Two New Cholinesterase Inhibitory and Antioxidative Constituents from *Syzygium cumini*

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ABSTRACT: *Phytochemical investigation on dichloromethane (CH_2Cl_2) fraction from fruit seeds of Syzygium cumini provided two new esters Syzygioate A (1) and Syzygioate B (2), along with known compounds dipropyl succinate and dioctyl phthalate. The isolated compounds were characterized via spectroscopic techniques such as ^1H, ^{13}C NMR, EI-MS spectrometry, and FT-IR. The bioassay studies were also conducted for the isolated compounds where the new compounds 1 and 2 exhibited significant inhibition potential against acetylcholinesterase (AChE), butylcholinesterase (BChE) & antioxidant activity against Diammonium 2,2'- azino -bis (3-ethyl benzo thiazoline-6-sulfonic acid (ABTS), superoxide anion radical scavenger & 2,2-di phenyl-1-picryl hydrazyl (DPPH). The IC50 values of compounds 1 and 2 for their cholinesterase inhibition were 7.15, 4.54µM against AChE, and 9.21 & 6.31µM against BChE. The DPPH radical scavenging potential for compounds 1 & 2 exhibited IC50 values as 69.4µg/mL and 74.6µg/mL respectively.*

KEYWORDS: *Syzygium cumini; Isolation; Antioxidant activity; Cholinesterase Inhibitory.*

INTRODUCTION

The family *Myrtaceae* comprises about 140 genera and around 5000 species. *Syzygium* is one of the prominent genera of the *Myrtaceae* which comprises five hundred species [1]. Literature reveals that the plants of *Syzygium* is commonly found in the Asian subcontinent and Southern-Asian regions. Various species of the genera possess anti-diabetic properties. *S.cumini* plant of *Syzygium* is an ever-green, light greenish flower that transformed into bear berries-like fruit (almost black) that can be eaten [2-3]. Various parts of *S.cumini* act as an antioxidant, anti-hepatotoxicity, anti-inflammatory, and bronchodilator, to treat irritation of the throat, thirst, and ulcers and display many other activities [4-6]. It encourages apoptosis in human breast cancer cells [7]. The diverse ethnopharmacological applications of this species encouraged us to carry out a phytochemical investigation on *S. cumini*.

Various natural products such as glucosides,

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isoquercetin, myrecetin & and kaemferol have been profoundly indicated in Jambolan. The seeds of the plant has the potential to transform starch into sugars as they contain glycosides, alkaloids, and jambosine. The plant is reported for protein, calcium, and flavonoids which is the main reason for the antioxidant potential of the extracts from this plant [3]. Generally, plants are useful means of getting antioxidants. The antioxidant properties of plant tissues are associated with the actions of free radicals scavenging enzymes such as peroxidases, superoxide dismutases, catalases, and others. Additionally, the antioxidative properties of plants are linked to their phytochemical constituents including phenolic compounds, carotenoids, tocopherols, and ascorbic acids [3]. In Alzheimer's Disease (AD), the cholinesterase enzyme is considered a reasonable target for inhibitor drugs. It has also been reported that memory impairment in dementia patients is mainly due to disorders in brain cholinergic functions and therefore the AChE has long been considered a promising target for the treatment of Alzheimer's disease [8-10].

Literature suggests that the crude extracts from *S.cumini* plant have been recognized for their great antioxidant potential [11]. Leaves and buds-based extracts of *S.cumini* have also been reported for their great antioxidant as well as for their cholinesterase inhibitor potential [12]. It is assumed that the plant is enriched with compounds having such properties as antioxidants and cholinesterase inhibition potential as extracts of the plant collectively exhibit this kind of activities which have been discussed previously. Since both the antioxidants and cholinesterase inhibitors are effective classes of drugs used to prevent various related diseases like Alzheimer's etc., therefore a bioassay-guided isolation of the potent natural antioxidants and cholinesterase inhibitors attracted great interest of the scientific community. Based on these facts and to properly affirm the antioxidant and cholinesterase inhibitory functions of the plant, the present work aimed to isolate natural products possessing antioxidant and cholinesterase inhibitory properties.

EXPERIMENTAL SECTION

General procedure

The column chromatography method was adopted for the purification/isolation of the natural substances. Silica gel (High purity grade obtained from E Merk) of mesh size about 70 to 230 was applied as a stationary phase while a mixture of *n*-hexane and dichloromethane was used as a mobile phase during purification. For identification purposes, pre-coated aluminium-supported silica gel (60F 245; 20cm, 0.2mm thick, E Merk) TLC cards were for identification and analysis. The solvents (*n*-hexane, dichloromethane, and methanol) of analytical grade were obtained from Sigma Aldrich. UV Vis light of 245nm to 366nm wavelength was to visualize TLC plates where $Ce(SO4)_2$ reagent (obtained from Sigma Aldrich, >99% purity) was applied as a spraying agent. Shimadzu made 460 FT-IR spectrophotometer and Bruker made 300 MHz spectrophotometer was used to record NMR and FT-IR spectrum respectively. The FABMS & EIMS were performed on JMS-DA 500 & JMS HX110 MS spectrometers respectively.

Plant material

The leaves, bark, and fruits of *S. cumini* were obtained from the District D.I.Khan of KPK province, Pakistan. A taxonomist (Professor Dr. Saddiq Khan) from Mufti Mehmood Agriculture University, Pakistan recognized the plant.

Extraction and isolation

The *S. cumini* fruit seeds (12 kg) material (initially dried under shade) was extracted through MeOH (2L × 60L) at room temperature. Under reduced pressure, the MeOH extract was concentrated via evaporation to get a gummy residue (400 g). Furthermore, the suspended residue in H_2O was successfully extracted with different solvents i.e. *n*-Hexane (50 g), DCM (208 g), EtOAc (57 g), and *n*-butanol (76 g). The DCM fraction was processed through a silica gel-packed chromatographic column, eluting with n-hexane (C_6H_6), *n*-hexane(C_6H_6)/ DCM, DCM, DCM/Methanol, and pure methanol in ascending polarity order. The *n*-hexane/DCM (4:6) exhibited a major spot. The obtained fraction was further subjected to flash chromatography using an eluting system *n*-hexane/DCM 5:5 which furnished Syzygioate A (1) (23 mg). Additionally, the column using *n*-hexane/DCM into 4:6 provided Syzygioate B (2) in a semi-pure form. Further, flash chromatographic purification by using *n*-hexane/DCM in 6:4 ratio furnishes a pure compound in an amount of 20 mg. The re-chromatographed *n*-hexane/DCM (4:6) fraction was further chromatographed using a silica column and *n*-hexane/DCM (3:7) eluent system which provided known natural product 3 in the amount of 24 mg while

the mentioned fraction upon passing through a silica gel column (*n*-hexane/DCM 8:2) provided a known compound **4** in the amount of 25 mg.

Syzygioate A (1)

Gummy solid (purity > 99%). $[\alpha]_D^{25}$ =+32(c = 0.12, MeOH). IR (KBr): ν_{max} = 1720-1735 (ester), 1610 (C=O), 1465-1500cm^1 (aromatic). For ^{1}H, and ^{13}CNMR see Table 1. HREI-MS m/z = 306.312 (calculated $[M]^+$ 306.423) assigned molecular formula $C_{16}H_{18}O_6$.

Syzygioate B (2)

Gummy solid (purity > 99%). $[\alpha]_D^{25}$ =+32 (c = 0.11, MeOH). IR (KBr): ν_{max} = 1715-1729 (ester), 1620 (carbonyl) cm^1. For ^{1}H, and ^{13}C NMR see Table 2. HREI-MS: m/z = 258.11 (calculated 258.27, $[M]^+$) corresponded to molecular formula $C_{12}H_{18}O_6$).

Known compounds (3 and 4)

Gummy solid (purity > 98%). $[\alpha]_D^{25}$ =+32 (C=0.12, Methanol). FT-IR (KBr): ν_{max}= 1715-1729 (ester), 1620 (carbonyl) cm^1. HR-FABMS: m/z = 202.12 (calcd. 202.25 for $C_{10}H_{18}O_4$, [M]+). Gummy solid (purity > 99%). $[\alpha]_D^{25}$ =+32 (c = 0.11, MeOH). IR (KBr): ν_{max}= 1715-1729 (ester), 1463-1502 (aromatic) 1620 (carbonyl) cm^1. HRFABMS: m/z = 390.28 (calculated 390.56 for $C_{24}H_{38}O_4$, [M]+). The spectral data is coincide with (*Deveci et al*., 2019).

Acetyl and Butyl cholinesterase inhibitory assay

Following a standard protocol, all the require chemicals (5,5'-dithio bis-2-nitrobenzoic acid), AChE (EC 3.1.1.7), BChE (EC 3.1.1.8), acetyl thiocholine iodide, galanthamine and butyryl thiocholine chloride of analytical grade were purchased from Sigma. Slightly modified spectrophotometric protocol (previously described by *Ellman et al*., in 1961 and reported in a study in 2020) [13] was followed in order to perform the best AChE and BChE inhibitory assays. The assay conditions & and procedure were as per introduced by *Rocha et al*., 1993 [14]. Following the procedure about 0.2 mM DTNB in a 62mM sodium phosphate buffer, pH 8.0 in the amount of 880µl was used. AChE/BChE solution was mixed with each sample compound solution of 40µl and then incubated at around 258°C for 15 to 20 minutes. By adding 40µl of acetyl thiocholine or butyryl thiocholine (substrate to AChE & BChE respectively) reaction was started. The DTNB reaction with thiocholine (ACh or BCh) resulted in a yellow 5-thio-2-nitrobenzoate which indicates enzymatic hydrolysis. The hydrolysis was monitored at 412nm of wavelength under a spectrophotometer. The experiment was repeated thrice. The effect was observed for the test compounds in their increasing concentrations (25-100) µg/mL (Table 3) in the inhibitory assay 50% (IC50 Values).

Antioxidant property and determination of IC_{50} value

The DPPH, Ascorbic acid, antioxidant assay finding kit (Catalog number # CS0790), 2,2'-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid (ABTS), Trolox, Nitro blue tetrazolium (NBT) and DMSO were used to perform the Antioxidant activity. The antioxidant activity of the isolated natural products was examined by following a reported radical scavenging procedure with some modifications [15-16]. The DPPH & and sample compound mixture (9µL:1µL) was shaken and subsequently left in the dark for 1h. The absorbance(A) was observed at a wavelength of 517nm via a spectrophotometer. The experiment for all the compounds were repeated thrice and IC50 values were calculated.

Additionally, free radical scavenging property was also examined through ABTS radical cations method with some changes if needed [17]. For this purpose, 7mM of ABTS was added to 2.45mM of K_3PO_4 (10 mL:10 mL) in H_2O at 25°C to prepare ABTS reagent, and then it was left in the dark for 16h. Then this reagent was diluted with MeOH up to its adjusted absorbance (*A*) of 0.007 at 734nm. Accordingly, a range of different concentrations of the sample compound (10 to 50)µg/mL were added to $ABTS^{\cdot+}$, and the absorbance (A) was examined at 734nm after 30 minutes of incubation at 25°C. All the experiments were repeated thrice and Trolox was used as a standard.

Superoxide radical scavenging activity was also performed according to a reported procedure using NBT [18]. About 3mL of 100mM sodium phosphate buffer at a pH of 7.4 having 1mL (150 mM) of NBT solution, about 1mL (468mM) NADH solution, and different concentrations of the sample isolated natural products in H_2O, 1mL (60mM) of phosphomethazone sulfate was added initiating reaction mixture. The Absorbance was observed at a wavelength of 530nm after incubation for 5 min. at 25 °C. The experiment was repeated thrice. Ascorbic acid was used as a standard and %age of DPPH, ABTS, &

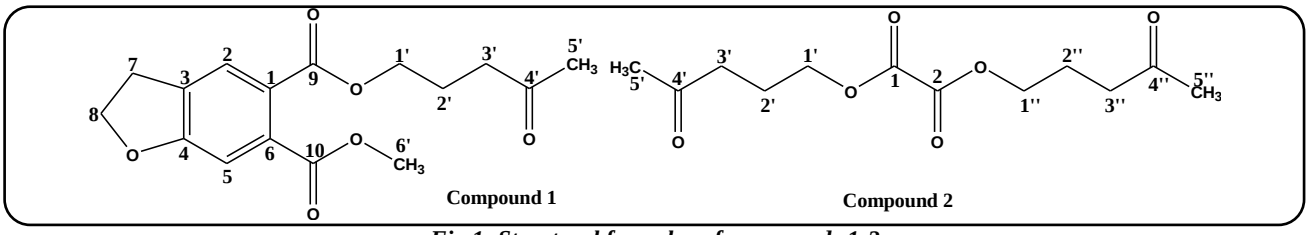

Fig 1: Structural formulae of compounds 1-2

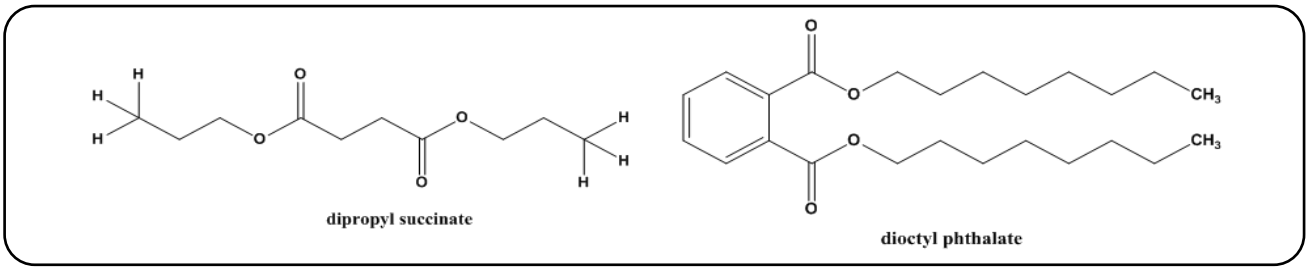

Fig 2: Structural formulae of the known compounds 3-4

superoxide radical scavenging was calculated by applying the formula:

$$\% \text{ scavenging activity} = [(AB - AA)/AB \times 100)] \quad (1)$$

Where AB is the absorbance of the control and AA is the absorbance of sample isolated compounds.

RESULTS AND DISCUSSION

Extraction and Structural Elucidation

The methanolic extract of the *S.cumini* seeds was distributed into n-hexane (C_6H_6), Dichloromethane (DCM), Ethyl-acetate (EtOAc), and Methanol (MeOH) fractions. The dichloromethane soluble fraction was passed through phases of column and thin-layer chromatography techniques. This resulted in two new esters, an aromatic one was named Syzygioate **A** bearing a molecular formula as 6-methyl 5-(4-oxo pentyl)2, 3-dihydro benzofuran-5,6-di carboxylate (1), and the second ester was aliphatic in nature which was named as Syzygioate B (bis(4-oxopentyl)oxalate) (2). The study resulted in two known phytochemicals, di-propyl succinate (3) and dioctyl phthalate (4) from its DCM fraction. Their structures were established by interpreting the data of their FT-IR, ^{1}H, ^{13}C NMR, and EIMS techniques. Their structures are presented in Figures 1 and 2.

Syzygioate A (1) was isolated as a colorless solid having a melting point in a range of 154-156°C. The FT-IR spectrum displays a signal at around 1465-1500 cm^{-1} corresponding to aromatic unsaturation and the signal at 1610 cm^{-1} showed the C=O moiety. Additionally, the peak observed on a scale of 1720 to 1735 cm^{-1} was due to ester functionality. An additional peak responsible for sp^2 CH stretching signal was observed at 2995 cm^{-1}. The HRMS technique confirms the M^+ peak at m/z= 306.31, suggesting its Mol. formula $C_{16}H_{18}O_6$ where its calculated mass was 306.42. It shows seven degrees of unsaturation (four for the benzene ring, two for ester group, and one for C=O). Linked scan measurement of the M^+ for syzygioate A suggested that ions at m/z 291, 275, 263, 247, 205, and 177 emerged directly from it.

The ^{1}HNMR spectrum corresponding to Syzygioate A exhibited a pair of overlapping peaks at a chemical shift of 7.1 to 7.5ppm; suggesting benzene moiety in the skeleton of the Syzygioate A. A clear triplet due to two protons was observed at δ4.5 ppm (*J*= 6.6 Hz) was assigned to oxy-methylene protons. Considering the oxolane part, the signal at δ3.8ppm (*J* = 6.2 Hz) was assigned to -OCH_2 protons, and the neighboring methylene protons exhibited signal at δ3.4ppm (*J*= 6.2 Hz) as a triplet. The –OMe protons were resonated at δ3.5ppm as a singlet, while the terminal acylated CH_3 showed a singlet at 2.7ppm. The acylated –CH_2- protons in a chain exhibited a tripet at 2.4ppm (*J*=6.4 Hz). The signal appeared at δ1.6-1.9ppm corresponded –CH_2- protons of the long chain and of the branches of Syzygioate A.

The ^{13}CNMR spectrum via a DEPT experiment indicated two CH_3, five –CH_2-, two methines, and 7 quaternary carbons (Table # 1). The highly downfield peak at δ 207.5ppm are corresponds to carbonyl (ketonic) carbon while the signals observed at δ168.0 and 167.71 ppm suggesting the presence

Table 1: The ^{1}HNMR and ^{13}CNMR data of Syzygioate A (δ chemical Shift in ppm, $CDCl_3$, J in Hz)

H/C	δ for H	δ for C
1	Quaternary	130
2(CH)	7.17	129
3	Quaternary	131
4	Quaternary	164
5 (CH)	7.5	120
6	Quaternary	119
7 (CH_2)	3.4	29
8(CH_2)	3.8	79.8
9	Quaternary	167.7
10	Quaternary	168
1'(CH_2)	4.5	80
2'(CH_2)	1.9	81
3'(CH_2)	1.6	64
4'	Quaternary	207.5
5'(CH_3)	2.7	51.5
6'(CH_3)	3.5	29.8

of ester C=O functional group. Additional peaks in the spectrum at 164.81ppm and δ131.51 ppm were attributed to C4 and C3 respectively. The signals appearing at 130.53ppm, δ129.19ppm, and δ120.53ppm were attributed to benzene carbon atoms. The –OCH_2- carbon was observed at 80.17ppm and the other –CH_2- carbon of alkyl group confirmed their presence based on the peaks appearing at 81.01ppm and δ 64.55ppm. The highly up-field peaks at 51.5ppm and 29.98ppm were assigned to the terminal –CH_3 carbon atom. The peaks for C7 and C8 resonated at 79.8ppm and 29.5ppm ppm respectively.

Syzygioate B (2) compound was obtained as a colorless solid having a melting point in a range of 152-155°C. The IR spectrum display bands at 1605 cm^{-1} showed the carbonyl moiety. Furthermore, the ester functionality showed a band at 1715 to 1729cm^{-1} and the sp^3 CH stretching showed a peak at 2989cm^{-1}. The HRMS technique confirmed the M^+ at m/z =258.11, which suggested its formula as $C_{12}H_{18}O_6$ where the calculated molecular mass was 258.27. This shows four degrees of unsaturation where two were attributed to C=O and two to the ester moiety. Linked scan measurements about M^+ of syzygioate B suggested that the ions at m/z 243, 215, 173, 129, and 85 were generated directly from it.

The ^{1}HNMR spectral data indicated all relevant peaks, one at 3.77ppm suggesting the presence of -OCH_2- and

Table 2: The ^{1}H and ^{13}CNMR values for compound 2 ($CDCl_3$ as a solvent, δ(chemical shift) in ppm and J in Hz)

H/C	δ for H	δ for C
1	Quaternary	156
2	Quaternary	156
1'(OCH_2)	3.7	64
2'(CH_2)	2.0	21.5
3'(CH_2)	2.3	41
4'	Quaternary	202
5' (CH_3)	2.8	29.8
1'' (OCH_2)	3.3	64
2''(CH_2)	2.0	21.5
3'' (CH_2)	2.3	41
4''	Quaternary	202
5''(CH_3)	2.8	29.8

the oxymethylene protons exhibited a triplet at 3.38ppm (t, *J*= 3.3 Hz). The peak for the terminal methyl group appears as a singlet at δ2.82 ppm. For H-3'' and H-3' a triplet peak was observed at around 2.39-δ2.35 ppm. Additional multiplets observed in up field region at 2.04-1.96ppm were due to the presence of methylene protons. The 12 peaks for carbon atoms in ^{13}C NMR (BB) spectrum were resolved through DEPT experiments into two CH_3, six –CH_2-. The highly downfield peak at 202.1ppm was attributed to the ketonic C=O atom. Additional peaks appearing at δ156.18 was attributed to the C=O carbon while the peak appeared at 64.14ppm is attributed to oxy metylene carbon. The upfield carbons appeared in the region of 41.0ppm attributed to C3'- 3'' whereas alkyl group was observed at 21.5ppm. NMR results for compounds 1 and 2 are presented in Tables 1&2 respectively.

Considering the ester functionality of the isolated compounds, the literature reveals that due to their lipophilic nature, the ester moiety has been widely studied in the medicinal field, as this characteristic is an attractive function of compounds for their diffusion across a semipermeable membrane. Additionally, these types of compounds found a suitable place in the field of medicines due to their various biological applications including antioxidant, cholinesterase inhibitory activity, antimicrobial, anti-inflammatory, antitumor, anti-leishmanial, and anti-hypertensive [19]. In this context antioxidant, cholinesterase inhibitory activity studies performed for the isolated products are described in the following sections.

Table 3: Kinetic parameters of inhibition of AChE and BChE of the sample products 1-4 (IC50, µM), The values are obtained through triplicate experiments

Sample Compounds for test	AChE± SEM[a]	BChE± SEM[a]
1	7.15 ±0.04	9.21 ±0.033
2	4.54 ±0.02	6.31 ±0.023
3	4.84 ±0.02	7.12 ±0.042
4	6.43 ±0.04	8.32 ±0.054
Allanzanthane[b]	5.45 ±0.45	11.69 ±0.054
Galanthamine[b]	2.94 ±0.41	8.73 ±0.011

[a]Standard error of the mean of thrice assays. [b]Positive control was used in the assay.

Cholinesterase Inhibitory properties of the isolated products

All four isolated natural products Syzyogiate A (1), Syzygioate B (2), dipropyl succinate (3), and dioctyl phthalate (4) were tested for their inhibition potential against AChE and BChE. The results obtained are presented in Table 3.

In-vitro Antioxidant assay of the sample isolated products

The isolated products were examined for their antioxidant properties. Assessment of antioxidant potential follows different mechanisms due to which it is difficult to rely on results obtained from only one assay. In this context, we have applied a set of different standard antioxidant evaluation methods to achieve more reliable results. In the present work, the commonly practiced laboratory assays such as DPPH, ABTS, and superoxide scavenging methods were performed to analyze the antioxidant potential of the isolated products [20]. The results demonstrated a significant % scavenging actions against DPPH, ABTS, and Superoxide anion radical (Table # 4).

In the above table 4, the values were obtained through triplicate experiments, and the % scavenging activity was calculated by applying the following formula;

$$\% \text{ scavenging activity} = [(AB - AA)/AB \times 1700)] \quad (2)$$

Where AB is the absorbance of the control, AA is the absorbance of the test compound/standard sample. The antioxidant potential results for the four isolated natural products can be presented graphically in Figure 3;

Studies have found that excess reactive oxygen species (ROSs) such as peroxides, superoxides, and hydroxyl radicals are the main source agents in the development of the majority of the diseases. It has been observed that ROSs radicals are highly reactive in nature which may result from oxidative metabolism and can oxidize cellular components like DNA, proteins & and lipids [21-22]. Therefore, the tested products can be considered as potent antioxidants & and anti-Alzheimer's agents.

CONCLUSIONS

The phytochemical research on dichloromethane soluble fraction of *S. cumini* was performed according to standard protocol following purification *via* chromatography and relevant spectroscopic techniques. Two new natural products along with two known products (dipropyl succinate and dioctyl phthalate) were achieved. The newly isolated products were recognized as Syzygioate A (an aromatic ester) and Syzygioate B (an aliphatic ester). All the compounds were found active against AChE and BChE whereas the newly isolated compound Syzygioate A showed the highest activity against both of AChE and BChE, even higher than the standard. The compound 1 and 2 also exhibited strong antioxidant potential of DPPH scavenger, ABTS & Superoxide anion radical scavenging of compounds 1 and 2, as IC50 values were 69.4 to 74.6µg/mL, 34.02 to 29.04µg/mL and 82.01 to 82.60µg/mL respectively. It is recommended that further in-vivo investigations on the isolated products and bioassay-guided studies on different parts of this medicinal plant can exploit the hidden medicinal importance of *S. cumini* against various diseases.

Acknowledgment

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Table 4: DPPH, ABTS and Superoxide radical scavenging assay

Concentration in µg per mL	Standard	(a) DPPH antioxidant activity			
	Ascorbic acid	Compound 1 (Syzygioate A)	Compound 2 (Syzygioate B)	Compound 3	Compound 4
25	30.2 ±0.02	26.2 ± 0.2	12.2 ±0.7	18.1 ±0.5	28.1 ±0.2
50	65.3 ±0.01	45.8 ±0.01	29.8 ±0.4	35.4 ±0.3	42.0 ±0.02
75	70.3 ±0.01	55.5 ±0.11	51.5 ±0.3	62.6 ±0.02	68.2 ±0.23
100	83.2 ±0.02	60.1 ±0.41	69.2 ±0.02	75.3 ±0.1	79.2 ±0.05
IC50, (µg/mL)	43.8±0.02	69.4±0.03	74.6±0.04	65.2±0.6	56.4±0.1
Concentration In µg per mL	Standard	(b) ABTS antioxidant activity			
	Trolox	Compound 1 (Syzygioate A)	Compound 2 (Syzygioate B)	Compound 3	Compound 4
10	18.1 ± 0.2	09.01 ± 0.09	12.02 ± 0.04	15.04 ± 0.08	06.04 ± 0.1
20	27.03 ± 0.52	16.03 ± 0.04	27.03 ± 0.07	23.09 ± 0.03	13.02 ± 0.3
30	55.02 ± 0.12	47.08 ± 0.01	49.08 ± 0.08	48.06 ± 0..01	29.08 ± 0.04
40	77.05 ± 0.4	63.02 ± 0.03	77.07 ± 0.01	66.03 ± 0..07	44.01 ± 0.08
50	95.01 ± 0.01	76.03 ± 0.91	95.06 ± 0.31	79.01 ± 0.05	59.03 ± 0.02
IC50, (µg/mL)	27.82 ± 0.02	34.02 ± 0.04	29.04 ± 0.08	32.19 ± 0.15	44.4 ± 0.05
Concentration In µg per mL	Standard	(c) Superoxide antioxidant activity			
	Ascorbic acid	Compound 1 (Syzygioate A)	Compound 2 (Syzygioate B)	Compound 3	Compound 4
25	37 ± 0.09	08 ± 0.06	15 ± 0.01	12 ± 0.03	18 ± 0.02
50	65 ± 0.05	27 ± 0.15	23 ± 0.03	21 ± 0.05	32 ± 0.22
75	69 ± 0.12	39 ± 0.13	41 ± 0.22	38 ± 0.25	51 ± 0.21
100	82 ± 0.02	67 ± 0.02	66 ± 0.30	51 ± 0.08	66 ± 0.05
IC50, (µg/mL)	38.6±0.20	82.01±0.13	82.60±0.11	98.8±0.25	78.22±0.04

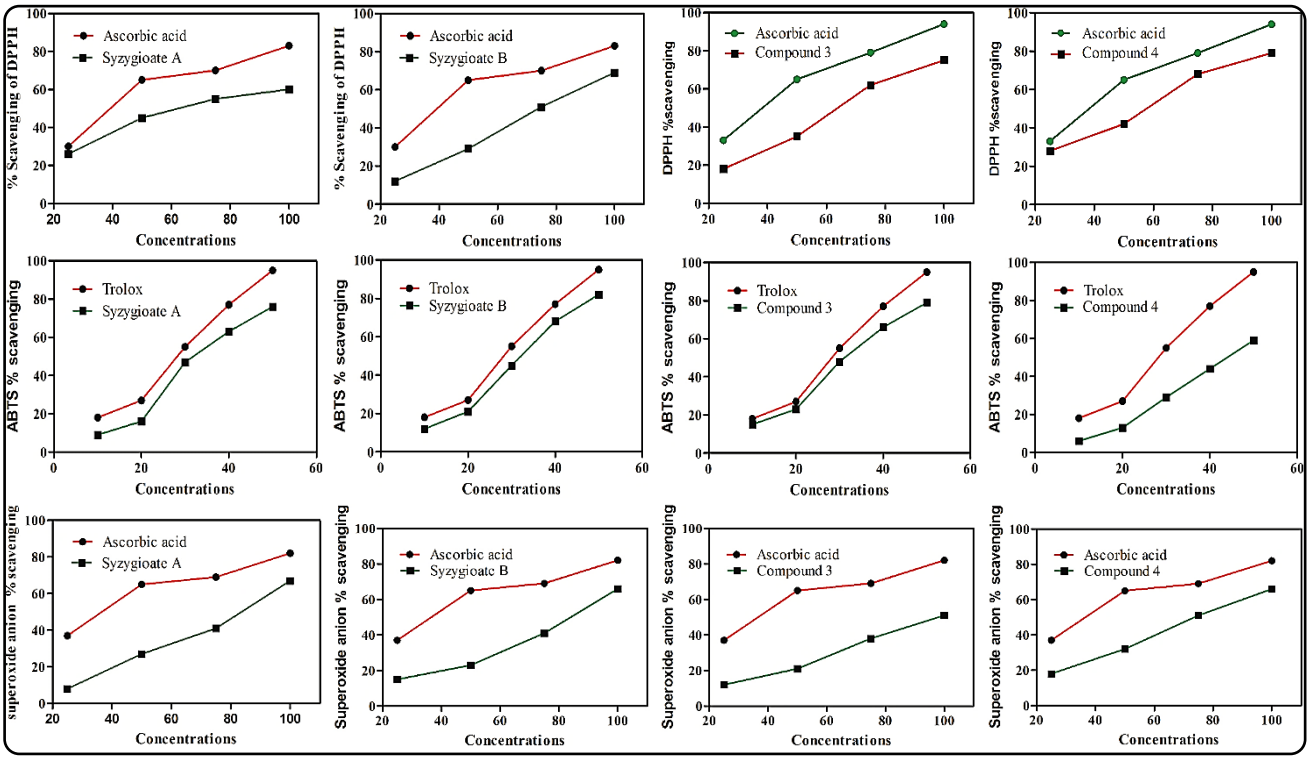

Fig. 3: DPPH, ABTS and Superoxide radical scavenging assay

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