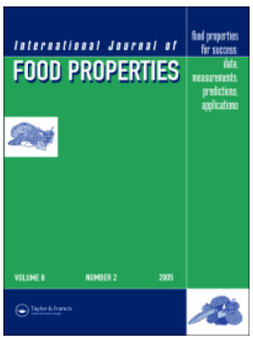

International Journal of Food Properties

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ljfp20

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To cite this article: Mumtaz Ali, Habib Ullah, Wasim Ul Bari, Noor Ul Islam, Muhammad Zahoor, Riaz Ullah & Ahmed Bari (2021) Phytochemical isolation and biological screening of *Cotoneaster microphyllus*, International Journal of Food Properties, 24:1, 1318-1334, DOI: 10.1080/10942912.2021.1963770

To link to this article: https://doi.org/10.1080/10942912.2021.1963770

Published online: 19 Aug 2021.

INTERNATIONAL JOURNAL OF FOOD PROPERTIES
2021, VOL. 24, NO. 1, 1318–1334
https://doi.org/10.1080/10942912.2021.1963770

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Phytochemical isolation and biological screening of *Cotoneaster microphyllus*

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ABSTRACT

The root and aerial parts of *Cotoneaster microphyllus* were subjected to extraction and isolation of phytochemicals. The extracts were evaluated for their antioxidant anthelmintic, antimicrobial, and anticholinesterase potentials using standard protocols. Crude extract of aerial parts and roots, more potently scavenged DPPH free radicals with IC_{50} values of 83 and 66 µg/mL while ABTS with 92 and 90 µg/mL respectively. Chloroform fraction exhibited highest anthelmintic activity followed by ethyl acetate fraction. Ethyl acetate fraction produced high zone of inhibition against selected bacterial and fungal strains. Maximum phenolic contents and vitamin C were found in the ethyl-acetate and chloroform fractions and were therefore, biologically the most potent fractions. Ethyl acetate fraction exhibited highest anticholinesterase potential and was therefore subjected to silica gel column chromatography which resulted in the isolation of one new (**1**) and four known **(2–5)** compounds. The isolated compounds were also screened for anticholinesterase potentials. Compound **3**, most potently inhibited acetyl cholinesterase and butyryl cholinesterase with IC_{50} value of 66 and 114 µg/mL, respectively. The extracts exhibited antioxidant, anticholinesterase, anthelmintic and antimicrobial potentials that should be further subjected for the isolation of other responsible compounds in pure state. As anticholinesterase compound **3** is a good candidate to be tested in animal models.

ARTICLE HISTORY
Received 2 June 2021
Revised 27 July 2021
Accepted 30 July 2021

KEYWORDS
Phenolic compounds; flavonoid; Antioxidant activity; antibacterial; *Cotoneaster microphyllus*

INTRODUCTION

The therapeutic potentials of plants have been utilized by human since the beginning of his life on the earth.[1] Plants being the complex chemical industries, prepare large variety of natural products.[2] Plants medication is preferred due to low side effects and inexpensive as well as abundantly available. There are approximately 308,312 plant species on planet earth out of which 450,000 are vascular plants.[3–7] According to World Health Organization report, 80% of population in the third world countries still relies on plant medication.[8] According to a recent survey about 5700 species of medicinal plants are growing in Pakistan and 82% of Pakistanis are frequently using traditional plant based medicines to cure different diseases.[9,10] The *Cotoneaster* genus (family Rosacea) is comprised of 84 species found mostly in Turkey, Syria, Iran, Pakistan and Europe. .[11] In Himalayas from Kuman to Kashmir, Pakistan, *Cotoneaster microphyllus* species is abundantly found. A number of species of the genus *Cotoneaster* are used as laxative, aperient, styptic, and

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expectorant. They are also used treat eye and bronchi infections, strangury, thirst, itch, fever, lesion, hemorrhoid, and urinary calculi.[12–15]

Methanolic extracts and isolated compounds of *Cotoneaster orbicularis* have shown lipo oxygenase inhibition and antioxidant activities. Biologically active flavonoids and their glycosides have been isolated from *Cotoneaster orbicularis*.[16] Furthermore, phenolic constituents like protocatechuic acid, anisic, *p*-coumaric, catechin, epicatechin, 2-O-α- rhamnopyranosylvitexin, vitexin, routine, isoquercetin, hyperin and naringenin have also been reported from *Cotoneaster simmonsii*.[16–18] A lignan with high antioxidant properties have been isolated from the ethyl acetate soluble fraction of *Cotoneaster racemiflora*.[19] The literature survey demonstrated that there is no phytochemical work reported so far on *Cotoneaster microphyllus*. Therefore, the present study was designed to isolate compounds from this plant and evaluate its antioxidant, antimicrobial, anthelmintic, and anticholinesterase potentials.

MATERIALS AND METHODS

Plant materials for phytochemical investigation

Cotoneaster microphyllus was collected from local hilly area, Kotigram District Dir (Longitude: 71.9045649, Latitude: 34.8453312, Elevation: 1112 m/3648 feet, Barometric Pressure: 89 KPa), Pakistan in 2012 and a voucher specimen was deposited with No. UOM-111. The collected samples (aerial parts + roots) were cleaned and shade-dried. The dried samples were grounded into fine powder through mechanical grinder.

Extraction and fractionation

The dried powdered samples were dipped into 85% methanol at room temperature and kept for two week with constant shaking. After filtration through cloth the extract was condensed into a semi solid mass through a rotary evaporator at 40°C which was then fractionated into ethyl-acetate, n-hexane and chloroform sub-fractions. The fractions were also condensed into semi solid mass through a rotary evaporator at 40°C and after complete drying in open air they were weighed. The resulting mass (aerial parts) of *n*-hexane, chloroform, and ethyl acetate fractions were 35, 22.1, and 21.1 g respectively. The crude extract of roots was also fractionated into ethyl acetate, chloroform, and *n*-hexane fractions.

Total phenolic contents

Antioxidant activities of plant samples are mostly due to phenolic compounds present in them.[20] Working dilutions of all extracts were prepared in range 62.5–1000 µg/mL. From each dilutions, 1 mL was further diluted into 10 mL by the addition of water. Then 1 mL of Folin-Ciocalteu reagent was added to the diluted reaction mixtures and incubated for 6 min. After incubation, 10 mL of 7% Na_2CO_3 was added and final volume was made 25 mL. After 1.5 h the absorbance of the mixture was recorded at 760 nm. Phenolic contents were estimated as mg of GAE/g of dry sample.

Total flavonoid contents

To estimate the flavonoid contents, the method devised by Park *et al* (2008) was followed.[21] From working dilutions (as mentioned in above step), 1 mL was further diluted to 10 mL through the addition of water. Then 1 mL of 5% $NaNO_3$ was also added and allowed to stand for 6 min. The mixture was incubated for 5 min after the addition of 2 mL of 10% $AlCl_3$ and 2 mL of 1 M NaOH was sequentially added. The absorbance of the reaction mixtures were noted at 510 nm using UV-visible

spectrophotometer. Total flavonoid contents were estimated as quercetin equivalent (mg QE/g) of dry sample from a standard quercetin curve (0 to 100 mg/mL).

DPPH free radical scavenging assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activities of the extracts were determined using previously reported method.[22] DPPH stock solution (20 mg/100 mL methanol) was prepared and kept for 24 h in dark in order to produce free radical in them. About 2 mL from the working dilutions were mixed with 2 mL of DPPH solution and after incubation for 15 min in dark the absorbance was noted at 515 nm using UV/visible spectrophotometer. The percent radical scavenging activity (%RSA) was calculated using the following formula:

$$\%RSA = \frac{Blank\ sample\ absorbance - sample\ absorbance}{Blank\ sample\ absorbance} \quad (1)$$

ABTS radical scavenging activities

The antioxidant potentials of the extracts were also determined against ABTS (2,2-azinobis [3-ethylbenzthiazoline]-6-sulfonic acid) free radical using Re *et al* method.[23] About 100 mL each ABTS (7 mM) and K_2SO_4 (2.45 mM) were prepared and after mixing were kept in dark for 24 h in order to develop free radical in it. From the working dilutions 2 mL were mixed with 3 mL of ABTS solution and incubated for 25 min at room temperature. The absorbance of the mixture was measured at 745 nm using a double beam spectrophotometer. The same procedure described above was used for ascorbic acid that was used as a positive control. Equation 1 was used to estimate the %RSA.

Anthelmintic activities

The anthelmintic activity of the extracts were determined against adult earthworms (*Pheretimaposthuma*) using a previously reported method.[24] Earthworms were collected from marshy muddy area. Different concentrations i.e. 10, 20, and 40 mg/mL of *Cotoneaster* microphyllus extract solution were prepared. The same size *Pheretima posthuma* worms were kept in Petri dishes having 35 mL fraction solution of the desired concentration. Albendazole was used as standard drug.

Antimicrobial activities

The antibacterial activity of *C. microphyllus* (roots and aerial parts), extracts were evaluated against two bacterial strains; *Pseudomonas aeruginosa* and *Streptococcus pyogenes* while antifungal potentials were evaluated against two fungal strains; *Candida albicans* and *Fusarium solani* using agar well diffusion method.[25]

Anticholinesterase assays

Acetyl cholinesterase (AChE) from electric eel and butyrylcholinesterase (BChE) from equine serum was used to evaluate the enzymes inhibitory potential of the crude extract, sub- fractions and isolated compounds using Elman's assay.[26] AChE and BChE solutions were prepared in phosphate buffer of pH 8. AChE (518 U/mg solid) and BChE (7–16 U/mg) were diluted in freshly prepared buffer (pH 8) until a final concentration of 0.03 U/mL and 0.01 U/mL respectively. Solutions of DTNB (0.0002273 M), acetyl and butyryl choline iodide (0.0005 M) were prepared in distilled water and were kept in Eppendorf caps in the refrigerator. For each analysis, an enzyme solution of 100 µL was added to the cuvette, followed by addition of plant extract dilutions and isolated compounds solution

(1 mL). Finally DTNB reagent (100 µL) was added to mixture, incubated at 25°C for 15 min in an incubator, and subsequently the substrate solution (100 µL) was added to them. A double beam spectrophotometer was used to measure the absorbance of reaction mixture at 412 nm. A negative control contained all components apart from the extract/compounds, whereas the positive control contained galantamine (5 mg/5 mL) along with all the mentioned components. Percent enzyme activity and percent inhibition were calculated as follows:

$$V = \frac{\Delta \text{Abs}}{\Delta \text{t}} \tag{2}$$

$$\%\ enzyme\ activity = \frac{V}{V_{max}} x100 \tag{3}$$

$$\%\ enzyme\ inhibition = 100 - \%\ enzyme\ activity \tag{4}$$

Where: V indicates the rate of reaction in the presence of inhibitor and V_{max} is the rate of reaction in absence of inhibitor.

Isolation of phytochemicals

Column chromatography was used for the isolation of different phytochemicals from ethyl acetate fraction (root). Silica gel was used as an adsorbent. The desired extract was mixed in silica gel slurry which was then loaded to column with care. The developed column was eluted with *n*-hexane-chloroform mixtures, mixed in different proportions in order of increasing polarities (1 to 100%). Several fractions were obtained which were then mixed together on the basis of TLC profiling and as result 11 sub-fractions designated as D-1 to D-11 were obtained.

Compound 1

Sub-sub fractions C-4 and C-5 were obtained from D-2 subfraction after elution with ethyl acetate-chloroform. Based on TLC analysis the fractions C-4 and C-5 were combined and further subjected to fresh silica gel column. In the obtained fraction impure crystals were obtained. The crystals were washed with *n*-hexane successively and 18 mg of compound **1** was obtained.

Melting point; 144°C

Molecular formula $C_{19}H_{36}O_2NCl$

IR (KBr) max; 3406, 2905 and 1640 cm^{-1}

^{1}H NMR (300 M Hz, $CDCl_3$): δ 2.25 (2 H, m, -CH_2-, 1), 1.57 (2 H, m, -CH_2-, 2), 7.51 (1H, m, -CH-, 3), 7.69 (1H, m – CH-, 4), 1.42 (1H, m, -CH_2-, 5), 4.21 (2 H, m, -CH_2-, 7), 1.31 (1H, m, -CH-, 8), 1.71 (2 H, m – CH_2-, 9), 0.9 (3 H, m, -CH_3, 10), 1.39 (2 H, m, -CH_2-, 11), 1.38 (2 H, m, -CH_2-, 12), 1.39 (2 H, m, -CH_2-, 13), 1.39 (2 H, m, -CH_2-, 14), 1.39 (2 H, m, -CH_2-, 15), 1.39 (2 H, -CH_2-, 16), 1.39 (2 H, m, -CH_2-, 17), 1.69 (2 H, m, -CH_2-, 18), 0.7 (3 H, m, -CH_3, 19)

^{13}C NMR (125 M, Hz $CDCl_3$)): δ 168.2 (C-6), 130 (C-3), 128.8 (C-4), 68.2 (C-7), 47.8 (C-1), 38.8 (C-8), 30.4 (C-5), 29.7 (C-11), 29.7 (C-13), 29.7 (C-14), 29.7 (C-15), 29.7 (C-17), 29.3 (C-16), 28.9 (C-12), 23.7 (C-2), 23.7 (C-9), 23.0 (C-18), 14.0 (C-19), 11.0(C-10)

EI-MS; *m/z* 345.4376 [M+] (calcd. 345.4763 for $C_{19}H_{36}O_2NCl$

Compound 2

Using an *n*-hexane-chloroform solvent system sub-sub fraction C-6 was obtained from subfraction D-4. Compound **2** weighing about 23 mg was obtained from this fraction.

Melting point; 144°C

Molecular formula $C_{29}H_{50}O$
IR (KBr) max; 3406, 2905 and 1640 cm-1
EI-MS; *m/z* 414.0111 [M+] (calcd. 414.0141for $C_{29}H_{50}O$)

Compound 3

About 13 mg of compound **3** was obtained from D-6 fraction after washing with various solvents like *n*-hexane, dichloromethane, and ethyl acetate.
Physical state:White amorphous solid
Melting point: 236–238°C
IR ν_{max}: 1379, 3450, 1642 and 1065 cm^{-1}
EI-MS (70 eV) *m/z* 414.0141($[C_{29}H4_{50}O]^{+}$)

Compound 4

Sub-sub fraction C-8 was obtained from D-7 subfraction after elution with n-hexane-ethyl acetate (2:8) which was then re-chromatographed over fresh silica gel column using n-hexane-ethyl acetate (7:3) solvent system. The compound **4** was obtained weighing about 17 mg.
Melting point; 142°C
Molecular formula $C_{29}H_{48}O$
IR max; 2906, 3406 and 1644 cm-1
EI-MS; *m/z* 414.0414 [M+] (414.0141 for $C_{29}H_{48}O$)

Compound 5

The sub-sub fraction C-7 was obtained from a subfraction D-9 using *n*-hexane-chloroform (7:3) gradient which was further re-chromatogrammed over fresh silica gel column and upon elution with *n*-hexane-chloroform (4:6) solvent system in rising order of polarity produced compound **5** weighing about 14 mg.
Melting point; 212–213°C
Molecular formula; $C_{30}H_{50}O$
FT-IR max; 3406, 1495, 1381, 940, 985,1183, 1104, 1645, 1039 cm-1
EI-MS *m/z*: 426.1099 [M+] (426.1042 for $C_{30}H_{50}O$)

Statistical analysis

All the experiments have been performed in triplicates. The results are presented as Mean ± SEM. *P* values were determined using Two-way ANOVA followed by Bonferroni posttest to establish the statistical differences between standard drug and test samples using Graph Pad Prism software. The value of $P < .05$ was considered as significant. The medium inhibitory concentration (IC_{50}) of DPPH, ABTS, AChE, and BChE has been calculated using linear regression (MS Excel program 2007).

RESULTS

Total phenolic and flavonoid contents

The Phenolic contents in various extracts of *Cotoneaster microphyllus* were investigated. The phenolic contents were found in range from 33.00 to 83.96 mg GAE/g of dried extract (Gallic acid equivalent) in aerial parts of the plants. Highest contents (83.96 mg/g) were present in ethyl acetate fraction, whereas *n*-hexane fraction exhibited the lowest contents (33.00 mg/g) amongst the fractions. The total flavonoid contents were expressed as quercetin equivalent (mg of QE/g of dry sample). Ethyl-

Table 1. Total phenolic and flavonoid contents in *C. microphyllus*(aerial parts) crude extract and their different subtractions.

Samples		Total phenolic contents (mg GAE/g of dry sample)	Total flavonoid contents (mg QE/g of dry sample)
Aerial parts	Crude	70.65 ± 0.82	68.70 ± 0.56
	Ethyl acetate	83.96 ± 1.67	78.40 ± 2.33
	Chloroform	65.49 ± 2.23	60.10 ± 0.90
	n-hexane	33.00 ± 0.88	22.94 ± 1.42
Roots	Crude	73.65 ± 0.71	62.70 ± 0.44
	Ethyl acetate	85.96 ± 2.80	80.40 ± 1.33
	Chloroform	58.49 ± 2.48	50.10 ± 1.98
	n-hexane	24.00 ± 1.34	16.94 ± 0.82

GAE = gallic acid equivalent, QE = Quercetin equivalent each value in the table is represented as mean± SEM (n = 3).

acetate fraction was the richest fraction with 78.40 mg QE/g of dry sample followed by crude, chloroform, and n-hexane fractions with values of 68.70, 60.10, and 22.94 mg QE/g of dry sample respectively (Table 1).

Phenolics contents were also determined in different fractions of roots (Table 1) which were ranged from 24.00 to 85.96 mg GAE/g of dry extract (Gallic acid equivalent). Ethyl acetate fraction of roots parts showed the highest phenolic contents (85.96 mg GAE/g), whereas *n*-hexane showed the lowest (24.00 mg GAE/g). Similarly, the total flavonoid contents were determined as quercetin equivalent (mg of QE/g of dry sample). Ethyl acetate fraction with flavonoid contents value of 80.40 mg QE/g was the richest fraction followed by crude, chloroform, and n-hexane fractions (62.70, 50.10, and 16.94 mg QE/g of the dry sample respectively).

Antioxidant potential of the extracts

Crude extract and their sub-fractions of both aerial and root parts were screened out for free radical scavenging potentials against DPPH and ABTS radicals (Tables 2 & 3). The crude extract and ethyl-acetate fractions showed the highest free radical inhibitions which were 89.87, and 84.17% for aerial part while 88.62, and 81.60% for root part respectively against DPPH. The n-hexane fractions exhibited a minimum inhibition of 58.27 and 50.77% for aerial and root parts. The activity of the chloroform fraction was found lower than the activity of ethyl acetate fraction. As ethyl-acetate fractions and crude extract of both parts showed the highest phenolics compounds, therefore its ABTS and DPPH radical scavenging potential are also high. While n-hexane fraction contained lower phenolic contents and hence showed the lowest %RSA activity.

The crude extract and ethyl-acetate fraction of both parts (aerial and root) of the mentioned plant showed promising antioxidant activity against ABTS free radical as well with IC_{50} values of 92/90 and 178/240 µg/mL respectively. The rest of the fractions like chloroform and n-hexane exhibited good scavenging activities with IC_{50} values of 220/320, and 880/1060 µg/mL respectively as shown in Tables 2 & 3.

Anthelmintic activity

Anthelmintic activity of *C. microphyllus* various extracts tested against earthworm, are presented in Table 4. Chloroform fraction of both parts showed high activity at all concentrations, while the lowest activity was observed with *n*-hexane fraction of both parts. Paralysis and death time of the earthworm for chloroform fraction (aerial parts) were 15 and 28 min respectively, while 19 and 31 min were for root parts at 30 and 40 mg/mL concentrations. In the case of ethyl acetate fraction time of paralysis and time of death of worms were 19 and 32 min respectively for both aerial parts and roots. When n-hexane fraction (aerial parts) was used against the worm after 34 min paralysis was observed and all the worms died after 66 min at a concentration 10 mg/mL, whereas for n-hexane fraction of roots the time of paralysis and death of warms were 41 and 72 min respectively at concentration of 10 mg/mL. The positive controlalbendazole showed paralysis and death time 10 and 16 min respectively.

Table 2. Percent DPPH and ABTS radical scavenging potential of crude extract and their sub fractions of *C. microphyllus*(aerial parts) using ascorbic acid as standard.

Samples	Concentrations (µg/mL)	DPPH Percent inhibition (mean ± S.E.M)	DPPH IC_{50} (µg/mL)	ABTS percent inhibition (mean ± S.E.M)	ABTS IC_{50} (µg/mL)
Crude	1000	89.87 ± 2.80*	83	87.37 ± 3.41^{ns}	92
	500	80.09 ± 1.28^{ns}		79.33 ± 0.60 ^{ns}	
	250	66.35 ± 0.70^{ns}		65.00 ± 2.10 ^{ns}	
	125	52.76 ± 3.40^{ns}		54.33 ± 0.82 ^{ns}	
	62.5	46.48 ± 0.79^{ns}		44.45 ± 0.92 ^{ns}	
Ethyl acetate	1000	84.62 ± 2.74***		80.50 ± 2.22***	
	500	72.32 ± 1.68**	140	70.05 ± 0.77***	178
	250	60.00 ± 1.93***		55.39 ± 1.33***	
	125	45.22 ± 0.96***		40.97 ± 0.99***	
	62.5	36.30 ± 3.88***		30.10 ± 1.68***	
Chloroform	1000	80.63 ± 1.45		75.43 ± 2.88***	
	500	70.07 ± 2.56***	180	64.69 ± 0.85***	220
	250	58.33 ± 0.46***		52.92 ± 0.49***	
	125	40.08 ± 2.70***		37.41 ± 1.62***	
	62.5	30.50 ± 3.45***		26.60 ± 3.59***	
n-hexane	1000	58.27 ± 1.71***		54.80 ± 2.47***	
	500	47.31 ± 0.60***		34.33 ± 0.70***	
	250	33.87 ± 2.45***	580	28.22 ± 0.40***	880
	125	27.05 ± 0.50***		22.01 ± 2.72***	
	62.5	21.12 ± 2.66***		19.25 ± 0.98***	
Ascorbic acid	1000	92.94 ± 0.86		89.67 ± 0.73	
	500	81.29 ± 0.79	48	80.45 ± 0.96	68
	250	70.93 ± 0.45		68.34 ± 2.16	
	125	62.90 ± 0.48		58.90 ± 0.85	
	62.5	53.88 ± 0.32		46.78 ± 0.76	

Ascorbic acid was used as a positive control. Data is represented as mean ± S.E.M (n = 3). Values significantly different as compared to positive control,*:P < 0.05, ***:P < 0.001, ns: *P* > 0.05.

Antifungal activity of various fractions of aerial parts and roots of C. microphyllus

The highest antifungal activity was recorded for ethyl acetate fraction (root parts) which was 16.04 mm against C*andida albicans* while 15.88 mm against *Fusarium solani*. Chloroform and *n*-hexane fractions exhibited high inhibitions against the used strains as shown in Table 5.

Antibacterial activities of various extracts of aerial parts and root of C. microphyllus

Both parts were found to be active against selected bacterial strains. Ethyl acetate fraction aerial and root parts exhibited the highest zone of inhibition as 13 and 12.3 mm respectively against *Pseudomonas aeruginosa* while against *Streptococcus pyogenes* the mentioned fraction produced zone of inhibition as 11.4 and 11.8 mm (Table 6).

Anticholinesterase potential of extracts

The enzyme inhibition potential of crude extract and their different sub fractions of *C. microphyllus* (aerial and root part) were tested against acetyl cholinesterase (AChE) and butyrylcholinesterase (BChE) and the results are summarized in Tables 7 and 8. Against AChE the crude and ethyl acetate fractions of aerial parts were found more potent with IC_{50} values of 90 and 125 µg/mL respectively while the mentioned extract of the root produced IC_{50} values equal to 80 and 145 µg/mL. The remaining fractions like chloroform and n- hexane also showed moderate inhibition and their IC_{50} were 170 and 380 µg/mL respectively (aerial parts). Similarly, the extracts were also tested against BChE. The crude and ethyl acetate fractions of aerial and root parts were more potent with IC_{50} values of 105/150 and 110/210 µg/mL, respectively. Galantamine was used as a positive control.

Table 3. Percent DPPH and ABTS radical scavenging potential of crude extract and their sub fractions of *C. microphyllus*(roots) using ascorbic acid as standard.

Samples	Concentrations (µg/mL)	DPPH Percent inhibition (mean ± S.E.M)	DPPH IC_{50} (µg/mL)	ABTS percent inhibition (mean ± S.E.M)	ABTS IC_{50} (µg/mL)
Crude	1000	88.17 ± 2.83*		84.77 ± 3.79 ^{ns}	
vm	500	80.26 ± 1.48 ^{ns}		77.64 ± 0.42 ^{ns}	
	250	70.21 ± 2.60 ^{ns}	66	65.43 ± 0.70 ^{ns}	90
	125	59.16 ± 0.90 ^{ns}		55.60 ± 1.85 ^{ns}	
	62.5	46.29 ± 1.61 ^{ns}		43.19 ± 3.8^{ns}	
Ethyl acetate	1000	81.60 ± 0.74***		74.52 ± 1.24***	
	500	66.32 ± 1.68**		65.09 ± 2.83***	
	250	55.00 ± 0.90***	180	50.69 ± 0.38***	240
	125	39.22 ± 0.60***		36.92 ± 1.99***	
	62.5	28.03 ± 0.33***		23.10 ± 0.44***	
Chloroform	1000	74.62 ± 1.45***		70.40 ± 0.80***	
	500	62.07 ± 3.50***	200	58.90 ± 2.35***	320
	250	49.32 ± 0.44***		44.90 ± 0.41***	
	125	36.88 ± 0.70***		38.41 ± 0.60***	
	62.5	24.57 ± 2.41***		18.65 ± 2.49***	
n-hexane	1000	50.77 ± 2.71***		47.80 ± 0.48***	
	500	39.11 ± 0.63***	980	34.33 ± 2.72***	1060
	250	29.87 ± 1.45***		25.94 ± 1.35***	
	62.5	12.42 ± 3.60***		10.28 ± 0.90***	
Ooi9i ewwwwwwwwwwwwq	1000	90.92 ± 0.80		89.67 ± 0.73	
	500	84.20 ± 0.72	52	80.40 ± 0.92	74
	250	73.93 ± 0.45		71.64 ± 1.16	
	125	60.60 ± 0.49		56.90 ± 0.85	
	62.5	52.18 ± 0.37		45.88 ± 0.73	

Ascorbic acid was used as a positive control. Data is represented as mean ± S.E.M (n = 3). Values significantly different as compared to positive control,*: $P < 0.05$, ***: $P < 0.001$, ns: $P > 0.05$.

Table 4. Time taken for paralysis and death in minutes by different fractions of *C. microphyllus* (roots and aerial parts).

Sample/Fraction	Concentration (mg/mL)	Paralysis time (min)	Death time (min)
Aerial/n-Hexane	10	34	66
	20	27	57
	40	19	36
Aerial/Chloroform	10	28	60
	20	23	51
	30	15	28
Aerial/Ethyl acetate	10	30	63
	20	26	55
	40	19	32
Roots/n-Hexane	10	41	72
	20	35	64
	40	24	41
Roots/Chloroform	10	33	60
	20	28	59
	30	19	31
Roots/Ethyl acetate	10	34	69
	20	31	65
	40	22	40

Isolation of pure compounds

(Z)-2́-Ethylundecyl 6-(chloroamino) hex-3-enoate (1)

The novel compound **1** was isolated as white solid. The molecular formula was established as $C_{19}H_{36}O_2NCl$ by its EI-MS giving molecular ion peak [M+] at 345.4376. The ^{1}H -NMR spectrum of compound **1** showed two multiples at δ_H 2.25 and δ_H1.57 that were assigned to four protons of two -CH_2-groups (H-1 and H-2). The appearance of two broad signals (2 H) at δ_H 7.52 (m) and 7.69 (m) of (H-3 and H-4) were due to ethylenic (-CH = CH-) protons. A doublet appeared at δ_H 1.41 justifying

Table 5. Antifungal activities of crude and different fractions of *C. microphyllus* against *Candida albicans and Fusarium solani.*

	Zone of inhibition (mm)	
Sample	*Candida albicans*	*Fusarium solani*
Clotrimazole (50 µg)	26.50	24.50
Roots-n-Hexane	8.50	9.00
Root-Chloroform	11	9.75
Root-Ethyl acetate	16.04	15.88
Root-crude	9.36	8.82
Aerial-n-hexane	7.25	7.00
Aerial-Chloroform	8.15	7.60
Aerial-Ethyl acetate	8.63	7.88
Aerial – crude	8.36	6.86

Table 6. Antibacterial activities of extracts of C. microphyllus against Pseudomonas aeruginosa and Streptococcus pyogenes.

	Zone of inhibition (mm)	
Sample-fraction	Pseudomonas aeruginosa	Streptococcus pyogenes
Root-n-Hexane	7	4
Root-Chloroform	8	5
Root-Ethyleaccetate	13	11.4
Root-Crude	8	6.5
Aerial n-Hexane	7.5	3.5
Aerial-Chloroform	9	4
Aerial-Ethyl acetate	12.3	11.8
Aerial-Crude	7.2	6.8

the proton of H-5 that is attached to the carbonyl carbon. Two peaks resonating at δ_H 4.21 and δ_H 1.71 showed the presence of two -CH_2- at H-7 and H-9. A multiplet appearing at δ_H1.31 was attributed to one proton at H-8. The appearance of multiplets at δ_H 1.38 and δ_H 1.69 were assigned to the presence of eight methylenic protons (-CH_2-) from H-11 to H-18. Two multiplets were observed at δ_H 0.9 (3 H, m) and 0.7 (3 H, m) were attributed to two methyl groups (CH_3-10, CH_3-19) respectively.

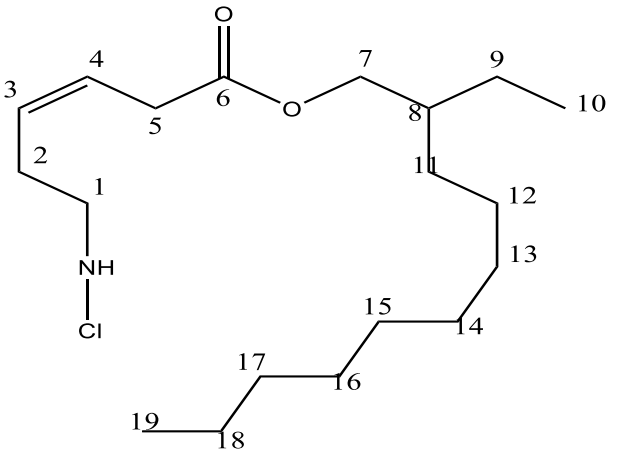
^{13}C and DEPT NMR spectral data of compound **1** showed that it contains 19 carbons, including 2 methyl, 12 methylene, 3 CH, and one quaternary carbon. The signal at δ_C 168.2 was assigned to C-6, the quaternary (carbonyl) carbon. The peaks at δ_C 128.8 and 130.9 were due to the presence of olefinic methene carbons C-3 and C-4. Peaks at δ_C68.2 and 23.7 were attributed to the aliphatic carbons C-7 and C-9 respectively. The peaks at δ_C 11.0 and 14.0 showed the presence of two methyl groups at position no 10 and 18 respectively. The other peaks resonating in between δ_C 23.0 (C-11) to δ_C 29.7 (C-18), were attributed to methylene carbon atoms. These assignments were confirmed by advanced techniques of 2D-NMR (HMBC, HMQC, and COSY).

From the HMQC and HMBC spectra data, the connectivity was determined and clear-cut assignments were made for all of the protons and carbons. The structure of the new compound was established. The proton signal at δ_H 7.52(H-3) is interrelated with the carbon at δ C 130.9 7 (C-4) in the HMBC spectrum (methylene) representing a direct connection to each other. The olefinic protons (H-3, H-4) correlated with the carbon signals at δ_C128. The proton at δ_H 1.42 (H-5) and δ_H 4.21(H-7) are correlated to the carbonyl carbon signal at δ_C 168.27(C-6) in the HMBC spectrum. Proton signals at δ_H 4.21 (H-7), δ_H 1.31(H-9), and δ_H0.9 (H-7) assigned to C-9 giving a signal at δ_C 23.7 protons at δ_H 1.71 (H-9) correlated with C-7 (δ_C 68.2). The proton at δ_H 1.39 (H-15), and δ_H 0.7 (H-19) correlated to C-17 which signaling at δ_C 29.7 and proton at δ_H 1.69 (H-18) correlate to C-16 (δ_C29.3) in the HMBC spectrum. These spectroscopic data including 1D-NMR and 2D-NMR established the structure as (*Z*)-2-ethylundecyl 6-(chloramine) hex-3-enoate, a novel compound, isolated for the first time from the aerial parts of *C. microphyllus*.

Table 7. Percent AChE and BChE inhibition potential of crude extract and their sub fractions of *C. microphyllus*aerial parts.

Samples	Concentrations (µg/mL)	AChE Percentinhibition (mean ± S.E.M)	AChE IC_{50} (µg/mL)	BChE percent inhibition (mean ± S.E.M)	BChE IC_{50} (µg/mL)
Crude	1000	87.97 ± 1.80*	90	85.37 ± 2.41^{ns}	105
	500	80.19 ± 2.28^{ns}		77.33 ± 1.60 ^{ns}	
	250	64.35 ± 1.70^{ns}		63.00 ± 3.10 ^{ns}	
	125	50.76 ± 2.40^{ns}		53.33 ± 1.82 ^{ns}	
	62.5	44.58 ± 1.79^{ns}		42.45 ± 1.92 ^{ns}	
Ethyl-acetate	1000	86.62 ± 1.74***		83.50 ± 2.22***	
	500	74.22 ± 0.68**	125	72.05 ± 1.77***	150
	250	63.10 ± 2.93***		60.39 ± 2.33***	
	125	49.12 ± 0.96***		58.97 ± 1.99***	
	62.5	35.30 ± 0.88***		44.10 ± 0.68***	
Chloroform	1000	78.63 ± 2.45		77.43 ± 2.88***	
	500	68.07 ± 1.56***	170	66.69 ± 1.85***	195
	250	57.33 ± 1.46***		54.92 ± 0.89***	
	125	46.08 ± 0.70***		41.41 ± 0.62***	
	62.5	30.50 ± 1.45***		29.60 ± 2.59***	
n-hexane	1000	65.27 ± 0.71***		60.80 ± 0.47***	
	500	56.31 ± 1.60***		48.33 ± 1.70***	
	250	47.87 ± 1.45***	380	38.22 ± 2.40***	450
	125	37.05 ± 0.50***		27.01 ± 0.72***	
	62.5	26.12 ± 0.62***		14.25 ± 1.98***	
Galantamine	1000	93.94 ± 0.82		90.65 ± 2.71	
	500	80.29 ± 0.89	45	81.43 ± 1.96	60
	250	72.93 ± 0.42		69.34 ± 2.16	
	125	62.90 ± 0.42		60.90 ± 0.85	
	62.5	52.88 ± 0.42		49.88 ± 0.76	

Galantamine was used as a positive control. Data is represented as (mean ± S.E.M) n = 3.Values significantly different as compared to positive control, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: $P > 0.05$.



(Z)-2́-Ethylundecyl 6-(chloroamino)hex-3-enoate (1)

(Z)-2́-Ethylundecyl 6-(chloroamino)hex-3-enoate (1)

β-Sitosterol (2)

The compound **2** was isolated from ethyl acetate fraction. The IR spectrum of compound **2** displayed bands at 3408, 1628, 1379, and 1065 cm^{-1} showing the presence of OH and unsaturation respectively. The molecular formula was determined as $C_{29}H_{50}O$ by its LR-EI-MS giving molecular ion peak [M+] at 414. 0141.

The ^{1}H -NMR spectrum of compound 2 displayed a multiplet at δ_H5.36 which is assigned to H-6 (exocyclic methylene protons). A broad signal (1H) present at δc 3.52 (m) was due to carbinol proton (H-3). The six methyl substituents appeared at 1.01 δc (3 H, s), 0.92 (3 H, d, $J = 6.82$ Hz), 0.86 (3 H, t, $J = 6.92$ Hz),

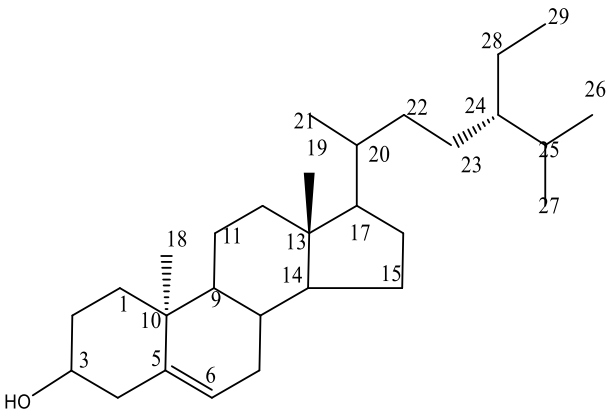
Table 8. Percent AChE and BChE inhibition potential of crude extract and their sub fractions of *C. microphyllus*(Root parts).

Samples	Concentrations (µg/mL)	AChE Percent inhibition (mean ± S.E.M)	AChE IC_{50} (µg/mL)	BChE percent inhibition (mean ± S.E.M)	BChE IC_{50} (µg/mL)
Crude	1000	86.17 ± 1.83*		83.77 ± 2.79 ns	
	500	78.26 ± 2.48 ns		75.64 ± 0.40 ns	
	250	70.21 ± 0.60 ns	80	62.43 ± 1.70 ns	110
	125	57.16 ± 1.90 ns		53.60 ± 1.80 ns	
	62.5	48.29 ± 0.61 ns		41.19 ± 1.80ns	
Ethyl-acetate	1000	83.60 ± 0.74***		77.52 ± 1.24***	
	500	71.32 ± 2.68**		67.09 ± 1.83***	
	250	60.00 ± 1.90***	145	56.69 ± 0.38***	210
	125	49.22 ± 2.60***		45.92 ± 0.99***	
	62.5	39.03 ± 1.33***		33.10 ± 1.44***	
Chloroform	1000	72.62 ± 1.45***		72.40 ± 1.80***	
	500	60.07 ± 2.50***	250	62.90 ± 1.35***	280
	250	47.32 ± 1.44***		50.90 ± 2.41***	
	125	34.88 ± 1.70***		39.41 ± 1.60***	
	62.5	22.57 ± 0.41***		27.65 ± 1.49***	
n-hexane	1000	55.77 ± 0.71***		57.80 ± 0.48***	
	500	43.11 ± 0.60***	700	47.33 ± 1.82***	830
	250	33.87 ± 2.45***		36.94 ± 0.45***	
	125	25.65 ± 1.50***		25.41 ± 1.66***	
	62.5	16.42 ± 1.60***		15.28 ± 0.90***	
Galantamine	1000	92.92 ± 1.80		90.67 ± 0.73	
	500	83.20 ± 0.72	50	81.40 ± 0.82	65
	250	75.93 ± 0.65		73.64 ± 0.66	
	125	62.60 ± 0.19		58.90 ± 0.55	
	62.5	53.18 ± 0.77		48.88 ± 0.93	

Galantamine was used as a positive control. Data is represented as (mean ± S.E.M) n = 3.Values significantly different as compared to positive control, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: $P > 0.05$.

0.83 (3 H, d, $J = 6.52$ Hz), 0.82 (3 H, d, $J = 6.52$ Hz), 0.65 (3 H, s, Me-18) were attributed to six methyl (CH_3 -19, CH_3-21, CH_3-29, CH_3-26, CH_3-27 and CH_3-18) respectively. The remaining methylene and methyne protons have appeared between δ 2.97 and 1.1.

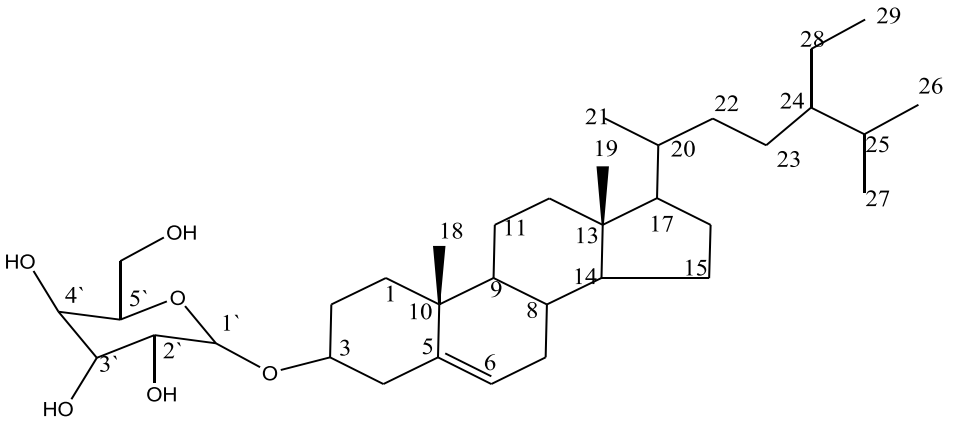
On the basis of the interpretation of its ^{13}C and DEPT NMR spectral data compound **2** contained 29 carbons, including six methyl, 11 CH_2, 9 CH, and three quaternary carbons. The signal at *δc* 140.76 was assigned to C-5, the quaternary (olifenic) carbon. The peak at δc 121.70 is assigned to the olefinic methene carbons C-6 while *δc*36.51 and 42.30 were attributed to the aliphatic carbons (C-10 and C-13 respectively). The peaks indicated at δc19.83(C-18), 19.40 (C-21), 19.03 (C-27), 18.79 (C-26), 11.99 (C-29), and 11.86 (C-19) were assigned to methyl of the respective carbons. These assignments were confirmed by advance 2D-NMR techniques (HMBC, HMQC, and COSY). The spectroscopic and physical data of compound **2** agreed with those reported in the literature as *β*-sitosterol.[24]



Structure of β- Sitosterol

Structure of β- Sitosterol

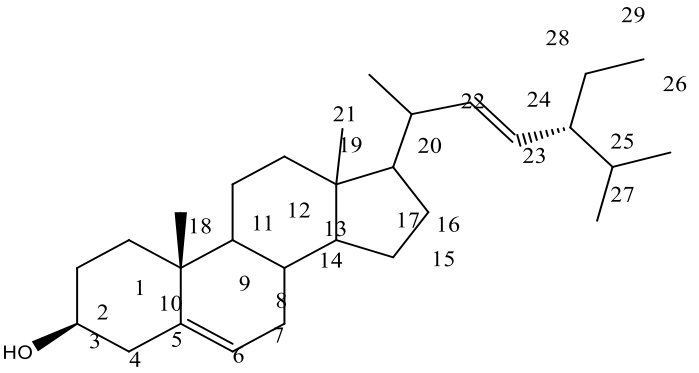
β-Sitosterol 3-O-β-D-glucopyranoside (3): The IR spectrum of compound **3** displayed bands at 3452, 1648, 1379 and 1065 cm^{-1} indicating the presence of OH and unsaturation respectively. The molecular formula was determined as $C_{29}H_{50}O$ by its EI-MS molecular ion peak [M+]+ at 414.0441 (as glycosides disappear in EI-MS). The ^{1}H and ^{13}C- NMR spectra of compound were found identical to compound **2** along with the additional peaks of sugar moiety resonated at δ_H 4.57 (3 H, d, J = 7.51 Hz, H-1́), 3.85 (^{1}H, dd, J = 11.81, 2.41 Hz, Ha-b'), 3.68 (1H, dd, J = 11.87, 5.71 Hz, Hb-b') and 3.24–3.45 (5 H, m, Glc-H), while in ^{13}C NMR the peak of anomeric carbon at C-3 with additional sugar moiety C-3́, C-4́, C-2́, C-5`. The spectroscopic and physical data of compound **3** in as β-sitosterol glycoside was showed good agreement with previously compiled data.[16–19]



β-Sitosterol3-*O*-β-D-glucopyranoside.

Stigma sterol (4)

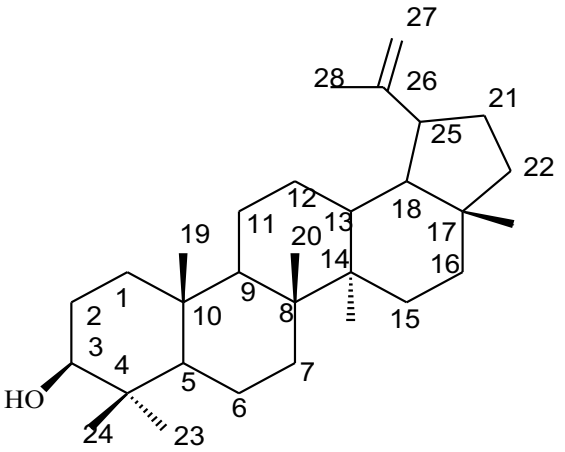
Compound **4** was obtained as an amorphous powder the molecular formula $C_{29}H_{48}O$, was established from ^{13}C – NMR, DEPT, and EIMS. Its steroidal nature was indicated by the appearance of six methyl groups. The IR spectrum showed characteristic peaks for hydroxyl (3412 cm^{-1}), CH stretching (2929, 2859 cm^{-1}), and aromatic CH stretching (1602, 1469 cm^{-1}). The UV spectrum showed a band at 241 nm (ε 4.2). The ^{1}H-NMR spectrum showed six methyl signals including two singlets at δ_H 0.76 (s, H-18) and 1.11 (s, H-19), one triplet at δ_H 0.90 (t, J = 5.8 Hz, H-29) and three doublets at δ_H 1.02 (d, J = 6.50 Hz, H-21), 0.89 (d, J = 6.5 Hz, H-26) and 0.94 (d, J = 6.4 Hz, H-27). Such protons have their corresponding carbons at δ_C 11.0 (C-18), 21.2 (C-19), 21.2 (C-21), 12.1 (C-29), 21.2 (C-26) and 19.0 (C-27). A carbonylic methyne proton resonated at δ_H 3.62 (H-3) as a clear seven-line pattern with diaxial splitting of 10.9 Hz and axial-equatorial splitting of 4.40 Hz which clarify the β-orientation (equatorial) of the hydroxyl group at C-3, which resonated at δ_C 71.81 in the ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum further showed olefinic protons at δ_H 5.43 (brs H-6), 5.20 (dd, J = 15.13, 8.50 Hz, H-22) and 5.20 (dd, J = 15.1, 8.50 Hz, H-23), which were attached to carbons δ_C 121.6 (C-6), 138.2.



Stigma sterol

Lupeol: β-Hydroxylup-20 (29)-ene (5)

Compound **5** was also obtained as an amorphous powder and its molecular formula $C_{30}H_{50}O$ was determined by HRMS which showed [M+] ion at m/z = 426.1542 a.m.u. The ^{1}H and ^{13}C NMR spectral data of the compound were assigned using reported values of 3β-Hydroxylup-20 (29)-ene (lupeol). The ^{13}C NMR showed thirty carbon signals at the broad band decoupled spectrum. Seven methyl, eleven methylene, six methyne and six quaternary carbon atoms were confirmed from DEPT experiments. The chemical shifts at δ_C 151.31 and 109.42 were the characteristic peaks for lupeol skeleton, assigned to C-20 and C-29 respectively. The oxygen de-shielding chemical shift in δ_C 78.08 was assigned to C-3. The resonances at δ_C 55.06, 50.12, 48.37, and 48.09 were assigned to C-9, C-18, and C-19 respectively. The signals at δ 43.08, 42.93, 40.90 and 40.07 were attributed to C-17, C-14, C-8, and C-22 respectively. The signals at δ_C 38.83, 38.86, 38.30, 37.50, 35.66, 34.24 and 29.40 were assigned to C-4, C-1, C-13, C-10, C-16, C-7 and C-21 respectively. The chemical shift resonated at δ_C 28.06, 27.08, 27.30, 25.77, and 21.84 was assigned to C-23, C-11, C-15, C-12, and C-2 respectively. The up field signals at δ_C 19.39, 18.73, 18.06 and 16.77 were attributed to C-30, C-6, C-18, and C-24 respectively. Further high field signals at δ_C 16.06, 15.14, and 14.03 were attributed to C-16, C-25 and C-27 respectively. Spectrum indicated two olefinic protons at δ_H 4.69 (1H, s, Ha-29, and at 4.57 (1H, s, Hb-29). One proton doublet of doublet for oxygen de-shielded proton H-3 and one-proton multiplets at δ_H 2.39 (1H, m, H-19) were assigned to H-19. All the spectral data (IR, UV, ^{1}H NMR ^{13}C NMR and mass spectrum) and other data are in agreement with the reported values for 3β-hydroxylup -20(29)-ene) (Lupeol) .[16–19] The structure was confirmed using 1D and 2D NMR techniques.



***β*-Hydroxylup-20 (29)-ene (lupeol)**

Table 9. Percent AChE and BChE inhibition potentials of the isolated compounds.

Sample	Concentration (µg/ml)	Percent AChE (mean ± SEM)	AChE IC_{50} (µg/mL)	Percent BChE (mean ± SEM)	BChE IC_{50} (µg/mL)
Compound-1	1000	72.87 ± 1.27		63.93 ± 0.67	
	500	60.95 ± 2.01		54.83 ± 1.21	
	250	49.08 ± 1.04	178	42.22 ± 1.28	320
	125	38.41 ± 0.99		30.76 ± 0.56	
Compound-2	1000	84.76 ± 0.61		80.98 ± 0.72	
	500	81.03 ± 0.86		73.65 ± 0.98	
	250	67.70 ± 0.92	80	57.93 ± 1.11	132
	125	57.32 ± 0.67		48.66 ± 2.43	
Compound-3	1000	90.12 ± 2.60		85.33 ± 0.40	
	500	81.09 ± 1.19		74.21 ± 0.88	
	250	68.17 ± 3.13	66	63.24 ± 1.64	114
	125	54.06 ± 0.50		51.07 ± 3.18	
Compound-4	1000	68.42 ± 0.70		65.20 ± 2.37	
	500	60.16 ± 2.22		56.51 ± 0.48	
	250	48.34 ± 1.49	268	44.014 ± 0.80	334
	125	35.81 ± 0.30		28.57 ± 0.65	
Compound-5	1000	75.012 ± 2.14		71.26 ± 0.62	
	500	62.45 ± 0.53		57.16 ± 2.42	
	250	50.83 ± 0.98	242	45.64 ± 3.73	316
	125	38.025 ± 1.31		32.58 ± 0.85	
Galantamine	1000	95.32 ± 0.88		94.50 ± 0.71	
	500	87.74 ± 0.55	44	84.66 ± 1.20	60
	250	76.44 ± 0.60		76.72 ± 0.72	
	125	64.58 ± 0.54		60.83 ± 0.69	

Galantamine was used as a positive control. Data is represented as (mean ± S.E.M) n = 3.Values significantly different as compared to positive control, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: $P > 0.05$.

Anticholinesterase effect of the isolated compound

The enzyme inhibition potential of the isolated compounds against acetylcholinesterase and butyrylcholinesterase are summarized in Table 9. The percent AChE inhibition observed for compound **1** were 72.87 ± 1.27, 60.95 ± 2.01, 49.08 ± 1.04, and 38.41 ± 0.99 at the concentration ranging from 125–1000 µg/ml respectively with the IC_{50} of 178 µg/mL while the compound **2** and **3** showed potent percent inhibitions with the IC_{50} of 80 and 66 µg/ml respectively as shown in Table 9. Similarly, compounds **4** and **5** showed moderate percent inhibition with IC_{50} of 268 and 242 µg/ml respectively. The BChE was inhibited by compounds; **1, 2, 3, 4** and **5** resulted with IC_{50} values of 320, 132, 144, 334, and 316 µg/ml, respectively. Galantamine was used as a positive control that has produced IC_{50} values of 44 and 60 µg/ml respectively against AChE and BChE.

DISCUSSION

In the current investigations, the medicinal aspects of *C. microphyllus* have been evaluated to revive its medicinal importance. The quantitative analysis of secondary metabolites in *C. microphyllus* showed the presence of significant amounts of phenolics and flavonoids. The phenolics and flavonoids have been the focus of research for decades due to their beneficial role in the management of the various types of health anomalies. One of the best role of phenolics and flavonoids, which have been reported from time to time, is the free radical scavenging role which is very important from human physiological point of view and their accumulation inside body leads to high oxidative stress which may cause the peroxidation of lipid membranes of various cells in the body and neuronal membranes as well. They also cause damage to DNA and other biologically important molecules. As mentioned above the high oxidative stress also causes neuronal damage, which may lead to a decreased amount of various neurotransmitters within the body. The decreased amount of the neurotransmitters i.e., acetylcholine or butyrylcholine leads to the demonstration of various symptoms including the memory impairment and movement disorders collectively known as Alzheimer's disease. As far as the role of phenolics, flavonoids, and other secondary

metabolites are concerned, they are capable of scavenging the free radicals in the body thereby minimizing the effects of oxidative stress and being from plant origin are considered nontoxic.[27–29] The scavengers of the free radicals are compounds mostly containing phenolic ring that prevent or ameliorate the symptoms of oxidative stress including Alzheimer's disease. The presence of phenolics and flavonoids in a considerable amount along with significant antioxidant activities as demonstrated by extracts of the selected plant signifies the beneficial role of *C. microphyllus* that would be helpful in the prevention of Alzheimer's disease and other oxidative stress related complications in human.[27–29] DPPH and ABTS free radicals were more potently inhibited by crude extract of aerial parts with IC_{50} values of 83 and 92 µg/mL respectively followed by ethyl acetate fraction (IC_{50} = 140 and 178 µg/mL respectively) while against the same free radicals crude extract exhibited IC_{50} values of 66 and 90 µg/mL respectively. Against AChE and BChE good inhibitions were observed for crude and ethyl acetate extracts. As anthelminthic agent chloroform fraction was more potent than other extracts followed by ethyl acetate fraction.

High zone of inhibitions were formed by ethyl acetate fraction of aerial parts against the selected fungal strains (16.04 mm against *Candida albicans* and 15.88 mm against *Fusarium solani*). Similar trend was observed for the root extracts. However, the intensity of inhibition of root extract was lower than that of the aerial parts. Against *Pseudomonas aeruginosa* zone of inhibitions equal to 13 and 12.3 mm respectively was produced by ethyl acetate fraction of aerial and root parts while against *Streptococcus pyogenes* the zone of inhibitions produced by aerial and root parts, ethyl acetate fractions were 11.4 and 11.8 mm respectively. Similar role of *C. microphyllus* as a significant antimicrobial agent has been reported previously.[27–29]

Although all the extracts exhibited good antibacterial, antifungal, antioxidant, anthelmintic and anticholinesterase activities. However, among them comparatively good activities were observed for ethyl acetate fraction which was then subjected to isolation of phytochemicals. Five compounds mentioned above were isolated in pure state. In neurodegenerative diseases the role of AChE and BChE is prominent, and to cure them the general strategy used is the inhibition cholinesterases. As these enzymes are responsible for the breakdown of acetylcholine and other neurotransmitters leading to its deficit results a number of neurodegenerative diseases. So by inhibiting the AChE and BChE, the acetylcholine level can be restored. Among the isolated compounds, compound **3** exhibited high percent inhibition of both of these enzymes as depicted in Table 7 with IC_{50} values of 66 and 114 µg/mL respectively against AChE and BChE followed by compound **2**.

CONCLUSION

The results of the current investigational study and the previous literature reveals the importance of *C. microphyllus*. Being a good source of phenolics and flavonoids the extracts exhibited high antioxidant, anthelminthic, anticholinesterase, and antimicrobial potential potentials. Among the extracts, ethyl acetate fraction due to its high phenolic and flavonoid contents was subjected to silica gel column isolation that resulted in isolation of one new and four known compounds. Among the isolated compounds, compound **3** exhibited promising anticholinesterase potentials. Further investigation are needed to isolate other biologically active compounds from this plant and investigate the anticholinesterase potential of compound **3** in animal models along with its toxicological evaluations.

Acknowledgments

Authors wish to thanks Research Supporting Project Number: RSP-2021/346 King Saud University Riyadh Saudi Arabia for their financial support

Disclosure statement

The authors declare that they have no competing interests.

Author contributions

M.A., H.U., W.B. and N.I. conducted experimental work and wrote the paper. M.Z. revised the paper for submission and after the reviewer comments. RU, and AA helped in analysis and will pay the APC. All authors have read and agreed to the published version of the manuscript.

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