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Research article

Chemical and biological characterize of some species from Mahdadh Dhahab region

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Abstract

The present study aims to detect the flavonoid and phenolic constituents of six selected species belonging to different families represented in Mahd adh Dhahab region as well as screen their antioxidant and cytotoxic activities. These are *Senna alexandrina* Mill. and *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Fabaceae), *Vitex trifolia* L. (Lamiaceae), *Moltkiopsis ciliata* (Forssk.) I. M. Johnston (Boraginaceae), *Salicornia europaea* L. (Chenopodiaceae) and *Acanthus spinosus* L. (Acanthaceae).The phenolic and flavonoid profiles were carried out using HPLC technique. In addition, *in vivo* antioxidant activity was performed using DPPH assay while the cytotoxicity was summarized by MTT test. Thirty three compounds were detected using HPLC technique and comparison with standards. They were detected as 14 phenolic acids and 19 flavonoids. The flavonoids were represented as five *C*-glycosyl flavones, five flavones, six flavonols and three flavanones. The DPPH assay showed moderate activities with % activity 40.67±0.8, 45.23±0.34 and 43. 55± 1.1 for *M. ciliata*, *V. nilotica and A. spinosus*, respectively and weak activity for the rest investigated plants. *V. nilotica* showed significant cytotoxicity against breast adenocarcinoma (MCF-7) cells, lung carcinoma (A549) and colon carcinoma with % inhibition 96.3±0.7, 97.2±0.24, 96.1±0.2, respectively. Among the studied species, *V. nilotica* showed the highest content of phenolic acids and flavonoids as well as a highly antioxidant and anticancer activities.

Key words: Mahd Ad Dahab region, HPLC, flavonoids, phenolics, Anitioxdant, cytotoxicity.

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Introduction

Mahd adh Dhahab (Cradle of Gold) is situated in the western region of the country known as the Hejaz in the Al Medinah province of Saudi Arabia [1]. Samples from different plants species which grown around Mahd Adh Dhahab Mine have ability to accumulated heavy metals [2].

Phenolics are widely distributed and important class of plant secondary metabolites, which possess aromatic ring with

one or more hydroxyl substituent [3]. Phenolic compounds are including flavonoids and phenolic are most frequently occurring in combination with sugars as glycosides [4]. Flavonoids and phenolics are very important for growth development and play key role in defense against microbial activities and infections. They provide oxidative stabilities to the plants in case of injuries [5]. Phenolics have attracted great attention and get a high importance due to their antioxidant activity [6].

Samples from different plants species, which grown around Mahad AD`Dahab region, have been selected to study the flavonoid and phenolic constituents as well as screen their antioxidant activity and cytotoxic effect. These are *Senna alexandrina* Mill.and *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Fabaceae), *Vitex trifolia* L. (Lamiaceae), *Moltkiopsis ciliata* (Forssk.) I.M.Johnston (Boraginaceae), *Salicornia europaea* L. (Chenopodiaceae) and *Acanthus spinosus* L. (Acanthaceae).

Materials and methods

Plant material

The plant materials were collected from Mahad Al-Thab region in October 2015 by Prof. Dr. Salwa Aly Kawashty and identified as *Senna alexandrina* Mill. and *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Fabaceae), *Vitex trifolia* L. (Lamiaceae), *Moltkiopsis ciliata* (Forssk.) I.M.Johnston (Boraginaceae), *Salicornia europaea* L. (Chenopodiaceae) and *Acanthus spinosus* L. (Acanthaceae).

Extraction

The air-dried powdered leafy branches of six studied species were extracted with 70% MeOH three times. The solvent was evaporated under reduced pressure and temperature at 60°C [7, 8]. The dried 70% aqueous methanol extracts were defatted with petroleum ether. The defatted aqueous extracts were subjected to the phytochemical analysis using HPLC analysis and Co-PC with authentic samples as well as the biological screening for antioxidant and anticancer activities.

HPLC profiles of phenolics and flavonoids.

Five gram of each dried powdered leafy branches of the studied species were mixed separately with methanol and centrifuged at 1000 rpm for 10 min and the supernatant was filtered through a 0.2 mm millipore membrane filter before injection. Chromatographic separations were performed using HPLC Hewlett Packard (series 1050) (Hewlett Packard Inc., Palo Alto, CA) equipped with auto-sampler injector, solvent degasser, ultraviolet (UV) detector set at (280 nm for phenolics determination and 330 nm for flavonoids determination), and quaternary HP pump (series 1050). The column temperature was kept at 35°C. Gradient separation was carried out using methanol and acetonitrile (2:1) as a mobile phase at a flow rate of 1 ml/min. Authentic phenolics and flavonoids were dissolved in the mobile phase and injected into HPLC. The retention time and the peak area were used to calculate the phenolic and flavonoids concentrations by the data analysis of Hewlett Packard software (Hewlett Packard Inc., Palo Alto, CA) [9].

Determination of free radical scavenging activity (DPPH assay)

Quantitative measurements of radical scavenging properties of six extracts were carried out according to [10]. Briefly, 0.1 mM solution of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) in methanol was prepared and 1 ml of this solution was added to 3 ml of each methanolic extract at different concentration (50-200 µg/mL). Butylated hydroxytoluene (BHT) was used as a positive control. Discoloration was measured at 517 nm after incubation for 30 min. The capacity to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = ADPPH – AS /ADPPH x100 Where, ADPPH is the absorbance of the DPPH solution and AS is the absorbance I solution when the sample extract is added.

Cytotoxic assay

Cell culture

A549 human lung carcinoma and prostate cancer (PC3) were maintained in DMEM medium, HCT-116 (colorectal carcinoma), HepG2 (hepatocellular carcinoma) and MCF-7 (breast adenocarcinoma) were maintained in RPMI. All media was supplemented with 10% fetal bovine serum and incubated at 37°C in 5% CO_2 and 95% humidity. Cells were sub-cultured using trypsin versene 0.15 %. All cell lines were purchased from Vacsera (Giza, Egypt).

Cell viability assay

After 24 h of seeding 20000 cells per well in case of A-549, HCT-116 and PC3, 10000 cells per well in case of HepG2 and MCF-7 cell lines (in 96 well plates), the medium was changed to serum-free medium containing a final concentration of the extracts of 100 µg/ml in triplicates. The cells were treated for 24 h. 100 µg/ml doxorubicin was used as positive control and 0.5 % DMSO was used as negative control. Cell viability was determined using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) [11, 12]. The equation used for calculation of percentage cytotoxicity: (1- (Av(X) / (Av(NC)))* 100

Where Av: average, X: absorbance of sample well measured at 595 nm with reference 690 nm, NC: absorbance of negative control measured at 595 nm with reference 690.

Result and Discussion

Results

Phenolic acids and flavonoids compositions

In the present work 14 phenolic acids were detected; gallic, protocatechuic, chlorogenic, caffeic, vanillic, p-coumaric, ferulic, 1so-ferulic, ellagic, salycilic, o-coumaric, e-vanillic acid and p-OH-benzoic acids (Table 1). Among the detected phenolic acids; e-vanillic acid was the major compound in *S. alexandrina*, *V. nilotica, V. trifolia*, *M . ciliate* and *S. europaea* while o-coumaric acid was the major compound in *A. spinosus* (21.23±0.35 mg/100g DW). The highest content of e-vanillic acid has been detected in *S. alexandrina* (115.73±0.58 mg/100g DW).

Table 1. Phenolic acids detected by HPLC and their amounts for investigated species (mg/100g DW)

Phenolics	*Rt*	*C. senna*	*V. nilotica*	*V. trifolia*	*M ciliata*	*S.europaea*	*A. spinosus*
Gallic acid	7.14	0.53±0.03*	19.33±0.31*	1.92±0.05	0	1.45±0.07	1.35± 0.14*
Protocatechuic acid	8.50	1.38±0.17	3.97±0.18	2.21±0.12	0.91±0.01	1.74±0.24	2.95±0.09
Chlorogenic acid	9.15	3.32±0.34	6.34±0.02	4.68±0.03	0	2.58±0.2*	3.29±0.37
Caffeic acid	10.28	1.03±0.02	2.91±0.04*	1.58±0.65	1.57±0.05	0.88±0.03*	2.43±0.05
Vanillic acid	10.39	2.27±0.19	14.44±0.62	8.56±0.04	1.97±0.43	8.38 ±0.16*	17.10±0.12
p-Coumaric acid	11.77	7.63±0.41	7.57±0.44	2.43±0.05	2.45±0.18	2.76 ±0.47*	6.33±0.55
Ferulic acid	11.94	8.95±0.05	9.77±0.24	6.00±0.11	1.40±0.06	2.78±0.09*	5.38±0.01
Iso-ferulic acid	12.32	7.77±0.31	2.36±0.07	2.12±0.02	0.92±0.04	1.72±0.03	9.80±0.48
Ellagic acid	13.13	6.14±0.07	23.13±0.71*	9.29±0.16	1.52±0.12	5.33±0.12	18.58±0.31
Salycilic acid	11.53	8.93±0.02	15.7 ±0.41	9.31±0.14	3.25±0.32	6.18±0.05	6.79±0.07
o-Coumaric acid	13.54	1.78±0.47	2.20±0.05	1.24±0.02	3.15±0.05	2.56±0.24	21.23±0.35
e-Vanillic acid	13.16	115.73±0.58	56.15±0.02	35.41±0.27	18.31±0.3	60.12±0.02	0
p-OH-benzoic acid	7.14	7.91±0.41	17.22±0.03	16.38±0.17	2.03±0.02	5.44±0.02	15.50±0.38
Total		**173.37**	**186.42**	101.13	37.48	101.92	110.73

* Phenolic acids detected previously from the investigated plants.

All results were expressed as mean ± standard deviations from replications n = 3.

The flavonoid compositions of the investigated species (Table 2) revealed the presence of 19 compounds (five *C*-glycosyl flavones, five flavones, six flavonols and three flavonones). The detected *C*-glycosyl flavones seem to be 6,8-di-*C*-glycosyl pattern of apigenin and luteolin; the major compounds were represented as luteolin 6-*C*-arabinose-8-*C*-glucose and luteolin 6-*C*- glucose-8-*C*- arabinose in *S. alexandrina* (808.79±0.14 and 424.62±0.21 mg/100g DW), luteolin 6-*C*-glucose-8-*C*-arabinose in *V. trifolia* (458.77±0.27 mg/100g DW) while apigenin 6-*C*-rhamnoside-8-*C*-glucoside in *V. nilotica* and *A. spinosus* with concentrations 409.70±0.05 and 329.44±0.32 mg/100g DW, respectively.

Flavones, flavanones and kaempferol derivatives were concentrated in *A. spinosus,* while quercetin and their derivative are concentrated in the two members of family Fabaceae; *S. alexandrina* and *V. nilotica* (Table 2).

Antioxidant activities

The *in vitro* radical scavenging activity of the 6 investigated extracts showed moderate activities with %inhibition 40.67±0.8, 43. 55± 1.1 and 45.23±0.34 for *M. ciliata*, *A. spinosus and V. nilotica*, respectively and weak activity for the rest investigated plants.

Cytotoxic activities

The results indicated that *V. nilotica* extract showed significance highly activity against A549, MCF7and HCT116 at 100 mg/mL with % cell viability of 97.2, 96.3 and 96.1, respectively. The rest extracts exhibited moderate effect against four cell line with cell viability ranged from 9.2% to 40.5%.

Discussion

In the present work, among the investigated extracts, the two species of family Fabaceae (*V. nilotica* & *S. alexandrina*) have been shown the highest concentrations of phenolic acids and flavonoids. They have been showed the highest content of various phenolic acids. The highest contents of gallic, protocatechuic, chlorogenic, caffeic, ferulic, ellagic, salicylic and p-Hydroxybenzoic acids has been observed in the extract of *V. nilotica,* while *S. alexandrina* is characterized by the highest contents of p-coumaric and e-vanillic acids (Table 1). From the flavonoids point of view, both species have the highest concentrations of *C*-glycosyl pattern; *V. nilotica* is characterized by the highest contents of *C*-glycosyl apigenin (apigenin 6-*C*-arabinoside-8-*C*-glactoside and apigenin 6-*C*-rhamnoside-8-*C*-glucoside), while *S. alexandrina* is characterized by the highest contents of *C*-glycosyl luteolin (luteolin 6-*C*-arabinoside-8-*C*-glucoside and luteolin 6-*C*-glucoside -8-*C*-arabinoside) (Table 2).

V. nilotica, the accepted name of *Acacia nilotica* Lamarack, is a wide medicinal plant of family Fabaceae. Its stem bark, leaves, flowers and pods are used against several diseases such as bleeding piles cancer, cold, congestion, cough, diarrhea, dysentery, fever, hemorrhoid, leucoderma, leprosy, ophthalmia, tuberculosis and sclerosis [13-15]. This plant contains a wide range of bioactive constituents; phenolic acids, flavonoids, terpenoids, tannins, alkaloids, saponins and glycosides. The genus also has anti-cancer, antihypertensive, antispasmodic, antimutagenic, antioxidant, anti-inflammatory and antimicrobial activities [14-22].

In the present work, except of kaempferol, quercetin and its glycoside derivative (rutin), all flavonoids were detected for the first time from the plant. Additionally, the phenolic acids (protocatechuic, chlorogenic, salicylic and p-Hydroxybenzoic) were also observed firstly. This species showed the highest antioxidant and cytotoxic activities (Tables 3-4), confirmed its medicinal properties.

S. alexandrina the accepted name of *Cassia senna* L. occurs naturally from Mali to Kenya and Somalia. It is also native in Asia and commercially cultivated in China, Egypt, India, Korea, Sudan and Pakistan [23]. The plant extracts reported to presence of flavonoids, phenolics, saponins, tannins, carbohydrates

Table 2. Flavonoids detected by HPLC and their amounts for investigated species (mg/100g DW)

Flavonoids		*Rt*	*C. senna*	*V. nilotica*	*V. trifolia*	*M. ciliata*	*S. europaea*	*A. spinosus*
***C*-glycosyl flavones**	Apigenin 6-*C*-arabinoside-8-*C*-glactoside	11.55	147.52±0.06	231.18±0.25	56.64±0.02	9.23±0.03	95.61±0.12	112.23±0.03
	Apigenin 6-*C*-rhamnoside-8-*C*-glucoside	11.97	329.44±0.32	409.70±0.05	164.24±0.61	43.50±0.16	135.13±0.07	128.86±0.17
	Apigenin 6-*C*-glucoside 8-*C*-rhamnoside	12.13	130.62±0.67	0	71.88±0.12	19.57±0.27	15.26±0.02	24.8±0.33
	Luteolin 6-*C*-arabinoside-8-*C*-glucoside	9.33	808.79±0.14	226.31±0.08	188.62±0.17	62.40±0.11	225.74±0.18	33.02±0.04
	Luteolin 6-*C*-glucoside 8-*C*-arabinoside	10.56	424.62±0.21	363.82±0.44	158.77±0.27	40.05±0.02	126.75±0.63	289.17±0.12
Flavones	Apigenin	16.6	3.02±0.02	8.09±0.06	0	7.97±0.43	5.55±0.07	162.45±0.18
	Apigenin 7-*O*-β-glucoside	13.28	173.78±0.63	45.02±0.02	24.58±0.09	4.80±0.16	9.14±0.08	156.66±0.05
	Acacetin	18.92	12.44±0.05	84.79±0.02	24.63±0.24	7.476±0.05	16.06±0.02	186.53±0.03
	Luteolin	12.17	220.89±0.05*	56.16±0.16	90.03±0.01*	0	0	253.92±0.07
	Luteolin 7-*O*-β-glucoside	12.20	101.48±0.07	73.81±0.02	38.31±0.05	20.13±0.04	28.47±0.42	117.45±0.14
Flavonols	Kaempferol	16.36	8.04±0.11*	25.42±0.04*	6.13±0.05	41.75±0.21	3.12±0.03 *	123.24±0.18*
	Kaempferol 3,7-di-*O*-rhamnoside	13.04	69.29±0.47	72.07±0.02	75.02±0.01	78.64±0.07	509.88±0.53	554.84±0.34
	K 3-(2´´-p-coumaroyl) glucoside	15.05	0	0	0	0	0	112.58±0.37
	Quercetin	15.01	38.58±0.02*	83.44±0.17*	24.46±0.12	23.15±0.03	11.05±0.16*	43.46±0.02*
	Quercitrin	13.44	124.61±0.33	70.28±0.24	6.16±0.02	0	14.48±0.07	61.10±0.32
	Rutin	12.46	483.69±0.24*	1138.54±0.08 *	118.43±0.17	13.57±0.24	35.23±0.16*	257.58±0.44
Flavanones	Hesperetin	16.60	3.77 ±0.11	4.64±0.02	12.16±0.05	0	2.49±0.02	170.27±0.12
	Hesperidin	12.55	120.57±0.12	0	91.99±0.18	30.33±0.24	46.37±0.21	153.29±0.03
	Naringenin	12.36	159.98±0.07	10.53±0.08	53.63±0.24	78.60±0.14	85.98±0.36*	380.84±0.46
Total			3361.13	3779.08	2941.768	441.11	1366.31	3322.92

* Flavonoids previously isolated from the investigated plants.

All results were expressed as mean ± standard deviations from replications n = 3.

Table 3. *In vitro* cytotoxic activity of the six studied extracts using MMT assay.

Plant extract	% Inhibition of cell viability at 100 mg/mL			
	HCT116	**A549**	**HePG2**	**MCF7**
C. senna	13.2±0.1	12.8±0.17	3.5±0.3	0
V. nilotica	96.1±0.2	97.2±0.24	45.4±0.18	96.3±0.7
V.trifolia	13.8 ±0.07	0	0	0
M.ciliata	12.9±0.2	23.8±0.11	0	0
S.europaea	9.2±0.03	11.8±0.2	0	16.3±0.2
A. spinosus	12.3	14.3±0.04	12.5±0.07	40.5±0.12
DMSO	1	5	1	3
Negative control	0	0	0	0

Table 4. LC_{50}(µg/ml) and LC_{90} (µg/ml) of *V. nilotica*

	HCT116	**A549**	**HePG2**	**MCF7**
IC_{50} (µg/ml)	44.1	55.7	-	49.5
IC_{90} (µg/ml)	75.5	84.5	-	81.7

IC_{50}: Inhibition concentration of the sample, which causes the death of 50% of cells in 48 hrs

IC_{90}: Inhibition concentration of the sample, which causes the death of 90% of cells in 48 hrs

and glycosides [24-26]. This species also observed antioxidant, anticancer and antimicrobial properties [27-31].

V. trifolia is a tropical shrub widely distributed in Pacific-Asian countries and East Africa [32]. This species contains various bioactive components, including flavonoids, iridoids, phenolics, diterpenoids and steroids [33, 34]. The leaves are used internally or externally in baths to cure Ciguatera fish poisoning-related pruritus [32]. It is used as an anti-pyretic, antioxidant, anti-tumour, anti-microbial, anti-inflammatory, and insecticidal agent [35-41].

S. europaea is a member of family: Chenopodiaceae grows as halophyte in salt fields and high salt marshes [42]. *S. europaea* contains various bioactive constituents, including phenolics mostly flavonoids and phenolic acids, sterols and polysaccharides [42-45]. The herb has been used as a folk medicine for treatment several diseases such as asthma, cancer, diabetes, hepatitis and gastroenteric disorders [46-48] and also used as antioxidant, antimicrobial agents [49-52]. In the present study, *S. europaea* showed moderate concentrations of phenolic acids and flavonoid contents compared with the other investigated species. Also, no antioxidant and cytotoxic activities were observed through the biological screening.

A. spinosus is a large, attractive, hardy plant belonging to the Acanthaceae family. It is mainly found in Mediterranean Europe and in North Africa. It is usually grown as an ornamental plant.

A. spinosus includes phenolic acids (caffeic acids, chlorogenic acid), methoxylated flavones (hispidulin), glycoproteins and amino acids [53, 54].

In the present research, *A. spinosus* is characterized by the highest contents of vanillic, iso-ferulic and o-coumaric acids, flavones (apigenin, apigenin 7-*O*-β-glucoside, acacetin, luteolin and luteolin 7-*O*-β-glucoside), flavanones (naringenin, hesperetin and hesperidin) and kaempferol and its derivatives (kaempferol 3,7-di-*O*-rhamnoside and kaempferol 3-(2′′-p-coumaroyl) glucoside) reported for the first time.

M. ciliata is belonging to family Boraginaceae, a perennial shrub with woody base, up to 25cm high with white younger branches, the plant covered with stiff white hairs.*M. ciliata* exposed carbohydrates, terpens, sterols, flavonoids, condensed tannins, nitrogenous bases, and saponins [55,56]. In the present study, *M. ciliata*

contains the lowest phenolic and flavonoid constituents as well as the extract showed a weak antioxidant and cytotoxic activities.

Conclusion

The present study demonstrated that all studied species collected from Mahd adh Dahab region share some phenolic acids and flavonoids with different concentrations although they are belonging to different families. It has been reported that the antioxidant activity of plants might be due to their phenolic and flavonoid compounds.

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