gallic (3,4,5-trihydroxybenzoic acid), vanillic (4-hydroxy-3-methoxybenzoic acid), syringic (4-hydroxy-3,5-dimethoxybenzoic acid), and 4-hydroxybenzoic (p-hydroxybenzoic acid). The structure, substituents, or side chains of phenolic acids have a significant impact on their activities. Although some publications dispute the idea that conjugated double bonds have any impact on antioxidant capability, it is suggested that the ethylenic side-chain plays a function in the resonance-stabilization of peroxyl radicals. Numerous writers have described the considerable biological activity of various botanical materials in addition to their pharmacological properties, which have been discovered as powerful sources of phenolic acids.[33] In this study, different solvent fractions of *P. aphylla* were studied for phenolic contents by HPLC and the findings of phenolic acids are outlined in Table 1. Both hexane and water fractions were found containing 8 phenolic acids each. Phenolic acids present in hexane fraction were in minute quantity.

Table 1

Phenolic composition of *P. aphylla* solvents fractions

Phenolic Acids	Hexane fraction (mg/g)	Chloroform fraction (mg/g)	Ethyl Acetate fraction (mg/g)	Butanol fraction (mg/g)	Water fraction (mg/g)
Quercetin	0.17	0.03	0.15	2.42	0.36
Gallic acid	0.11	0.55	1.58	8.51	1.42
Ferulic acid	0.52	–	–	2.18	7.41
Sinapic acid	0.86	–	0.23	–	–
Vanilic acid	0.49	0.15		7.25	–
4-hydroxy-3-methoxy benzoic acid	0.03	–	6.36	23.94	10.19
Chlorogenic acid	–	–	–	0.37	–
p-Coumaric acid	0.48	–	1.27	–	0.44
m-Coumaric acid	–	0.22	–	–	0.42
Trans-4-hydroxy 3-methoxy cinnamic acid	–	–	0.81	20.94	1.09
Caffeic acid	–	–	1.42	0.34	4.46
Syringic acid	0.33	–	–	–	–

On the other hand, substantial quantities of 4-hydroxy-3-methoxy benzoic acid, ferulic acid, and caffeic acid were discovered in the water fraction. Four and six phenolic acids, respectively, were present in the fractions of chloroform and ethyl acetate, but there was not sufficient amount of these phenolic acids to exert any detectable antioxidant activity. There were eight phenolic acids identified in the butanol fraction, and this fraction had the highest concentration of phenolic acids.

The butanol fraction had the highest concentration of 4-hydroxy-3-methoxy benzoic acid (23.94 mg/g), while trans-4-hydroxy 3-methoxy cinnamic acid had the lowest concentration (20.94 mg/g). The outcomes of the HPLC are consistent with those of the DPPH• and ABTS•+ scavenging assays. The largest percentage of inhibition against both free radicals was shown by the butanol fraction, and this activity can be related to the butanol fraction's abundant phenolic

acids. The results of recent research point to *P. aphylla* butanol fraction as a potential source of natural antioxidants.

Antioxidant activity of sub-fractions from P. aphylla butanol fractions

DPPH radial scavenging potential of sub-fractions of** P. aphylla **butanol fraction

The *P. aphylla* sub-fractions (PSF), which are made from the plant's butanol fraction, might individually contain compounds from the same class. The DPPH radical scavenging assay was used to distinguish between these sub-fractions for their antioxidant capability. Data is showed in Fig. 4 revealing that all sub-fractions have significant DPPH radical scavenging activity. Out of all the sub-fractions studied, sub-fractions 12 and 13 showed the strongest potential to scavenge DPPH radicals. Sub-fractions 12 and 13 had the highest capability for scavenging free radicals, inhibiting them up to 88.16% and 79.14%, respectively, in the system. Therefore, PSF12 can be taken into account for further isolating and purifying molecules with potent antioxidant activity.

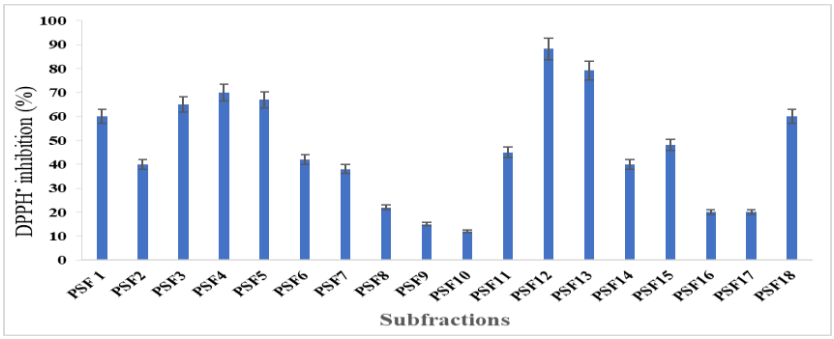

Fig. 4 – DPPH radical scavenging potential of *P. aphylla* sub-fractions.

ABTS$^{•+}$ *scavenging activity of sub-fractions of P. aphylla* *butanol fraction*

All sub-fractions exhibited varying levels of ABTS•+ radical scavenging activity, as illustrated in Fig. 5, however it is obvious that sub-fractions PSF2, PSF12, and PSF13 are the most effective antioxidants and should be taken into consideration for the isolation and purification of important antioxidant compounds.

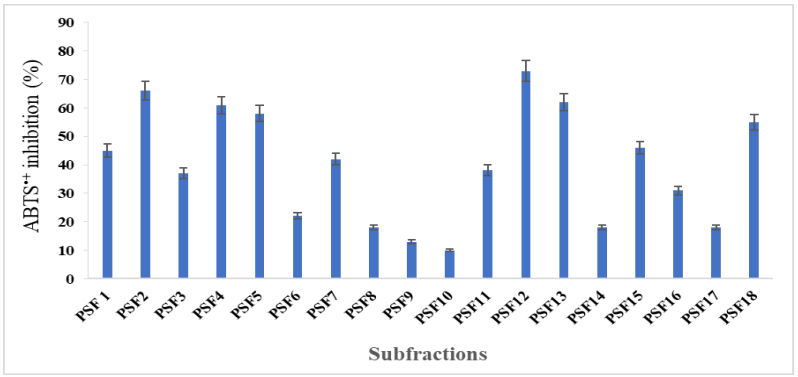

Fig. 5 – ABTS$^{•+}$ radical cation scavenging potential of *P. aphylla* sub-fractions.

Determination of bioactive compounds in sub-fraction 12 by GC-MS

After derivatization, 30 mg of each sample's extract was injected into the GC-MS. Figure 6 presents the total ion chromatogram with its peak details from sub-fraction 12. The entire run duration was 39.5 min, and the oven ramping was from 50°C to 150°C at 5°C per min and hold for 2 min at 150°C, followed by from 150°C to 250°C at 8°C per min and hold at 250°C for 5 min.

The existence of additional carboxylic acids is indicated by the molecule 2-propenoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester. Silane [(3,7-dimethyl-6-octenyl) oxy] was one of the substances. Trimethyl- was an alcohol that was reported to be present in alcohols. This chemical has also been noted to be an excellent anti-diabetic drug. An ester of long chain alcohol is silane, which is [1-(5-

ethenyltetrahydro-5-methyl-2-furanyl)-1-methylethoxy] trimethyl. Its presence in Nigella sativa oil was reported with positive biological effects.

P. aphylla included the compound silanamine, 1,1,1-trimethyl-N-(1-methyl-2-phenylethyl)-N-(trimethylsilyl)-, ()-. Certain biological activities have reportedly been found in the butanol fraction. Different plant extracts have also been shown to contain numerous similar alcohols.

These alcohols have been reported effective for treating different diseases.[35.] 2,6-dimethyl-3,4-bis(trimethylsilyloxymethyl)pyridine, another substance with that name, is an ester of 3,4-bis-hydroxymethyl-2,6-dimethyl-pyridine (Council, 1987). 1,2-bis(trimethylsiloxy)ethane is an ester of 1,2-dihydroxyethane or ethylene glycol, while 7-trimethylsilyloxytridecane is an ester of iso alcohol. Six macromycetes growing in sub Antarctic woodlands in southern Chile's culture broth contain ethylene glycol compounds, which were identified.[36,37] Trimethylsilyl ether of glycerol and free glycerol appeared to be the major precursor of monochloropropane-1,2-diol (MCPD) in leavened dough as described earlier.[38] An ester of 4-hydroxy benzoic acid is 4-[(trimethylsilyl)oxy]-benzoic acid, which is methylated. It was also noted in numerous other species, including grapes, carrots, and palm oil. Additionally, ortho hydroxyl benzoic acid has antibacterial properties.[39] Wedelia chinensis (Osbeck) Merrill's leaf has been found to contain benzoic acid, 2-hydroxy-, and methyl ester, which has antibacterial properties.

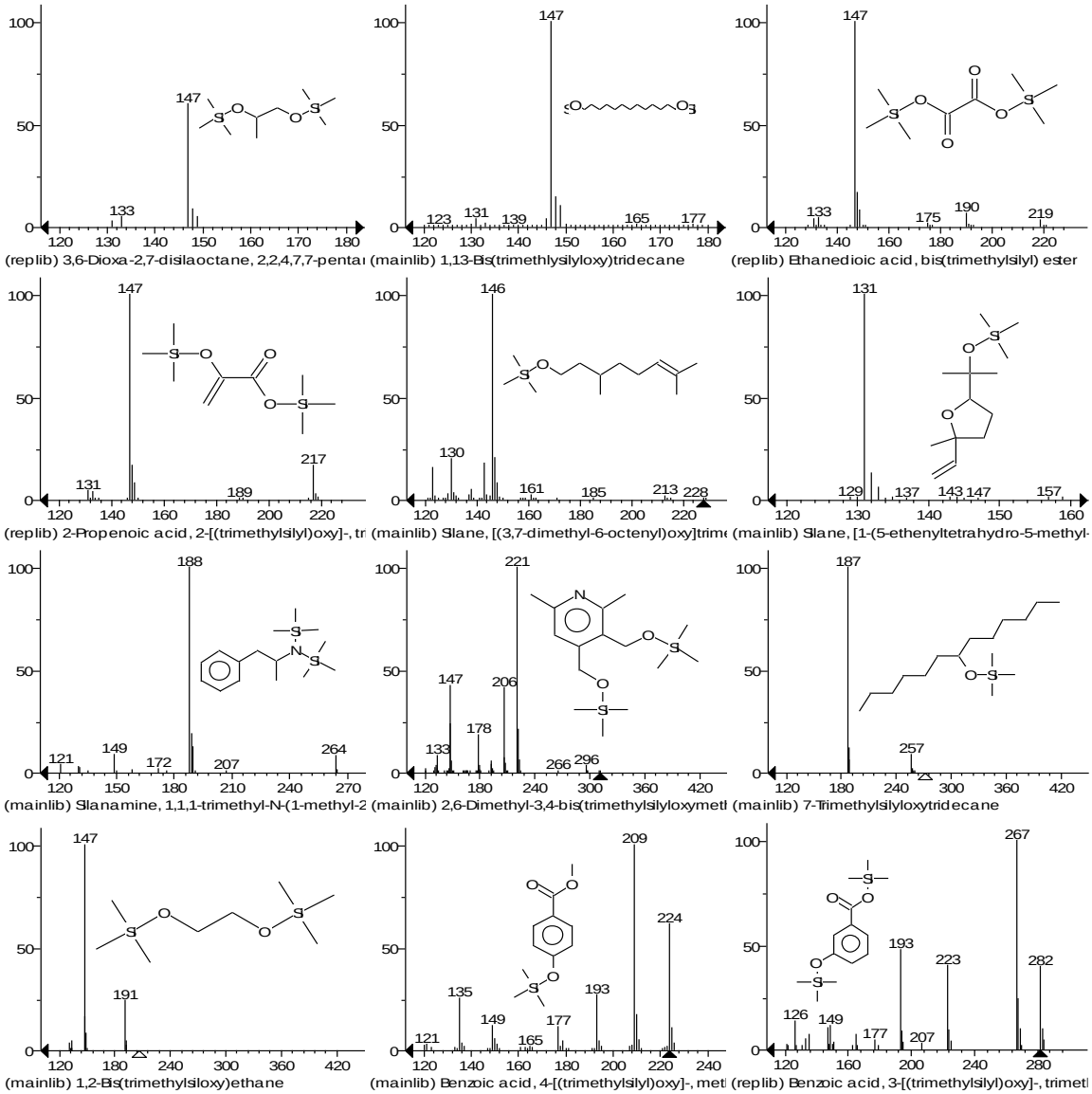

Fig. 6 – Structures of compounds identified and quantified in *P. aphylla* sub fraction 12 from ButOH fraction.

Table 2

GC-MS analysis of *P. aphylla* butanol sub-fraction 12

Compounds	Compound name (formula)(MW)	RT (ng/ mg of sub-fraction)	Biological activities/ Reference
A	3,6-Dioxa-2,7-disilaoctane, 2,2,4,7,7-pentamethyl- ($C_9H_{24}O_2Si_2$) (220)	2.757 (0.79)	Antimicrobial [34, 40]
B	1,13-Bis(trimethlysilyloxy)tridecane ($C_{19}H_{44}O_2Si_2$) (360)	2.83 (0.84)	
C	Ethanedioic acid, bis(trimethylsilyl) ester ($C_8H_{18}O_4Si_2$) (234)	4.011 (0.71)	Anti-oxidant & Anticancer
D	2-Propenoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester ($C_9H_{20}O_3Si_2$) (232)	4.083 (0.07)	[41, 42]
E	Silane, [(3,7-dimethyl-6-octenyl)oxy]trimethyl- ($C_{13}H_{28}OSi$) (228)	4.166 (0.10)	[43]
F	Silane, [1-(5-ethenyltetrahydro-5-methyl-2-furanyl)-1-methylethoxy]trimethyl-, trans- ($C_{13}H_{26}O_2Si$) (242)	4.262 (0.87)	[44]
G	Silanamine, 1,1,1-trimethyl-N-(1-methyl-2-phenylethyl)-N-(trimethylsilyl)-, (ñ)- ($C_{15}H_{29}NSi_2$) (279)	5.251 (1.16)	[45]
H	2,6-Dimethyl-3,4-bis(trimethylsilyloxymethyl)pyridine ($C_{15}H_{29}NO_2Si_2$) (311)	5.647 (0.002)	Antibacterial & Antioxidant [46, 47]
I	7-Trimethylsilyloxytridecane ($C_{16}H_{36}OSi$) (272)	7.163 (0.03)	[48]
J	1,2-Bis(trimethylsiloxy)ethane ($C_8H_{22}O_2Si_2$) (206)	8.298 (0.02)	Antioxidant [37, 38, 49, 50]
K	Benzoic acid, 4-[(trimethylsilyl)oxy]-, methyl ester ($C_{11}H_{16}O_3Si$) (224)	23.13 (0.07)	Antioxidant & Antibaterial [39, 51, 52]
L	Benzoic acid, 3-[(trimethylsilyl)oxy]-, trimethylsilyl ester ($C_{13}H_{22}O_3Si_2$) (282)	26.126 (5.00) [used as internal standard]	Antioxidant & Antibaterial

CONCLUSIONS

In current work, antiradical potential of different solvents fractions and sub-fractions of *P. aphylla* have been determined. Extract of *P. aphylla* was prepared in methanol followed by preparation of different solvent fractions. Butanol fraction with promising antioxidant activity was further sub-fractionated and sub-fraction 12 was analyzed using GC-MS and it was found carrying 12 different compounds. All the compounds obtained in sub-fraction 12 can be claimed as antiradical agents. Therefore, authors recommend the use of *P. aphylla* butanol fraction or aforementioned sub-fraction as additive in functional foods and nutraceuticals. Keeping in view the results of the current study, botanical materials of medicinal importance should be processed to obtained bioactive rich fractions. This will help in improving the efficacy of functional foods and extract based medicines.

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