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Chemical Constituents, Antischistosomal and Antioxidant Activities of the Methanolic Extract of *Azadirachta indica*

M.M. El-Sayed, M.A. Mahmoud[*], Hanan, A. El-Nahas, S.A. El-Toumy[**], Eman, A. El-Wakil and M.A. Ghareeb[#]

Laboratory of Medicinal Chemistry, Theodor Bilharz Research Institute, Giza, [*]Department of Chemistry, Faculty of Science, Ain-Shams University, Cairo, and[**]Chemistry of Tannins Department, National Research Center, Giza, Egypt.

THE PRESENT study was performed to evaluate the *in vitro* schistosomicidal effect of 70% methanolic extract of the leaves of *Azadirachta indica* and its derived ethyl acetate and n-butanol fractions against adult *Schistosoma mansoni* worms. Also, antioxidant properties of these extracts were determined. Owing to the high antischistosomal and antioxidant activities of EtOAc and n-BuOH fractions, they were subjected to chromatographic isolation using different chromatographic techniques. Two flavonoid compounds; (1,2) were isolated from EtOAc fraction whereas one flavonoid; (3) quercetin-3-*O*-α-L-rhamnopyranoside and two bidesmosidic saponins; (4,5) were isolated from the butanolic fraction. Their structures were established using spectroscopic methods. Compounds 1-3 showed high antioxidant activity whereas compounds 4 and 5 were inactive. It is the first time that antischistosomal activity of *A.indica* and isolation of compounds 2- 5 from this plant were carried out.

Keywords: *Azadirachta indica*, DPPH scavenging activity, Antischistosomal, Flavonoids and Triterpenoid saponins.

Human schistosomiasis caused by trematode flatworms of the genus *Schistosoma* is considered one of the most common tropical diseases in the world .It affects the liver and intestine causing granuloma formation, hepatic fibrosis and causes certain necrotic changes in liver tissues[1,2]. Praziquantel (PQZ) is the only drug currently effective against all species of schistosome, however praziquantel does not prevent re-infections and it is expensive. Moreover, the reliance on one antischistosomal drug is alarming, therefore the scientific community has called for research and development of novel and inexpensive drugs against schistosomiasis[3,4]. In this respect, compounds from natural sources mainly plants, which continue to be a major source of biologically active metabolites may lead to the development of new drugs[5-7]. Plants are still a large source of natural antioxidants that might serve for the development of novel drugs[8]. Several studies showed that the antiproliferative, anti-inflammatory, antischistosomal

[#]Corresponding author. Tel.: +2 018 65 44 906; Fax: +2 02 35 40 8125
mosad_tbri@hotmail.com Email: (M. Ghareeb)

activities and liver protection properties of plant extracts are attributed to their abilities as antioxidant agents[9-11].

Azadirachta indica, commonly known as neem, has attracted worldwide prominence in recent years owing to its wide range of medicinal properties. All parts of the neem tree have been used traditionally for treatment of inflammation, infection, fever and skin diseases[12]. In our previous studies, it appeared that the methanolic extract of *A.indica* leaves has antioxidant properties[13]. These results promoted us to evaluate the plant extracts as antischistosomal agents and isolate certain chemical constitutes of the active extracts.

Materials and Methods

General

Melting points were determined on an electrothermal apparatus and were uncorrected. ^{1}H-NMR (300 and 500 MHZ, DMSO-d_6) and ^{13}C-NMR (75 and 125 MHZ, DMSO-d_6) spectra were recorded on a varian Mecauy 300 or 500 JEOL GX-Spectrometer. The chemical shifts in δ were referenced to using TMS as internal standard. UV spectra (max) were determined in methanol after addition of different reagents on a UV-601 UV-VIS recording spectrophotometer. ESI-MS was performed on a Micromass Q-TOF Micro-instrument. Silica gel (70-230 mesh, Merck) and Sephadex LH-20 (25-100 µm, Sigma) were used for column chromatography. Thin-layer chromatography and preparative TLC were performed on silica gel GF_{254} precoated plates (Merck). Paper chromatography was carried out on Whatmann No.1 or No. 3 paper sheets (Whatmann, England). Spots were visualized by absorption of UV radiation and spraying with ethanolic $AlCl_3$ (2%) or 10% H_2SO_4 for flavonoids and glycosides whereas aniline phthalate for sugars.

Plant materials

Leaves of *Azadirachta indica* A. juss (Meliaceae) were collected from Faculty of Agriculture, Cairo University, Giza, Egypt. The plant was kindly identified by Prof.Dr. Wafaa Amer, Professor of Plant Taxonomy, Faculty of Science, Cairo University, Giza, Egypt. The fresh leaves of the plant were dried in shade place at room temperature and then powdered by electric mill.

Extraction and fractionation

The air-dried powdered leaves of *A.indica* (2.4 kg) were extracted with 70% methanol at room temperature for several times. The methanolic extract was concentrated under reduced pressure using rotatory evaporator to yield 320 g. The methanolic extract was defatted with petroleum ether (60-80 ^{0}C) and the defatted material was diluted with distilled water and partitioned with different organic solvents such as $CHCl_3$, EtOAc and n-BuOH. The obtained fractions were concentrated under vacuum using rotatory evaporator to give 10, 30 and 40 g, respectively.

Chromatographic isolation of EtOAc fraction

The ethyl acetate soluble fraction (25 g) was subjected to silica gel column chromatography. Elution was started with $CHCl_3$ followed by gradient mixtures of $CHCl_3$: MeOH and ending with pure methanol. Two major fractions A and B

were obtained by elution with $CHCl_3$: MeOH; 85:15 and 60:40 respectively. The two fractions exhibited DPPH radical-scavenging activity (SC_{50} = 13.57 and 21.86 µg/ml). Fraction A was rechromatographed over silica gel column chromatography using $CHCl_3$, gradient mixtures of $CHCl_3$: MeOH and pure MeOH as eluents. Elution with $CHCl_3$: MeOH; 95:5 gave compound 1. Fraction B was submitted to other silica gel column eluted with $CHCl_3$, mixture of $CHCl_3$: MeOH. Elution with $CHCl_3$: MeOH; 75:25 gave impure compound 2 which was further purified by sephadex LH-20 column using methanol as eluent to give pure compound 2.

Chromatographic isolation of n-BuOH fraction

The butanolic soluble fraction (25 g) was applied to silica gel column chromatography and eluted with $CHCl_3$, gradient mixtures of $CHCl_3$:MeOH and ending with MeOH. Elution with $CHCl_3$:MeOH; 85:15; 65:35 gave two major fractions I and II. Fraction I showed high DPPH -scavenging activity SC_{50} = 17.45 µg/ml whereas fraction II was less active (SC_{50} =56.79 µg/ml). Fraction I was applied to preparative TLC using solvent system $CHCl_3$: MeOH; 4:1 to yield compound 3 which was further purified by Sephadex LH-20 column and methanol as eluent. Fraction II was applied to the silica gel column chromatography eluted with $CHCl_3$, gradient mixture of $CHCl_3$: MeOH and finally with pure methanol. Elution with $CHCl_3$: MeOH; 90:10 and 85:15 gave two impure compounds 4 and 5. Each compound was separately submitted to preparative TLC using $CHCl_3$: MeOH: H_2O; 7:3:1 and 7:3:2 as eluent to give two pure compounds 4 and 5, respectively.

Compound 1, m.p. (276-278 °C), R_f 0.71 [TLC, $CHCl_3$: MeOH; 95:5] and 0.79 [n-BuOH: AcOH: H_2O; 4:1:5]. UV_{max} nm (MeOH); 267, 293^{sh}, 367; (NaOMe) 272, 300^{sh}, 418; ($AlCl_3$) 274, 345^{sh}, 430; ($AlCl_3$+HCl) 271, 350^{sh}, 420; (NaOAc) 275, 318^{sh}, 395; (NaOAc+H_3BO_3) 267, 315^{sh}, 370.

Compound 2, m.p. (189-191 °C), R_f 0.55 [15% AcOH] and 0.48 [BAW]. UV_{max} nm (MeOH); 267, 326^{sh}, 358; (NaOMe) 275, 347^{sh}, 410; ($AlCl_3$) 273, 330^{sh}, 426; ($AlCl_3$+HCl), 302^{sh}, 401; (NaOAc) 274, 313^{sh}, 393; (NaOAc+H_3BO_3) 263, 308^{sh}, 375. ^{1}H-NMR (DMSO-d_6) δ: 12.55 (1H, s, 5-OH), 7.99 (2H, d, J=8.90 Hz, H-2',H-6'), 6.90 (2H, d, J=8.85Hz, H-3', H-5'), 6.43 (1H, d, J=2.0 Hz, H-8) 5.32 (1H, d, J=7.5 Hz, H-1", Glc), 4.39 (1H, d, J=1.25 Hz, H-1", Rha), 1.03 (3H, d, J=6.07 Hz, Rha-6"). ^{13}C-NMR (Table 1).

Compound 3, m.p. (192-194 °C), R_f 0.53 (15% AcOH). UV_{max} nm (MeOH) 256, 264,^{sh}, 358; (NaOMe) 271, 351^{sh}, 405; ($AlCl_3$) 274, 334^{sh}, 429; ($AlCl_3$) 274, 334^{sh}, 429; ($AlCl_3$+HCl) 271, 357^{sh}, 401; (NaOAc) 273, 324^{sh}, 390; (NaOAc+H_3BO_3) 260, 299, 385. ^{1}H-NMR (DMSO-d_6) δ: 7.28 (1H, d, J=2.1 Hz, H-2'), 7.23 (1H, dd, J=8.1 and 2.1 Hz, H-6'), 6.86 (1H, d, J=8.1 Hz, H-5'), 6.33 (1H, d, J=2.1 Hz, H-8), 6.15 (1H, d, J=2.1 Hz, H-6), 5.25 (1H, J=1.41 Hz, H-1"). ^{13}C-NMR (Table 1).

Compound 4, m.p. (205-207 °C), R_f 0.31 [TLC; $CHCl_3$: MeOH: H_2O; 7:3:1]. IR (KBr ν_{max}) cm^{-1} 3423, 2928, 1736, 1636, 1455, 1075, 820, 622. ^{1}H-NMR

(DMSO-d_6) δ: 0.78-1.18 (7 Me groups), 4.47 (1H, d, J=7.5 Hz), 0.33 (1H, d, J=7.5 HZ). ^{13}C-NMR (Table 1).

Compound 5, m.p. (220-222 °C), R_f 0.29 [TLC; $CHCl_3$: MeOH: H_2O; 7:3:2]. IR (KBr ν_{max}) cm^{-1}; 3427, 1735, 1637, 1075, 622. ^{1}H-NMR (DMSO-d_6) δ:0.84-1.24, 5.18 , 4.37, 4.48 and 5.23.^{13}C-NMR (Table 1).

Acid hydrolysis

Each isolated glycoside (5 mg each) was refluxed with 10% HCl in aqueous methanol for two hours. The reaction solution was concentrated and extracted with ethyl acetate. The ethyl acetate extract was evaporated to dryness. The obtained aglycones were identified by direct comparison with an authentic sample whereas the aqueous layer was neutralized, filtered and concentrated and the sugar moieties were identified with direct comparison with authentic sugars.

DPPH radical scavenging activity

The ability of each extract or compound to scavenge DPPH radicals was measured according to the procedure described by Mensor *et al.* [14]. Briefly; 3 ml of each plant extract at a concentration of 100 µg /ml were mixed with 1 ml of 0.1 mm DPPH in methanol. The mixture was then shaken and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as a reference standard. Control was prepared containing the same solvents and reagents without any extract and reference ascorbic acid. All experiments were carried out in triplicate.

The scavenging effect (antioxidant activity) of each extract was expressed and SC_{50} which was defined as the concentration of each extract required for 50% scavenging of DPPH radicals compared with that of ascorbic acid which was used as the standard. The lower SC_{50} value corresponds to a higher scavenging activity (higher antioxidant activity) of plant extract.

Determination of total antioxidant capacity

The antioxidant activity of each extract or compound was determined according to phosphomolybdenum method by Prieto *et al.* [15] using ascorbic acid as standard. In this method, 0.3 ml of each extract (100 µg /ml) in methanol was combined in dried vials with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials containing the reaction mixture were capped and incubated in a thermal block at 95 °C min. After the samples had cooled at room temperature, the absorbance was measured at 695 nm against a blank. The blank consisted of all reagents and solvents without the sample and it was incubated under the same conditions. All experiments were carried out in triplicate. The antioxidant activity of the extracts or compounds was expressed as the number of equivalents of ascorbic acid (AAE).

TABLE 1. ^{13}C-NMR (DMSO-d_6) of pure compounds (2-5) isolated from *Azadirachta indica* compounds.

Carbon	2	3	Carbon	4	5	Carbon	
1	-	-	1	39.20	39.20	1’’’	94.00
2	156.84	156.17	2	26.50	26.70	2’’’	74.20
3	133.53	133.33	3	88.45	88.65	3’’’	79.10
4	177.75	177.33	4	39.37	39.37	4’’’	71.30
5	161.54	161.22	5	55.48	55.48	5’’’	79.40
6	99.09	98.82	6	18.60	18.64	6’’’	62.40
7	164.53	164.60	7	33.65	33.60		
8	94.11	93.62	8	39.87	40.70		
9	157.20	156.17	9	48.10	47.80		
10	104.34	103.87	10	37.40	37.10		
1’	121.23	121.16	11	23.50	24.20		
2’	131.21	116.22	12	126.40	128.50		
3’	115.46	144.87	13	138.60	139.40		
4’	160.28	148.56	14	42.30	42.20		
5’	115.46	120.98	15	28.50	29.40		
6’	131.21	121.62	16	23.90	26.20		
1’’	101.70	100.97	17	48.10	48.70		
2’’	74.54	73.35	18	53.50	54.50		
3’’	76.11	70.70	19	39.70	72.70		
4’’	72.20	71.82	20	39.53	42.30		
5’’	76.75	69.97	21	30.60	26.80		
6’’	67.25	17.54	22	36.80	37.65		
1’’’	101.12	-	23	28.05	28.30		
2’’’	70.70	-	24	16.90	17.10		
3’’’	70.98	-	25	15.78	15.80		
4’’’	70.30	-	26	17.50	17.50		
5’’’	68.59	-	27	23.70	24.70		
6’’’	18.07	-	28	176.41	176.56		
			29	17.51	27.20		
			30	21.47	16.90		
			1’	103.99	106.00		
			2’	75.15	72.98		
			3’	78.60	84.20		
			4’	71.40	69.50		
			5’	78.89	67.20		
			6’	61.28	-		
			1’’	94.96	104.00		
			2’’	74.16	75.90		
			3’’	78.59	78.50		
			4’’	70.51	71.60		
			5’’	79.01	78.80		

Schistosoma mansoni worms and culture media

Adult *Schistosoma mansoni* worms were freshly obtained from Schistosoma Biological Supply Center (SBSC), Theodor Bilharz Research Institute, Giza, Egypt. The culture media used consists of 4.2 ml RPMI 1640 (Gibco,USA) supplemented with 0.3ml, sterilizing antibiotic (streptomycin 100 mg/ml + penicillin 100 µg/ml) and 0.5 ml fetal calf serum as nourishing agent.

In vitro antischistosomal assay

The *in vitro* antischistosomal activity of the *A.indica* leaves extract or the isolated compounds was performed according to the method described by Jiwahinda *et al*. [16]. Briefly, in polystyrene Petri dish (3.5 x 10 mm), ten adult *Schistosoma mansoni* worms were cultured in 10 ml sterile RPMI-1640 media with graduated extracts from 10 µg/ml to 100 µg/ml at 37 °C for 24 hr .The negative control consisted of 10 adult worms cultured in RPMI-1640 media and 10 % DMSO whereas the positive control consisted of 1 mmol PZQ and incubated for 24 hr at 37 °C. The efficacy of different concentrations of the extracts or compounds was evaluated by observing the motility and viability of the worms using a stereomicroscope after 24 hr of incubation in comparison with negative control .The active extracts were further subjected to determination of their LC_{50} and LC_{90} values based on Litchfield and Wilcoxon method[17].

Statistical analysis

All experiments were run in triplicate and statistical analysis were performed using SPSS software. Analysis of variance was performed by ANOVA procedures. Results were given as means ± standard deviation. Significance level was defined as $P< 0.05$.

Results and Discussion

Schistosomiasis is one of the most tropical diseases and its control is largely dependent on a single drug; praziquantel. In view of the possible development of resistance to this drug, research into alternatives for the prevention and cure of schistosomiasis has become justified[18]. Several studies on *A.indica* showed that the extracts of this plant have a wide range of biological activities that include antiviral, antibacterial, antitumer and antioxidant[19,20]. However, to our knowledge, the schistosomicidal activity of *A.indica* extracts is now being reported for the first time in this study.

The antischistosomal activity of 70% defatted methanolic extract of *A.indica* and its derived $CHCl_3$, EtOAc and n-BuOH fractions was carried out. The results in Table 2 showed that all extracts have activity against *Schistosoma mansoni* worms (LC_{90} for male were 36.74, 98.26, 24.69 and 20.95 whereas for female were 52.49, 112.85, 36.45 and 29.76, respectively). Although, the methanolic extract, ethyl acetate and butanolic fractions exhibited high activity but the butanolic fraction was the highest one. These results are full in agreement with our previous study which revealed that the methanolic extract of *A.indica* and its EtOAc and n-BuOH fractions have high antioxidant properties using two *in vitro* methods; DPPH radical scavenging activity method (SC_{50} = 32, 28.16 and 21.08

µg/ml) and phosphomolybdenum method (231.39, 279.79 and 393.93 Ascorbic acid equivalent /g extract) respectively. Also, the antioxidant activity of *A.indica* extracts are correlated with their phenolic contents[13]. Therefore, in the present study each of the EtOAc and n-BuOH fractions was submitted to isolation using different chromatographic techniques. Structure of the isolated compounds was established using certain spectroscopic analysis such as UV, IR, NMR and MS as well as comparison with authentic specimens. Compounds 1 and 2 were isolated from EtOAc fraction whereas compounds 3-5 have been isolated from n-BuOH fraction. Although, these compounds were previously isolated from other plant species[21,22] but compounds 2-5 are isolated for the first time from *A.indica* in this study.

TABLE 2. *In vitro* effective concentrations of some successive extracts of *A.indica* against *S.mansoni* worms.

Extract	LC_{50} (µg/ml)		LC_{90} (µg/ml)	
	Male worms	Female worms	Male worms	Female worms
70% defatted MeOH	22.56	30.48	36.74	52.49
Chloroform	80.72	92.89	98.26	112.85
EtOAc	12.54	21.18	24.69	36.45
n-butanol	10.82	16.58	20.95	29.76
PZQ (+ve Control) (1 µg/ml)	+++ ve			
Negative control	--ve			

+++ ve : Very active ---ve :Not active

Compound 1 was isolated as a yellow powder. Its UV spectral analysis with usual shift reagents revealed that all the absorption bands were identical to the reported kaempferol[21,22]. Also, compound 1 was identified as kaempferol by comparing its m.p., Co-PC behavior with authentic kaempferol.

Compound 2 was obtained as a yellow amorphous powder. Its UV spectrum in MeOH exhibited the characteristic bands of flavonol glycoside at 267, 326^{sh}, 358. Bathochromic shift (68 nm, Band I) was observed by addition of $AlCl_3$ indicating the presence of a hydroxyl group in position C-3. The Bathochromic shift (52 nm, Band I) on addition of NaOMe confirmed the presence of a free 4'-OH. The Bathochromic shift of 35 nm with NaOAc indicated the presence of free 7-OH group [21,23]. In ^{1}H-NMR; two signals were observed at δ 6.22 and 6.43 ppm assignable to H-6 and H-8 (J= 2.0 Hz) for A-ring of the flavonoid unit, respectively. Two doublets which one of them appeared at δ 6.90 (2H, d, J= 8.85 Hz, H-3', H-5') whereas the other δ at 7.99 (2H, d, J= 8.90 Hz, H-2', H-6') indicated the presence of 4'-hydroxylation of the B-ring. In addition, two anomeric protons were assigned at δ 5.32 (1H, d, J= 7.5 Hz, H-1") and 4.39 (1H, d, J= 1.25 Hz, H-1"') and one methyl protons at δ 1.03 (3H, d, J= 6.07 Hz) ppm of the rhamnose unit[22,24,25]. This was confirmed by presence of characteristic signals of kaempferol aglycone in ^{13}C-NMR as shown in Table 1, where the chemical shift of C-2 was observed downfield at δ 156.84 ppm as compared with

kaempferol which appeared at 146.80 ppm indicating 3-*O*-glycosylation[22,24]. Also, two anomeric carbon signals appeared at δ 101.70 and 101.12 ppm. The glycosylation at C-3 was confirmed by presence of signal at 133.59 ppm and downfield signal of C-2 at 156.84 ppm [25, 24]. The interglycosidic (1→6) linkage was also confirmed by the downfield shift of C-6 of the glucose moiety by δ 67.25 ppm relative to an unsubstituted glucose moiety (C-6 at δ 61.21 ppm)[25,22,24]. The sugar moiety and the aglycone were resulting from acid hydrolysis which identified as kaempferol, L-rhamnose and D-glucose by CO-TLC with authentic samples. ESI-MS spectrum of compound 2 showed a characteristic peaks of $[M+Na]^+$ at m/z 617.14 and $[2M+Na]^+$ at m/z 1237.08. Other peaks appeared at m/z 595.16 $[M+H]^+$, 449.16 $[M+H\text{-}Rha]^+$ and 287.05 $[M+H\text{-}Rha\text{-}Glc]^+$ reflected that one rhamnosyl and one glucosyl units were lost [24]. From the above data compound 2 was identified as kaempferol-3-*O*-α-L-rhamnopyronosyl (1→6) β-D-glucopyranoside.

Compound 3 was isolated as a yellow amorphous powder. UV spectral analysis of the compound in MeOH and with the usual shift reagents showed that the absorption band in methanol at 358 nm (band I) is comparable to 3-hydroxy substituted flavonol. The presence of a free 4'-OH group was confirmed by the bathochromic shift of band I (47 nm) with NaOMe. The 5-OH group was confirmed by the bathochromic shift of band I (43 nm) with $AlCl_3$/HCl. The presence of 7-OH group was indicated by the bathochromic shift (17 nm) with NaOAc (band II). The bathochromic shift (27 nm) band I with NaOAc/H_3BO_3 suggested the presence of *O*-dihydroxy groups in B-ring[25-30]. In ^{1}H-NMR spectrum; the characteristic pattern of quercetin proton resonances appeared at δ 6.15 (1H, d, J= 2.1 Hz) and 6.33 (1H, d, J= 2.1 Hz) ppm, respectively for H-6 and H-8 protons. Also, the signals of H-5', H-6' and H-2' were observed at δ 6.86 (1H, d, J= 8.1 Hz), 7.23(1H, dd, J= 8.1and 2.1 Hz) and 7.28 (1H, d, J= 2.1 Hz) ppm. In addition, the spectrum revealed an anomeric proton resonance as doublet signal at δ 5.25 ppm assignable to the rhamnoside proton H-1". While, the methyl rhamnose proton resonance appeared at δ: 0.82 ppm (d, J= 6.0 Hz)[31,32]. The structure of compound 3 was confirmed by the presence of twenty-one carbon signals in the ^{13}C-NMR spectrum of the compound (Table 1). Fifteen carbon signals were assigned to quercetin moiety and six carbon signals were assigned to rhamnose unit. The anomeric carbon of rhamnose appeared at δ 100.97, whereas the characteristic methyl carbon of rhamnose moiety at C-6" appeared at δ 17.54 ppm. The signal of the C-3 carbon of the flavonol moiety appeared at δ 133.33 ppm and C-2 at 156.17 showing the direct bonding between aglycone (quercetin) and sugar (rhamnose) moiety at the flavonol C-3 position[32,33]. Complete acid hydrolysis of the compound yielded quercetin as aglycone and L-rhamnose as sugar. The aglycone (quercetin) was identified by comparison with authentic sample and sugar moiety (rhamnose) was detected via CoTLC with authentic sugar markers. Consequently, compound 3 was identified to be quercetin-3-*O*-α-L-rhamnopyranoside.

Compound 4 was obtained as amorphous powder. Its IR spectrum (KBr) ν_{max} cm^{-1} showed bands for hydroxyl groups (3423 cm^{-1}), ester group (1736 cm^{-1}),

double bond (1636 cm^{-1}) and glycosidic linkage at (1075 cm^{-1})[34,35]. It gave positive reactions of triterpenoidal saponins[36]. ^{1}H-NMR spectrum of the compound exhibited resonances of seven methyl groups at the range δ 0.78-1.18 ppm, olefinic proton at δ 5.13 ppm which are characteristic of an oleanene-type triterpene and two anomeric protons of sugar moieties at δ 4.47 (1H, d, J=7.5 Hz) and 5.33 (1H, d, J=7.5 Hz) ppm[35,37]. The ^{13}C-NMR spectrum (Table 1) of the compound revealed 42 carbon signals of which 12 were assigned to two hexose units and the remaining 30 signals to a triterpenoid skeleton. The ^{13}C-NMR spectrum showed characteristic signals of two olefinic carbons at δ 126.40 and 138.60, ester carbonyl carbon at δ 176.41, the anomeric carbon signal at δ 94.96. The downfield position of C-3 signal of the aglycone moiety of compound 4 at δ 88.48 in comparing with ursolic acid which it appeared at 78.01 reflected that C-3 is the position of attachment of one glucose unit with aglycone. Also, the appearance of the signal of anomeric carbon of one glucose unit at δ 94.96 suggested that compound also has a 28-*O*-glycosidic linkage. This was supported by appearance of signal of C-28 at 176.40 and appearance of ester group at 1736 cm^{-1} in the IR spectrum of the compound[37,38]. All of ^{1}H and ^{13}C-NMR spectroscopic signals of the aglycone were identical with the reported signals of ursolic acid except for C-3 and C-28 which reflected that compound 4 is bidesmosidic saponin [35, 37, 38].

Acid hydrolysis of compound 4 gave ursolic acid as aglycone and D-glucose as sugar moiety which were identified by comparison with authentic samples. ESI-MS spectrum exhibited a peak at m/z 804.54 that originated from $[M+Na]^+$ and $[M+H]^+$ at 781.44. Also, it gave a diagnostic fragment ion peaks at m/z 619.66 $[M+H-Glc]^+$ and 455.67 $[M+H-2Glc]^+$. This reflected that successive elimination of two glucose units. Thus, the structure of compound 4 was assignated as 3-β-D-glucopyranosyl ursolic acid 28-*O*-β-D-glucopyranoside ester.

Compound 5 was obtained as amorphous powder. It gave s characteristic reactions of saponins[36]. Its IR spectrum (KBr) ν_{max} cm^{-1} exhibited a characteristic bands of hydroxyl group at 3427 cm^{-1}, ester carbonyl group at 1735 and double bond (C=C) at 1637 cm^{-1}[39,40]. The ^{1}H-NMR spectrum of the compound exhibited resonances for protons of the methyl groups of the aglycone part at the range δ 0.84-1.24, proton of olefinic double bond at δ 5.18 and three anomeric protons of the sugar moieties at δ 4.37, 4.48 and 5.23[40,41]. This was confirmed by presence of 47 carbon signals in ^{13}C-NMR spectrum of this compound (Table 1) of which seventeen were assigned to a pentose and two hexose units and the remaining 30 signals to a triterpenoid skeleton. The signals at 128.49 and 139.26 due to C-12 and C-13, respectively supported the identification of the aglycone as pomolic acid (19α-hydroxy ursolic acid)[39,41]. Acid hydrolysis of compound 5 gave L-arabinose and D-glucose which were identified by comparison with authentic samples. The signal of C-28 appeared at δ 176.87 ppm in ^{13}C-NMR spectrum as well as the appearance of the absorption band of the ester carbonyl group in IR spectrum at 1735 cm^{-1} indicated that one glycosidation linked at C-28 through carboxylic group. This was confirmed by

presence of signal of one anomeric carbon at δ 94.51[41,38]. Other glycosidation linkage was suggested at C-3 of the aglycone by downfield shifts of C-3 at δ 88.42. Also, the downfield shift of C-3 of the arabinose unit at δ 83.02 reflected that the C-3 is the position of the interglycosidation linkage between the sugar units[36]. These results supported that compound 5 has a bidesmosidic structure[41,38]. Also, the carbon and proton resonances for the aglycone moiety of compound 5 were identical with the reported signals of pomolic acid (19α-hydroxy ursolic acid) except for C-3 and C-28 which reflected that compound 5 is bidesmosidic glycoside [40,38]. The ESI-MS of compound 5 showed the $[M+Na]^+$ ion at m/z 950.68 and fragment ions at m/z 765.45 $[M+H-162]^+$ due to cleave of hexose unit, m/z 603.87 $[M+H-2x162]^+$ due to cleave of two hexose units and m/z 470.26 $[M+H-2x162-132]^+$ due to cleave of two hexose units and one pentose unit [40]. Therefore, the structure of compound 5 was characterized as 3-*O*-α-L-arabinopyranosyl (1→3)-β-D-glucopyranoside pomolic acid 28-*O*-β-D-glucopyranoside ester.

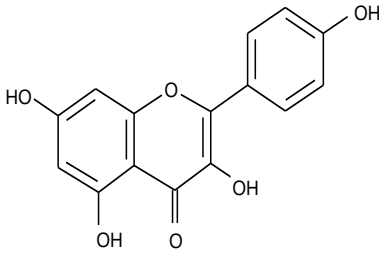
DPPH radical-scavenging activity and total antioxidant capacity of the compounds isolated from EtOAc and n-BuOH fractions were evaluated. The results in Table 3 showed that compounds 1-3 have antioxidant activity. Among the isolated compounds, the highest activity was observed for compound 1 in the two tests (SC_{50}= 4.07 with DPPH and 772.58 mg AAE/ g compound in case of phosphomolybdenum assay, respectively).

TABLE 3. DPPH-scavenging activity and total antioxidant capacity of compounds 1-3.

Compounds	**DPPH SC $_{50}$ [**μg/ml]	**Total antioxidant capacity (mg AAE/g compound)**
1	4.07 ± 0.0	772.58 ± 2.59
2	6.50 ± 0.03	409.61 ± 2.56
3	12.55 ± 0.04	239.97 ± 5.89
Ascorbic acid	7.90 ± 2.10	-

Conclusion

In recent years, much attention has been devoted to natural antioxidants and their association with health benefits. Our results indicate that the extracts of *A.indica* possess *in vitro* schistosomicidal activity against *S.mansoni* adult worms .This activity is correlated with the ability of these extracts as antioxidant agents and is due to the presence of various phytoconstituents. Finally *A.indica* extracts could be considered as a promising natural agent for the development of new schistosomicidal drug.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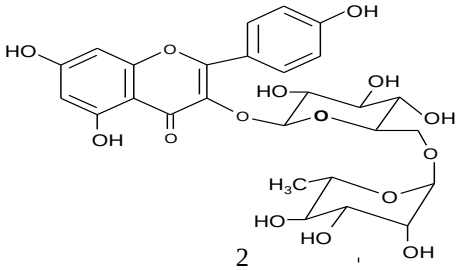
1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HO
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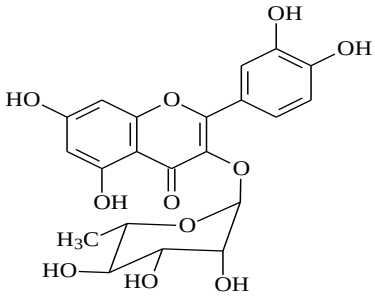
OH

2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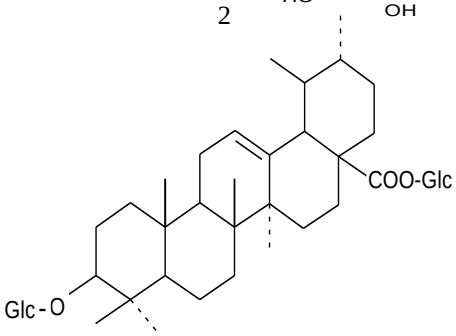
OH

3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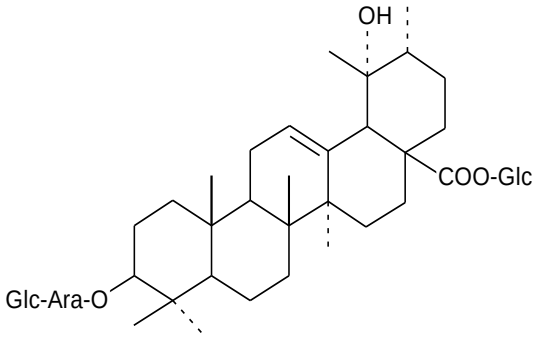
Glc-O

4


OH
COO-Glc
Glc-Ara-O

5

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المكونات الكيميائية للمستخلص الميثانولي لنبات ازدراختا انديكا وتقييمهم كمضادات للأكسدة وضد ديدان البلهارسيا

مرتضي محمد السيد ، ماهر عبدالعزيز محمود [*]، حنان عبد الخالق النحاس ، سيد عبد الحميد التومي[**] ،ايمان احمد الوكيل و مسعد احمد الغريب
معمل الكيمياء العلاجية -معهد تيودور بلهارس ، [*]قسم الكيمياء- كليه العلوم - جامعه عين شمس و [**]قسم كيمياء المواد الدابغة وتكنولوجيا الجلود- المركز القومي للبحوث – الجيزة – مصر.

أجريت هذه الدراسة لتقييم كفاءة المستخلص الميثانولي (٧٠٪) لأوراق نبات ازدراختا انديكا وكذلك مستخلص خلات الاثيل والبيوتانول المشتقين من المستخلص الميثانولي ضد ديدان البلهارسيا وكمواد مضادة للاكسده.ونتيجة للفاعلية العالية لكل من مستخلص خلات الاثيل والبيوتانول تم إجراء فصل كروماتوجرافي لكل من المستخلصين باستخدام طرق كروماتوجرافيه مختلفة وأمكن فصل مركبين فلافونيد(١،٢) من مستخلص خلات الاثيل بينما أمكن فصل مركب فلافونيد (٣) ومركبين صابونين (٤،٥) من المستخلص البيوتانولي .وتم إثبات التركيب الكيميائي الدقيق بواسطة بعض التحاليل الطيفية .أظهرت المركبات ١-٣ فاعلية كمواد مضادة للاكسده بينما المركبين ٤،٥ لم تظهر أي فاعليه .وهذه أول مره يتم فيها فصل المركبات (٢-٥) من نبات ازدراختا انديكا وأيضا أول مره يتم فيها تقييم فاعليه نبات الازدراختا ضد ديدان البلهارسيا.