ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 8, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

EXTRACTION, ISOLATION, AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS AND ESSENTIAL OIL FROM *SYZYGIUM JAMBOS*

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Received: 01 April 2017, Revised and Accepted: 01 May 2017

ABSTRACT

Objectives: Over the past few decades, phenolic compounds become important due to it has been associated with protection against different diseases and sensory point of vision. Hence, at the present study, there has been a growing interest to carry out structural elucidation and characterization of the pure isolates from *Syzygium jambos*.

Methods: *S. jambos* dried powder leaves were extracted by soaking in 85% methanol solvent at room temperature 25±2°C. The antioxidative activity of the isolates was assessed according to 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and phosphomolybdenum assays.

Results: A total of 8 compounds were isolated from the n-butanol extract of *S. jambos* (Family Myrtaceae) plant, they were identified as quercetin-3-O-rutinoside (1), prenylbenzoic acid 4-β-D-glucoside (2), morolic acid 3-O-caffeate (3), 5,4'-dihydroxy, 7-methoxy, 6-methyl-flavone (4), 3,4,5-trihydroxybenzoic acid (5), quercetin (6), isoetin-7-O-β-D-glucopyranoside (7), and (4'-hydroxy-3'-methoxyphenol-β-D-[6-O-(4"-hydroxy-3",5"-dimethoxylbenzoate)] glucopyranoside) (8). Compounds 5 and 6 showed the most radical scavengers among the tested compounds with SC_{50} values of 5.50 and 4.30 µg/ml, respectively, compared to ascorbic acid as standard and the total antioxidant capacity (TAC) values of 605.0 and 680.59 mg ascorbic acid equivalent/g compound, respectively. *In vitro* antimicrobial activities of the isolated compounds were tested using disc agar plate method against four pathogenic microbial strains including Gram-positive, Gram-negative bacteria and yeast with inhibition zones from 9 to 19 mm. Gas chromatography-mass spectrometry analysis for the essential oil provides twenty four identified components representing 92% of its total oil composition.

Conclusion: The results supported that *S. jambos* could be attributed to sources of natural antioxidant and antimicrobial applications.

Keywords: *Syzygium jambos*, Antioxidant compounds, Flavonoids, Natural products, Phenols.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i8.18849

INTRODUCTION

Herbal medicine is the utilization of plants for their curative value. Plants contain a variety of chemical compounds that follow up on the body to eliminate and treat sicknesses [1,2]. Therapeutic plants are important for pharmacological research and medication development. Not only plant constituents are utilized specifically as therapeutic agent but also considered as beginning materials for the synthesis of drugs or as forms for pharmacologically active compounds [3]. The polyphenolic compounds are mostly exist in both edible and nonedible therapeutic plants; accordingly exhibited multiple biological and pharmacological activities, such as, antioxidant activity [4,5]. Antioxidants of natural products are compounds that capable of scavenging free superoxide radicals, anti-aging, and diminished the cancer risk. On the other hand, polyphenols are antioxidants within redox abilities which can be considered as hydrogen donators, reducing agents, singlet oxygen quenchers, and also metal chelators agents. In particular, the biological properties of the natural antioxidants have been strongly related to their large levels of numerous phenolic compounds, such as flavonoids and phenolic acids [6]. *Syzygium* species having considerable medicinal properties have drawn the consideration in recent times. Numerous pharmacological reviews have been done in various *Syzygium* species [7]. *Syzygium jambos* is an evergreen tree of the family Myrtaceae, it was reported that several phenolic compounds and triterpenes have been isolated from the leaves of the *S. jambos* [8-10]. Moreover, *S. jambos* showed antioxidant, antiviral [11], anti-inflammatory [12], and antimicrobial activity [13]. Consequently, in our study, there has been a growing interest to carry out structural elucidation and characterization of the pure isolates derived from *S. jambos*. Furthermore, the volatile oil from fresh leaves of the plant was isolated and identified by gas chromatography-mass spectrometry (GC/MS) analysis.

METHODS

Plant-materials

S. jambos leaves were given from Zoo Garden, Giza, Egypt at May 2014. Identification of the plant was by Dr. Threase Labib, consultant of plant taxonomy - Ministry of Agriculture - the Head of Taxonomist Specialists at the Zoo garden, a voucher specimen (No.S7/3/1 for *S. jambos*) was kept at the garden herbarium. The plant was air-dried in out of sun area at room temperature then powdered by electric mill and kept in a closed container in a dark laboratory until undergoes the extraction process.

Apparatus and equipments

Nuclear magnetic resonance (NMR) spectrometer: Jeol Delta 2 spectrometer (300/75 MHz), NMR Department, Faculty of Science, Cairo University. Microfine Cutting Grinder (IKA WERKE, Germany). Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS used for GC/MS analysis and TGSMS Fused Silica Capillary Column (30 m, 0.251 mm, 0.1 mm film thickness), National Research Center, Giza, Egypt. Ultraviolet (UV) lamp, Vilber Louremat (VL-6LC 254 and 365 nm). Sensitive electric balance, BP 2215 (Germany). UV-visible

spectrophotometer, Spectronic 601 (Milton Roy, USA) was used for measuring the absorbance in UV range. For measuring the melting points, SMP3 Stuart Scientific (UK). For performing GC/MS detection, use an electron ionization system with ionization energy for 70 ev as the carrier gas within a constant flow rate of 1 ml/minute. The chemistry works were done at the Medicinal Chemistry Department, Theodor Bilharz Research Institute.

Chemicals for biological studies

Reagents and solvents used were of analytical grade. 2,2'-diphenyl-1-picraylhydrzyl (DPPH) was purchased from (Sigma-Aldrich Co.). Acids and solvents (petroleum ether, chloroform, ethyl acetate, n-butanol (BuOH), acetic acid, sulfuric acid, and dimethyl sulfoxide (DMSO)) were purchased from (Sigma-Aldrich Co.). Paper chromatography (PC) was done on filter paper Whatman No. 1 (57×46 cm) and Whatman filter paper No. 3 (46×57 cm) Whatman Ltd., Maidstone, Kent, England, whereas pre-coated silica plates (GF_{254}, Merck) was used for thin layer chromatography (TLC). Ferric chloride, ammonium molybdate, aluminum chloride, sodium bicarbonate, sodium phosphate, and ascorbic acid were purchased from (Merck Chemical Co.), D-glucose and L-rhamnose (Merck, Germany). For column chromatography (CC), Silica gel (70-230 mesh) (Merck), Sephadex LH-20 (Sigma-Aldrich/ Germany) and polyamide 6S (Sigma) were used. Methanol, n-BuOH, methylene chloride, acetone, ethyl acetate, acetic acid and petroleum ether purchased from El-Nasr Pharmaceutical Chemicals Co. Adwic; Egypt. The absorbance measurements for antioxidant activity were registered using the UV-V is spectrophotometer Spectronic 601 (Milton Roy, USA).

Solvent systems

(S1) [$CHCl_3$:MeOH, 8.5:1.5]; (S2) [AcOH:H_2O (15% AcOH), 15:85]; (S3) [n-BuOH:AcOH:H_2O (BAW), 4:1:5 (upper phase)] and (S4) [$CHCl_3$:Me_2CO: MeOH:H_2O, 3:3:2:1].

Antioxidant activity

DPPH free radical scavenging activity

The scavenging activity of DPPH free radical was dictated by Marwah *et al.*, 2007 strategy. In brief, the reaction medium contained 2 ml of 100 μM DPPH purple solution in methanol and 2 ml of compound. The mixture was brooded in the dark for 20 minutes and the absorbance was recorded at 517 nm, examine was completed in triplicate. The decrease in absorbance on addition of tested compound was utilized to calculate the antiradical activity, as expressed by the inhibition percentage (%IP) of DPPH radical, according to the following equation:

$$\%IP = [A_c\text{-}A_s]/A_c \times 100$$

Where A_c and A_s are the absorbencies of the control and of the test sample, respectively, after 20 minutes. From a plot of concentration against %IP, a linear regression analysis was performed to determine the inhibitory concentration (compound concentration resulting in 50% inhibition) record for each compound. The scavenging reaction between ($DPPH^{\bullet}$) and an antioxidant (A-H) is as follows:

$$(DPPH^{\bullet}) + (A\text{-}H) \rightarrow DPPH\text{-}H + (A\bullet)$$

Antioxidants react with purple-colored $DPPH^{\bullet}$ radical, and is reduced to the yellow-colored DPPH-H, and consequently, the absorbances decreased from the $DPPH^{\bullet}$ radical to the DPPH-H form. The degree of discoloration marked the scavenging power of the antioxidant compounds according to its hydrogen donating capability [14].

Assessment of total antioxidant capacity (TAC)

Antioxidant activity of each (pure compound) was determined according to phosphomolybdenum assay [15] using a standard ascorbic acid. According to this assay, the reduction of Mo(VI) to Mo(V) occurs by the sample analyte followed by formation of a green colored [phosphate = Mo (V)] complex at acidic pH. In dried test tubes, 0.5 ml of each compound (100 μg/ml) in methanol was reacted with 5 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Capped test tubes containing the reaction mixture and incubated in a thermal block at 95°C for 90 minutes. The samples were cooled at room temperature, and then, the absorbance was measured at 695 nm against a blank. The blank composed of all reagents and solvents without the sample and it was incubated under the same conditions. All experiments here were carried out in triplicate. The antioxidant activity of the sample was expressed as the number of equivalents of ascorbic acid (AAE).

Antimicrobial activity

To evaluate the antimicrobial activity, disc agar plate method was used according to the reported method [16].

Complete acid hydrolysis for compounds 1, 2, 7, and 8

The compound (4 mg) was hydrolyzed with aqueous methanol of 10% HCl (3.5 ml) at 100°C for 2 hrs; after the separation of the solvent, hydrolysate was exhaustively extracted by ethyl acetate using a separating funnel. Aglycone was determined using Co-PC with authentic sample. The aqueous phase was neutralized using 5% sodium bicarbonate and used for investigation of the sugar moieties by Co-TLC with authentic sugar markers in solvent system (S4) [17,18].

Statistical analysis

Data were presented as mean ± standard deviation of triplicates (n=3) according to Annegowda *et al*., 2010 using SPSS 13.0 program (SPSS Inc. USA) [19].

Volatile oil separation

Fresh leaves of *S. jambos* (850 g) were cut into small pieces and enforced to hydro distillation using Clevenger apparatus for 3 hrs [20], after the mixture had reached boiling point (90°C). The obtained light yellow oil was separated and the yield was determined. It was stored in a freezer in air-tight container at −20°C until analyzed. The obtained oil chemical composition was qualitatively through GC/MS by comparison between their retention times and mass spectral fragmentation patterns within the previously announced data [21].

GC/MS analysis

The injector and MS transfer line temperature was adjusted at 280°C. The oven temperature was programmed initially at 40°C (for 3 minutes) to 280°C final at an increasing rate of 5°C/minutes (for 5 minutes). The components were determined using a percent relative peak area. A temporal identification of the compounds was completed based on the comparison of their relative retention time and mass spectral data with those of the NIST, WILLY Library data of the GC/MS system [21].

Extraction and fractionation

S. jambos dried powder leaves (2.5 Kg) were extracted by soaking in 85% methanol solvent at room temperature 25±2°C, and then repeated several times until the extraction process completed (3 l × 5 times). Using rotatory evaporator to concentrate the obtained extract at 40°C, to give 85% methanol extract as a dark brown residue (620 g). Then, 570 g methanol extract was defatted using petroleum ether (60-80°C) (3 l) this means; remove fats and sterols, to afford an oily extract (44.02 g). The defatted 85% methanol (480 g) was dissolved in distilled water and then successively fractionated with methylene chloride (3 l), ethyl acetate (2.5 l) and n-BuOH (3 l), to yielded methylene chloride extract (6.18 g), ethyl acetate extract (15.56 g), n-BuOH extract (77.1 g) and aqueous extract (90.50 g). The resulting fractions were compared chromatographically using 2D-PC using solvent systems (S_2 and S_3), which guide us to select the n-BuOH fraction for further chromatographic isolation of compounds.

Chromatographic isolation of the n-BuOH fraction

About 45 g of the n-BuOH extract were subjected to chromatographic isolation using polyamide CC (100×6 cm, 250 g). A gradient elution was applied firstly with 5% MeOH/H_2O and the polarity was gradually increased by methanol to pure MeOH at the elution end. Fractions

Table 1: ^{1}H-NMR spectral data of compounds 2-4 (300 MHz, DMSO-d_6; TMS as internal standard, d in ppm and J in Hz)

Compound 2		Compound 3		Compound 4	
Position	**δ_H (ppm)**	**Position**	**δ_H (ppm)**	**Position**	**δ_H (ppm)**
H-2	7.48, d, J=1.2 Hz	6-CH_3 protons	18H, 0.84-1.13, s	H-3	1H, 6.65, s
H-5	7.08, d, J=8.6 Hz	H-3	1H, 4.25, dd, J=11.5, 4.0 Hz	H-8	1H, 6.21, s
H-6	7.68, dd, J=8.6, 2.0 Hz	H-19	1H, 5.20, s	H-2',6'	2H, 7.69
H-7	3.87, 2H, brd, J=7.2 Hz	H-2'	1H, 8.0, br s	H-3',5'	2H, 6.83
H-8	4.13, t, J=7.2 Hz	H-5'	1H, 7.27	OH-5	1H, 11.13, br s
H-10	1.93, 3H, s	H-6'	1H, 7.27	OH-4'	1H, 10.87, br s
H-11	1.69, 3H, s	H-7'	1H, 8.05, d, J=16.0 Hz	-CH_3-6	3H, 1.92, s
H-1'	4.35, 1H, d, J=7.5 Hz	H-8'	1H, 6.65, d, J=16.0 Hz	-OCH_3-7	3H, 3.91, s

(250 ml each) were collected, concentrated and examined using TLC [S1], PC, [S_2 and S_3], 5% $AlCl_3$, 1% $FeCl_3$, and UV light for detection. The same fractions were collected according to their pattern on PC, to give 6 main fractions (1-6) suitable for the flavonoids and phenolic category.

Fraction 1 (3 g) eluted with 10% MeOH, which was then rechromatographed on Sephadex LH-20 (CC) to give compound 1 (15 mg) which eluted with H_2O:MeOH (40:60/v: v) and compound 2 (10 mg) eluted with H_2O:MeOH (20:80/v: v). Fraction 2 (1 g) eluted with 20% MeOH, then was rechromatographed over silica gel (CC) eluted with MeOH:CH_2Cl_2 (25:75/v: v) to afford compound 3 (12 mg). Fraction 3 (0.700 g) eluted with 30% MeOH, which was then rechromatographed over Sephadex LH-20 (CC) to produce compound 4 (13 mg) eluted with H_2O:MeOH (20:80/v: v). Fraction 4 (0.500 g) eluted through 40% MeOH, which was then rechromatographed on Sephadex LH-20 (CC) to afford compound 5 (9 mg) eluted through H_2O:MeOH (15:85/v: v), and compound 6 (11 mg) eluted through H_2O:MeOH (10:90/v: v). Fraction 5 (1 g) eluted with 50% MeOH, which was then rechromatographed on sephadex LH-20 (CC) to give compound 7 (16 mg) eluted via H_2O:MeOH (60:40/v: v).

Fraction 6 (1.5 g) eluted with 60% MeOH, which was then rechromatographed on Sephadex LH-20 (CC) to produce compound 8 (14 mg) eluted using H_2O: MeOH (30:70/v: v).

RESULTS AND DISCUSSION

Compound 1 was obtained as a yellow powder, m.p. 208-209°C, R_f 0.55 in (S3) solvent system. It gave dark purple-colored spot under long UV-light, converted to yellow color on using $AlCl_3$ as spraying reagent; it gave positive Molisch's test proved its glycosidic nature. The complete acid hydrolysis afforded D-glucose and L-rhamnose in the aqueous phase which detected by Co-TLC with authentic sugar samples and quercetin in organic phase; therefore, compound 1 was identified as quercetin-3-O-rutinoside (Fig. 1) [22,23].

Compound 2 was obtained as a white amorphous powder, m.p. 192°C, R_f 0.60 in (S1) solvent system. It gave fluorescent blue-colored spot under long UV-light. Its ^{1}H-NMR spectra (Table 1) showed the presence of three protons located in the aromatic region at δ_H 7.48 (1H, d, J=1.2 Hz, H-2), 7.08 (1H, d, J=8.6 Hz, H-5), 7.68 (1H, dd, J=8.6, 2.0 Hz, H-6), and a set of aliphatic protons including; 2 methylene protons at δ_H 3.87 (2H, br d, J=7.2 Hz, H-7), olefinic proton at δ_H 4.13 (1H, t, J=7.2 Hz, H-8), 6 methyl protons at δ_H 1.93 (3H, s, H-10), 1.69 (3H, s, H-11), and an anomeric proton at δ_H 4.35 ppm corresponding to glucosyl moiety. The complete acid hydrolysis afforded D-glucose in the aqueous phase which detected by Co-TLC with authentic sugar markers; therefore, compound 2 was identified as prenylbenzoic acid 4-β-D-glucoside (Fig. 1) [24].

Compound 3 was obtained as a white powder, m.p. 296°C, R_f 0.48 (S1) solvent system, it showed rose pink color after spraying with sulfuric acid/methanol reagent (40:60, v/v). Its ^{1}H-NMR spectra revealed the existence of a set of aliphatic protons with a characteristic resonances at δ_H 0.84-1.13 (6 CH_3, each 3H, s), 4.25 (1H, dd, J=11.5, 4.0 Hz, H-3), 5.20 (1H, s, H-19), as well as another set of aromatic resonances assignable to the caffeoyl moiety at δ_H 8.0 (1H, br s, H-2'), 7.27 (1H, overlap, H-5'), 7.27 (1H, overlap, H-6'), 8.05 (1H, d, J=16.0 Hz, H-7'), and 6.65 (1H, d, J=16.0 Hz, H-8') (Table 1); therefore, compound 3 was identified as morolic acid 3-O-caffeate (Fig. 1) [25].

Table 2: ^{1}H-NMR spectral data of compounds 7 and 8 (300 MHz, DMSO-d_6; TMS as internal standard, δ in ppm and J in Hz)

Compound 7		Compound 8	
Position	**δ_H (ppm)**	**Position**	**δ_H (ppm)**
H-3	1H, 6.90, s	H-2'	1H, 7.44, d, J=2.0 Hz
H-6	1H, 6.55, d, J=1.9 Hz	H-5'	1H, 6.93, d, J=3.0 Hz
H-8	1H, 6.62, d, J=1.9 Hz	H-6'	1H, 6.11, dd, J=3.0, 2.0, Hz
H-3'	1H, 6.86, s	H-2'',6''	2H, 6.64, s
H-6'	1H, 7.22, s	H-3'	3H, 3.62, s
OH-5	1H, 14.40, br s	H-3'',5''	6H, 3.70, s
Glc-H-1''	1H, 4.73, d, J=7.8 Hz	H-1	1H, 4.41, s
		Rest of glucose protons	3.48-4.02

Compound 4 was obtained as a dark yellow powder, m.p. 136°C, R_f 0.72 in (S3) solvent system, it showed a dark purple-colored spot under UV light. The ^{1}H-NMR spectra revealed the existence of a set of protons located in the aromatic region with a characteristic signals appeared at δ_H 6.21 (1H, s, H-8), 6.65 (1H, s, H-3), a characteristic AA'BB' spin system with four aromatic protons in the B-ring appeared at δ_H 7.69 (2H, H-2',6'), and 6.83 (2H, H-3',5'), three downfield aliphatic protons corresponding to one methoxy group 3.91 (3H, s, OCH_3), and another three upfield aliphatic protons corresponding to one methyl group 1.92 ppm (3H, s, CH_3), downfield signals corresponding to the presence of hydroxyl proton with intera molecular hydrogen bond at δ_H 11.13 (br s, OH-5), and 10.87 (br s, OH-4') (Table 1); therefore, compound 4 was identified as 5,4'-dihydroxy, 7-methoxy, 6-methyl-flavone (Fig. 1) [26].

Compound 5 was obtained as a white powder; m.p. 260°C, R_f 0.56 (S2) solvent system, it showed a violet-colored spot under UV light. Furthermore, it showed a positive result with $FeCl_3$ and positive acidity test with $NaHCO_3$ mentioned the presence of phenolic and carboxylic groups [17]. Based on the Co-PC with the standard authentic sample, compound 5 was identified as 3,4,5-trihydroxybenzoic acid (gallic acid) (Fig. 1) [22].

Compound 6 was obtained as a yellow powder, m.p. 315°C, R_f 0.07 in (S2) solvent system, it showed a yellow-colored spot under long UV light converted to green with $FeCl_3$ and not changed with $AlCl_3$ [17]. Based on the Co-PC with the standard authentic sample, compound 6 was identified as quercetin [22].

Compound 7 was obtained as a pale brown, m.p. 310-312°C, R_f 0.37 in (S3) solvent system, showed a dark purple-colored spot under long UV light. Its ^{1}H-NMR spectra (Table 2) revealed the existence of a set of protons located in the aromatic region with a characteristic signals appeared at δ_H 6.90 (1H, s, H-3) in C-ring, two meta coupled protons at

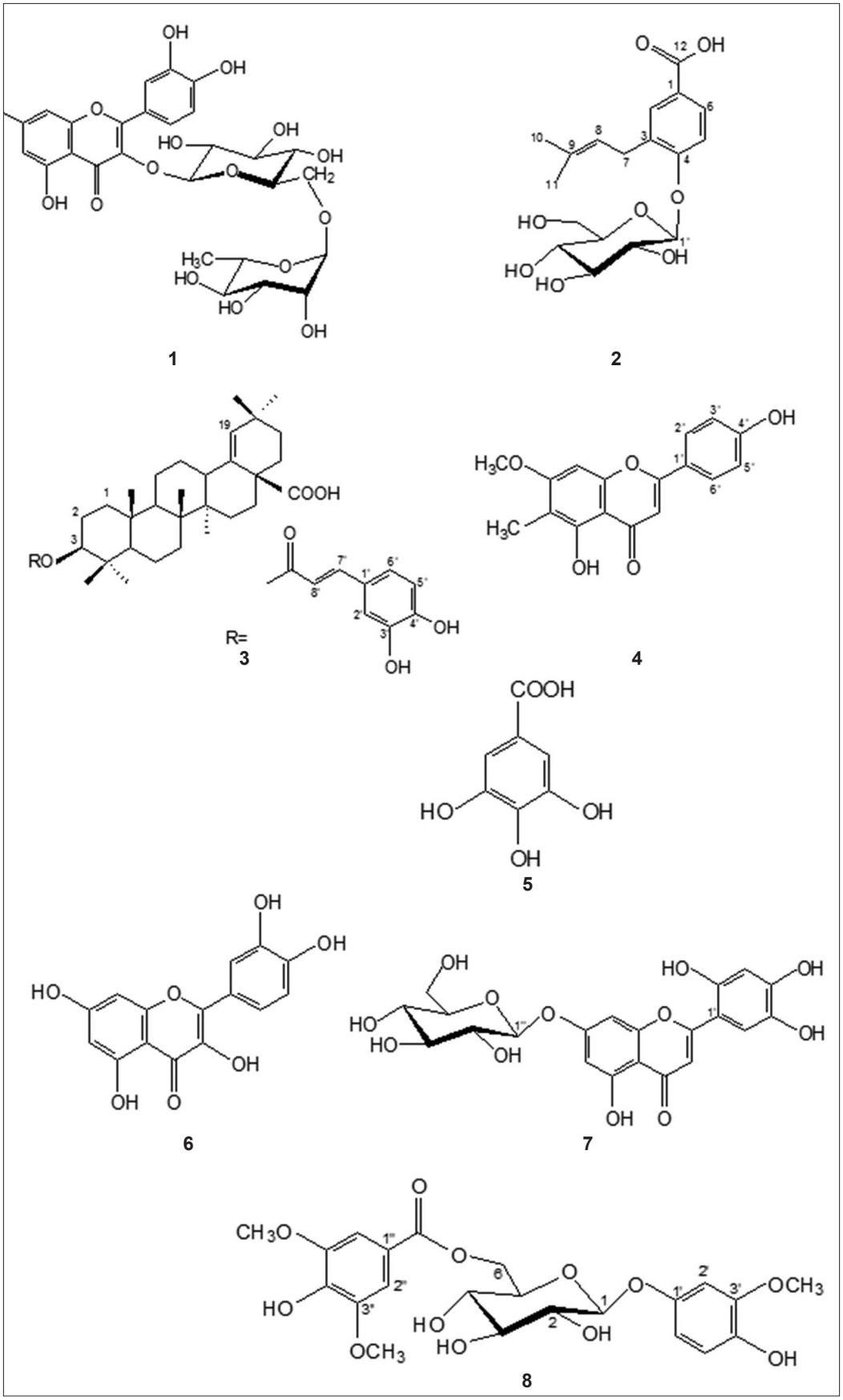

Fig. 1: Chemical structure of isolated compounds 1-8

δ_H 6.55 (1H, d, J=1.9 Hz, H-6) and 6.62 (1H, d, J=1.9 Hz, H-8) in A-ring, as well as two protons in B-ring at δ_H 6.86 (1H, s, H-3') and 7.22 (1H, s, H-6'), most downfield hydroxyl proton at δ_H 14.40 (5-OH) due to the effect of the intermolecular hydrogen bond, in addition anomeric

Table 3: Chemical constituents identified in the *S. jambos* leaves essential oil using GC/MS

Peak No.	R_t	Area %	M.F.	Identified compounds
1	12.78	0.14	$C_{15}H_{24}$	α-Ylangene
2	12.94	3.73	$C_{12}H_{18}$	Perilla acetate
3	13.08	3.59	$C_{15}H_{24}$	α-Longipinene
4	13.34	2.45	$C_{15}H_{24}$	α-Selinene
5	13.39	0.20	$C_{10}H_{12}O$	2-Adamantanol
6	13.65	10.75	$C10H_{12}O$	Cumaldehyde
7	13.71	6.39	$C_{15}H_{24}$	Isocaryophyllene
8	13.89	6.40	$C_{15}H_{24}$	β-Himachalene
9	13.95	0.56	$C_{15}H_{24}$	Copaene
10	14.12	5.41	$C_{15}H_{24}$	Alloaromadendrene
11	14.21	0.21	$C_{10}H_{14}$	Bicyclo[3,2,2]oct-2-ene, 3-methyl-4-methylene
12	14.32	10.85	$C_{15}H_{24}$	δ-Cadinene
13	14.36	3.14	$C_{13}H_{20}O_2$	Citroviol
14	14.44	4.84	$C_{15}H_{24}$	Ledene
15	14.62	5.63	$C_{15}H_{24}$	β-Cedrene
16	14.87	5.27	$C_{15}H_{24}$	γ-Gurjunene
17	15.04	4.84	$C_{15}H_{24}$	α-Guaiene
18	15.12	4.25	$C_{15}H_{24}$	Valencene
19	15.29	5.64	$C_{15}H_{24}$	Naphthalene, 1,2,3,4,6,8a-hexahydro-1-isopropyl-4,7-dimethyl
20	15.54	4.03	$C_{15}H_{24}$	α-Cubebene
21	15.62	0.41	$C_{15}H_{24}$	β-Chamigrene
22	15.79	0.98	$C_{10}H_{14}O_3$	5-Sec-butylpyrogallol
23	15.85	1.68	$C_{10}H_{16}O$	Camphor
24	16.06	0.26	$C_{11}H_{17}N_3O$	p-Mentha-6,8-dien-2-one, semicarbazone
Total %		92%		

S. jambos: Syzygium jambos, GC/MS: Gas chromatography-mass spectrometry, The determination of the components was based on comparison of their mass spectral fragmentations pattern with those of the data described in Wiley and NIST Libraries and those by reported [21]. M.F.: Molecular formula; R_t: Retention time

proton at 4.73 ppm (Glc-H-1, J=7.8 Hz) assignable to the sugar moiety. The complete acid hydrolysis afforded D-glucose in the aqueous portion which detected by Co-TLC with authentic sugar markers; accordingly, compound 7 was characterized as isoetin-7-O-β-D-glucopyranoside (Fig. 1) [27,28].

Compound 8 was obtained as a pale yellow powder, R_f 0.49 in (S1) solvent system, showed fluorescent green-colored spot under long UV light. Its ^{1}H-NMR spectra (Table 2) revealed the existence of a set of four aromatic protons appeared at δ_H 7.44 (1H, d, J=2.0 Hz, H-2'), 6.93 (1H, d, J=3.0 Hz, H-5'), 6.11 (1H, dd, J=3.0, 2.0 Hz, H-6'), 6.64 (2H, s, H-2'', 6''), 9 methoxy protons at δ_H 3.70 (6H, s, H-3'', 5'' OMe), 3.62 (3H, s, H-3' OMe), and anomeric proton at 4.41, and the rest of glucose protons were appeared from 3.48 to 4.02 ppm. Therefore, compound 8 was identified as (4'-hydroxy-3'-methoxyphenol-β-D-[6-O-(4''-hydroxy-3'', 5''-dimethoxylbenzoate)]-glucopyranoside) (Fig. 1) [29].

Identification of the essential oil constituents

The GC/MS analysis of *S. jambos* leaves essential oil revealed the presence of 24 identified components representing 92% of the total oil composition, which were identified based on their retention times and mass spectral fragmentation patterns into δ-cadinene (10.85%), cumaldehyde (10.75%), β-himachalene (6.40%), isocaryophyllene (6.39%), and β-cedrene (5.63%) as the major components, most of them belonging to sesquiterpenes (Table 3). Rezende *et al*. revealed that the essential oil from *S. jambos* leaves growing in Brazil contains 62 compounds, i.e., (E)-caryophyllene, α-humulene, α-zingibirene, hydroxytoluene butylated, caryophyllene alcohol, caryolan-8-ol, caryophyllene oxide, thujopsan-2-α-ol, and *n*-heneicosane were the major components [30].

***In vitro* antioxidant activities of the isolated compounds**

The *in vitro* antioxidant activities of the isolated compounds were evaluated using two antioxidant assays, namely, DPPH free radical scavenging (SC_{50} values) and phosphomolybdenum (TAC) assays. The results of the free radical scavenging activity revealed that the SC_{50} values of the tested compounds were 10.25, 17.75, 6.55, 14.0, 5.50, 4.30, 10.74, and 11.92 µg/ml, respectively, for the compounds 1-8 compared to ascorbic acid as standard with SC_{50} of 7.60 µg/ml (Table 4).

Table 4: *In vitro* antioxidant activities of the isolated compounds 1-8

Compound	DPPH free radical scavenging activity SC_{50} (µg/ml)[1]	Total antioxidant capacity (mg ascorbic acid equivalent AAE/g compound)[2]
1	10.25±1.28	545.0±1.23
2	17.75± 0.65	320.44±1.53
3	6.55±1.24	590.0±1.63
4	14.0±0.54	388.0±1.37
5	5.50±1.42	605.0±2.15
6	4.30±1.85	680.59±1.30
7	10.74±0.48	500.0±1.89
8	11.92±0.89	474.55±1.31
Ascorbic acid	7.60±0.45	-

AAE: Ascorbic acid equivalent, [1]SC_{50}: Concentration from sample required for scavenging of 50% of radical, [2]TAC was evaluated by the phosphomolybdenum assay in mg ascorbic acid equivalent AAE/g compound, TAC: Total antioxidant capacity, DPPH: 2,2'-diphenyl-1-picrylhydrazyl

Furthermore, the TAC values of the tested compounds were 545.0, 320.44, 590.0, 388.0, 605.0, 680.59, 500.0, and 474.55 mg AAE/g compound, respectively, for the compounds 1-8 (Table 4). From the structural-activity relationship point of view, the tested compound showed a wide range of antioxidant activities with large diversity in each of SC_{50} and TAC values, this phenomena may be ascribed to the presence or absence of the characteristic structural criteria for effective free radical scavenging activity including; the presence of 5-OH (A-ring) free, ortho-dihydroxy groups, a 2, 3 unsaturated double bond, and 4-oxo group (ring-C); that is in accordance with the high scavenging activity of compound 6 in compared to the other flavonoid isolates. Moreover, mono- and di-glycosides were less active than their aglycones which may be due to steric hindrance given by a bulky glycosidic moiety [31,32]; that is in agreement with the less activity of compounds 1, 2, 7 and 8. Also the presence of three adjacent phenolic hydroxyl groups at the phenolic acids increases the free radical scavenging activity as shown in compound 5.

Concerning to compound 3, it has a cinnamoeyl moiety; that is responsible for its high antioxidant activity.

Table 5: *In vitro* antimicrobial activities of the isolated compounds 1-8

Compound	Clear inhibition zone (fmm)			
	Pseudomonas aeruginosa	*Staphylococcus aureus*	MRSA	*Candida albicans*
1	17.5	10.5	11.5	14.5
2	11.5	16	13	12
3	18	17	18	19
4	13	9	12	13
5	11.5	12.5	10.5	12.5
6	16	15	17	14
7	19	17	15	9
8	15	18	19	18

The results of compounds (1-8) against *Staphylococcus aureus* (Gram-positive bacteria); methicillin-resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa* (Gram-negative bacteria) and *Candida albicans* (yeast)

***In vitro* antimicrobial activities of the isolated compounds**

The compounds isolated were tested for their antimicrobial activity against four pathogenic microbial strains including Gram-positive, Gram-negative bacteria and yeast which are *Pseudomonas aeruginosa* with inhibition zones (17.5, 11.5, 18.0, 13.0, 11.5, 16.0, 19.0, and 15.0 mm, respectively, for the compounds from 1-8), *Staphylococcus aureus* with inhibition zones (10.5, 16.0, 17.0, 9.0, 12.5, 15.0, 17.0, and 18.0 mm, respectively, for the compounds from 1-8), Methicillin-resistant *Staphylococcus aureus* with inhibition zones (11.5, 13.0, 18.0, 12.0, 10.5, 17.0, 15.0, and 19.0 mm, respectively, for the compounds from 1 to 8), and *Candida albicans* with inhibition zones (14.5, 12.0, 19.0, 13.0, 12.5, 14.0, 9.0, and 18.0 mm, respectively, for the compounds from 1 to 8) (Table 5). Previous reported on the antimicrobial activity of major groups from plants, for example, simple phenols, polyphenols, phenolic acids, quinones, flavonoids, flavones, flavonols, coumarins, tannins, terpenoids, and alkaloids [16,33].

CONCLUSION

Medicinal plants used in folk medicine are of interesting vision so it is necessary to isolate and identify the active principals and characterized their activities for the advantageous of the human being. Our study provides a beneficial insight to research about the application of potential antioxidant and antimicrobial metabolites from *S. jambos*. Accordingly, the bioactivities showed by the isolates; compounds 5 and 6 could serve as a potential source for antioxidant and antimicrobial agents. The presence of phenolics and flavonoids in *S. jambos* which are drown from our results is responsible for realizing the antioxidative stress that reflects the causative factors of various diseases.

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