Antioxidant and antiviral activities of the aqueous alcoholic leaf extract of *Boscia angustifolia* A. Rich. (Capparaceae) and its major component 'ombuin'

Maha M. Salem[1], Sameh R. Hussein[1], Reham El-Sharawy[1], Ahmed El-Khateeb[1], Eman A. Ragab[2], Kamal M. Dawood[2], Sabry I.M. El Negoumy[1]

[1]Department of Phytochemistry and Plant Systematics, National Research Center, Cairo, [2]Department of Organic Chemistry, Cairo University, Giza, Egypt

Correspondence to Sameh R. Hussein, PhD, Department of Phytochemistry and Plant Systematics, National Research Center, Dokki, Cairo 12622, Egypt
Tel: +20 011 102 38700; Fax: (202) 33370931
e-mail: sameh_reda@hotmail.com

Received 25 January 2016
Accepted 16 February 2016

Egyptian Pharmaceutical Journal
2016, 15:1–5

Background and objectives
Boscia angustifolia A. Rich. is an endemic African species and has various folk medicinal uses. The present study aimed to investigate the polar compounds in *B. angustifolia* leaves and evaluate the antiviral and antioxidant activities of its extract and its major compound.
Materials and methods
The isolated compound (ombuin) was identified using chemical and spectroscopic tools (UV, ^{1}H, and ^{13}C NMR), and the polar constituents were characterized using gas chromatography mass spectrometry after silylation.
Results and conclusion
B. angustifolia A. Rich. leaves yielded 7,4′-dimethoxy quercetin 'ombuin'. Both the aqueous alcoholic extract and ombuin showed moderate antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl assay with IC_{50} values 41.2 and 16.5 μg/ml, respectively. Remarkable inhibition against H5N1 virus was found at concentration 80 μg/μl (63 and 68%, respectively). The gas chromatography mass spectrometry analysis revealed the presence of a complex mixture of 42 compounds, mainly acids, sugars, and their derivatives.

Keywords:
antioxidant, antiviral, *Boscia angustifolia*, gas chromatography mass spectrometry analysis

Egypt Pharm J 15:1–5

1687-4315

Introduction

Boscia angustifolia A. Rich. (Capparaceae) is commonly called rough-leaved shepherd's tree growing up to 25 feet high, with glabrous branches and fragrant greenish flowers [1]. The leaves are shortly lanceolate and the fruits are pitted and rugulose berries. It is distributed in the savanna throughout the east and south tropical Africa and Arabia [2]. The presence of the plant in Gabal Elba, Egypt, was reported [3].

Different parts of the plant have various folk medicinal uses. The leaves and roots are used for the treatment of diarrhea, pneumonia, boils, chest pain, wound infection, typhoid fever, urinary infection, and also to protect cells of the skin [1]. The berries are crushed in water and given to cause purging [4]. The leaves are used as a cholagogue. The dried barks are applied as a fumigation for opthalmias and neuralgia as well as to cure malaria [5,6].

The plant has been subjected to few pharmacological and phytochemical studies, which reported antiplasmodial and GABA (A)-benzodiazepine receptor binding activities [7], and antibacterial and hepatorenal effects of different root extracts and the alkaloidal content [8,9]. Protoalkaloids have also been reported previously [10]. The phytochemical studies focused mainly on the nonpolar fractions of the root extracts, and merely nothing was reported on the chemical composition or the biological activities of the leaves. It was, therefore, found interesting to measure the antioxidant and antiviral activities of the leaf extract and ombuin as the literature shows interesting reports on the antioxidant and antiviral activities of quercetin derivatives [11,12]. A partial characterization of the composition of the extract after derivatization was carried out using gas chromatography mass spectrometry (GC/MS) analysis as a part of an ongoing study to fully investigate the polar constituents of the plant.

Materials and methods

General experimental procedures

^{1}H NMR spectra were measured using a Jeol ECA 500 MHz NMR (JOEL Inc., Tokyo, Japan), spectrometer, at 500 MHz. ^{1}H chemical shifts (δ) were measured in

 DOI: 10.4103/1687-4315.184025

parts per million (ppm), relative to tetramethylsilane (TMS) and ^{13}C NMR chemical shifts to dimethyl sulfoxide (DMSO)-d_6 and converted to the TMS scale by adding 39.5. Ultraviolet (UV) recordings were made on a Shimadzu UV-Visible-1601 spectrophotometer (Shimadzu Inc., Tokyo, Japan). The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS (Thermo Fisher Scientific Inc., Waltham, MA) for mass spectral data.

Plant materials

B. angustifolia leaves were collected from Gabal Elba, Egypt, on April 2013. Authentication was performed by Professor Dr Salwa Kawashty. Voucher specimen (SN. SK742) was deposited at the herbarium of the National Research Center (CAIRC).

Preparation of the extract

The fresh leaves of *B. angustifolia* were dried in the shade in an air draft at room temperature. The powdered leaves (1574 g) were refluxed in MeOH/H_2O (3:1) mixture (three extractions, each for 8 h with 1.25 l). The collected extract was filtered on and dried in vacuum to yield a dark brown amorphous powder (305 g).

Isolation and identification of ombuin

Crystallization of a light-yellow powder from the concentrated solution of the aqueous methanol extract followed by purification using a Sephadex LH-20 column gave rise to the pure ombuin (3.4 g), which was then identified through R_f-values, UV, NMR spectroscopic techniques, and mass spectrometry data.

R_f-values: 0.02 (H_2O), 0.07 (HOAc), and 0.95 (BAW). UV λ_{max} nm in MeOH: 255, 268sh, 321sh, and 368; +NaOMe: 268 and 471; +NaOAc: 257, 269sh, and 371; +NaOAc–H_3BO_3: 269, 305, 374, and 424; +$AlCl_3$: 269, 305, 360, and 423; $AlCl_3$–HCl: 269, 305, 354, and 424. EIMS m/z=330. ^1H NMR: δ ppm (500 MHz, DMSO-d_6): 7.64 (^1H, *d*, *J* = 8 Hz, H-6′), 7.66 [^1H, *s*(*br*), H-2′], 7.03 (^1H, *d*, *J* = 8 Hz, H-5′), 6.64 [^1H, *s*(*br*), H-8], and 6.27 [^1H, *s*(*br*), H-6]; 3.89 (^3H, *s*, OCH_3 at 4′); 3.89 (^3H, *s*, OCH_3 at 7). ^{13}C NMR: δ ppm 147.2 (C-2), 136.9 (C-3), 176.5 (C-4), 160.8 (C-5), 97.9 (C-6), 165.4 (C-7), 92.3 (C-8), 156.6 (C-9), 104.5 (C-10), 123.8 (C-1′), 115.2 (C-2′), 146.6 (C-3′), 149.9 (C-4′), 112.19 (C-5′), and 120.3 (C-6′); 56.11 (CH_3 at 4′) and 56.50 (CH_3 at 7).

Derivatization and GC/MS analysis

The dried extract (5 mg) was subjected to silylation with 50 µl pyridine and 75 µl bis (trimethylsilyl) trifluoroacetamide. The mixture was heated at 80°C for 30 min [13]. The GC/MS analysis was performed using TG-5MS fused silica capillary column (30 m, 0.25, and 0.1 mm film thickness). For GC/MS detection, an electron ionization system with an ionization energy of 70 eV was used. Helium gas was used as the carrier gas at a constant flow rate of 1 ml/min. The injector and MS transfer line temperature was set at 280°C. The oven temperature was programmed at an initial temperature of 50°C (hold 2 min) to 150°C at an increasing rate of 7°C/min and then to 270 at an increasing rate of 5°C/min (hold 2 min), and then to 310 as a final temperature at an increasing rate of 3.5°C/min (hold 10 min). The quantification of all identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their mass spectra with those of the NIST, WILLY library data of the GC/MS system.

Biological methods

Antioxidant activity (DPPH assay)

Determination of radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay radical scavenging activity of plant extract against the stable free radical DPPH (Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound that can donate hydrogen, it is reduced. The changes in color (from deep-violet to light-yellow) were measured at 517 nm. Radical scavenging activity of the extract was measured using a slightly modified method of Brand-Williams *et al.* [14], in which the extract solution was prepared by dissolving 0.025 g of the dry extract in 10 ml of methanol. The solution of DPPH in methanol (6×10^{-5} mol/l) was freshly prepared, before UV measurements. Three milliliters of this solution was mixed with nine different concentrations of the samples. The resulting solutions were kept in the dark for 30 min at room temperature and then the decrease in absorbance was measured. Absorbance of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula: Inhibition (%)=[(AB–AA)/AB]×100, where AB is the absorbance of blank sample and AA is the absorbance of the tested samples. IC_{50} value, the concentration of the substrate that causes 50% loss of the DPPH activity (color), was calculated for the standard and the extract from a graph plotted for the % inhibition against the concentration in µg/ml.

Antiviral activity against H5N1 virus

Cells and virus

Madin-Darby canine kidney (MDCK) cells were maintained in the Center of Scientific Excellence for influenza viruses at the National Research Center. The cells were propagated until confluence in multiwell plates. The highly pathogenic avian influenza virus A/Chicken/Egypt/M7217B/2013 (H5N1) used in this study was isolated from the infected chickens in Egypt in 2013 and characterized at immunologic and molecular levels.

Preparation of extracts for bioassay

Stock solutions of the tested extract and compound were dissolved as 0.1 g in 1 ml of 10% DMSO in deionized water. The prepared extract solutions were used for both cytotoxicity and antiviral bioassays.

MTT cytotoxicity assay (TC_{50})

Samples were 10-fold serially diluted with Dulbecco's modified Eagle's medium. Stock solutions of the test compounds were prepared in 10% DMSO in ddH_2O. The cytotoxic activity of the extracts were tested in MDCK cells using the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method [15] with a minor modification. Briefly, the cells were seeded in 96-well plates (100 µl/well at a density of 3×10^5 cells/ml) and incubated for 24 h at 37°C in 5% carbon dioxide. After 24 h, the cells were treated with various concentrations of the tested compounds in triplicates. After further 24 h, the supernatant was discarded and cell monolayers were washed with sterile phosphate buffer saline three times, and MTT solution (20 µl of 5 mg/ml stock solution) was added to each well and incubated at 37°C for 4 h followed by medium aspiration. In each well, the formed formazan crystals were dissolved with 200 µl of acidified isopropanol (0.04 mol/l HCl in absolute isopropanol=0.073 ml HCl in 50 ml isopropanol). Absorbance of formazan solutions was measured at λ_{max} 540 nm with 620 nm as a reference wavelength using a multiwell plate reader. The percentage of cytotoxicity compared with the untreated cells was determined as follows: % of cytotoxicity=[(absorbance of cell without treatment–absorbance of cell with treatment)/absorbance of cell without treatment]×100. The plot of % cytotoxicity versus sample concentration was used to calculate the concentration that exhibited 50% cytotoxicity (TC_{50}).

Plaque reduction assay

Anti-H5N1 activity of the extract and ombuin was investigated using the plaque reduction assay with confluent 24-h-old monolayer of MDCK cells. The assay was carried out according to the method of Hayden *et al.* [16] in a six-well plate, in which MDCK cells (10^5 cells/ml) were cultivated for 24 h at 37°C. A/Chicken/Egypt/M7217B/2013 (H5N1) virus was diluted to give 10^4 plaque-forming unit/well and mixed with the safe concentrations of the tested compounds and incubated for 1 h at 37°C before being added to the cells. Growth medium was removed from the cell culture plates and virus-Cpd or virus-extract and virus-zanamivir mixtures were inoculated (100 µl/well). After 1 h contact time for virus adsorption, 3 ml of Dulbecco's modified Eagle's medium supplemented with 2% agarose was added into the cell monolayer, and the plates were left to solidify and incubated at 37°C until formation of viral plaques (3–4 days). Formalin (10%) was added for 2 h and then the plates were stained with 0.1% crystal violet in distilled water. Control wells were included, in which untreated virus was incubated with MDCK cells, and, finally, plaques were counted and percentage reduction in plaque formation in comparison with control wells was recorded as follows: % of inhibition=[viral count (untreated)–viral count (treated)/viral count (untreated)]×100.

Results and discussion

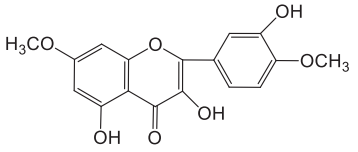
Identification of ombuin

Ombuin was isolated as faint yellow amorphous powder, which appeared as a yellow spot under short UV light and turned orange when exposed to ammonia vapors; the spot gave a dark gray color when sprayed with Fe_2Cl_3. The MS data revealed a molecular formula: $C_{17}H_{14}O_7$. These data together with the UV absorption spectral data (see the General Experimental Procedures section) indicated that the compound is a flavonol substituted with methoxyl groups at C-4′ and C-7. ^1H NMR and ^{13}C NMR spectroscopic analysis (see the General Experimental Procedures section) gave very close data to that reported previously for 7,4′-dimethoxy quercetin (Fig. 1), ombuin [17], which has been isolated for the first time from *B. angustifolia* and has not been reported before in this genus.

GC/MS analysis

The dried extract was silylated, and the polar constituents possessing the hydroxyl, amine, and

Figure 1



Chemical structure of Ombuin; 7,4'-dimethoxy quercetin.

carboxyl groups transformed into volatile TMS-ethers were analyzed using the GC/MS. In this complex mixture, 42 compounds were identified belonging to different groups, such as acid derivatives, sugar derivatives, aldehydes, amino acid derivatives, and other nitrogen and/or sulfur compounds.

The major compound detected is lactic acid, which is known for its antimicrobial activity [18]. Quinic acid was also found in remarkable abundance. The literature shows high antiviral activity for the latter [19].

As it was expected, sugars and sugar derivatives represented more than half of the total peak area percent of the silylated extract. Significant concentrations of maltose methyloxime well known for its use as antidote against organophosphorus poisoning [20], followed by lower yet high concentrations of turanose, fructofuranose, and sucrose, were found.

The data analysis revealed the presence of four nitrogen-containing compounds, the majority of which is 1-methyl imino thieno [3,4b] naphthalene. A detailed data for the composition of the silylated aqueous alcoholic leaf extract can be found in Table 1.

Table 1 Composition of the aqueous alcoholic leaf extract of *Boscia angustifolia* gas chromatography mass spectrometry after silylation (%)[a]

Compound	Abundance (%)
Carboxylic acids and their derivatives	
Lactic acid	18.11
Quinic acid	2.46
Malic acid	1.03
Hexadecanoic acid	0.7
Threonic acid	0.63
4-N, N dimethylaminobutyric acid	0.53
Hydracrylic acid	0.46
Glycolic acid	0.44
Gulonic acid lactone	0.37
Glucuronic acid lactone	0.33
2-Deoxy-erythro-pentonic acid	0.28
2-Hydroxy-4-methylpentanoic acid	0.19
2,3,4,5-Tetrahydroxypentanoic acid-1,4-lactone	0.18
Sugars and their derivatives	
Maltose methyloxime	15.19
Turanose	11.91
Fructofuranose	5.76
Sucrose	5.17
Glycerol	2.5
Lactulose	1.75
Mannitol	1.58
Talopyranose	1.36
Trehalose	1.33
Ribitol	1.12
1,5-Anhydro-d-sorbitol	1.09
Ribofuranose	0.83
Erythritol	0.59
Ribono-1, 4-lactone	0.53
Allofuranose	0.52
Xylose, methyloxime	0.46
Myo-inositol	0.4
Sorbose	0.35
Methyl galactoside	0.34
Glucopyranose	0.29
Methyl mannofuranoside	0.18
Aldehydes	
Pentenal	1.04
Tetrahydroxy-2-furanacetaldehyde	0.76
Amino acid derivatives	
Methyl leucine	1.48
N, N-dimethyl lysine methyl ester	0.17
Other sulfur and/or nitrogen-containing compounds	
1-Methyl imino thieno [3,4b] naphthalene	9.99
1, 2, 4-Trimethoxy-5,6-dihydrophenanthridin-6-one	2.42
1-H-9-methoxybenz [f] indole	1.9
Methyl-3-H pyrrolo [1,2a] indole-9-carboxylate	0.45

[a]The ion current generated depends on the characteristics of the compound and is not a true quantitation.

DPPH assay

On evaluation of the antioxidant activity, the aqueous methanol extract of *B. angustifolia* showed moderate values for absorbance inhibition at the different concentrations used; the extract completely inhibited DPPH absorbance at a concentration of 100 µl. Moreover, the IC_{50} (the concentration that inhibits 50% of the absorbance of DPPH) was determined from the graph plotted for the percentage inhibition against concentration in µg/ml for ascorbic acid, *B. angustifolia* extract, and the pure compound ombuin, which was found to be 1.93 ± 0.03, 41.2 ± 3.1, and 16.5 ± 1.8 µg/ml, respectively. Values are the average of triplicate experiments and represented as mean–SD. Although quercetin has a high antioxidant activity, its methoxylated derivative ombuin showed lower activity due to methoxylation at C-7 and C-4′ [21].

Cytotoxicity and plaque reduction assay

The toxicity of the extract and ombuin were determined using the MTT assay. The TC_{50} was found to be 151 and 81 µg/µl, respectively. Both showed a potential activity against influenza A virus (H5N1) infection up to 63 and 68.5%, respectively, at concentration 80 µg/µl. The pure compound ombuin showed higher activity inhibition compared with the extract at all used concentrations (Table 2).

Conclusion

B. angustifolia aqueous methanolic leaf extract was subjected for the first time to antiviral and antioxidant screening assays, which showed potential activities that suggest a further characterization of its composition. The compound ombuin isolated spontaneously through

Table 2 Antiviral activity measured using plaque reduction assay

Sample	Concentration (μg/μl)	Initial viral count	Viral count (PFU/ml)	Inhibition (%)
Extract	80	2.7×10^{10}	0.98×10^{10}	63
Ombuin	20	2.7×10^{10}	1.04×10^{10}	61
	40		0.85×10^{10}	69
	80		0.75×10^{10}	68.5

PFU, plaque-forming unit.

crystallization from the concentrated extract showed moderate antioxidant activity and remarkable antiviral activity against H5N1. The GC/MS analysis revealed the presence of 42 compounds composed mainly of acids, sugars, and their derivatives together with other aldehydes and nitrogen and/or sulfur-containing compounds.

Acknowledgements

This research was funded by the National Research Center, Cairo, Egypt. The antiviral activity assay was performed at the Center of Scientific Excellence for influenza viruses at the National Research Center by Dr Ola Bagato under the supervision of Professor Dr Mohamed A. Ali.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Keay RW, Onochie CF, Stanfield DP. *Nigerian trees*. Lagos; Ibadan: Federal Department of Forest Research; 1960.

2 Burkill HM. *The useful plants of West Tropical Africa*. Surrey, UK: Royal Botanic Gardens, Kew; 1985.

3 Boulos L. *Flora of Egypt*. Cairo, Egypt: Al Hadara Publishing; 1999:174–175.

4 Dalziel JM. *The useful plants of West Tropical Africa*. London, UK: Crown Agents for the Colonies; 1937.

5 Kerharo J, Adam JG. *Traditional Senegalese pharmacopoeia*. Paris, France: Vigot Frère; 1974.

6 Kokwaro JO. *Medicinal plants of East Africa*. Nairobi, Kenya: EA Lit Bureau; 1976.

7 Bah S, Jäger AK, Adsersen A, Diallo D, Paulsen BS. Antiplasmodial and GABA(A)-benzodiazepine receptor binding activities of five plants used in traditional medicine in Mali, West Africa. J Ethnopharmacol 2007; 110(3):451–457.

8 Hassan SW, Umar RA, Lawal M, Bilbis LS, Muhammad BY, Dabai YU. Evaluation of antibacterial activity and phytochemical analysis of root extracts of *Boscia angustifolia*. Afr J Biotechnol 2006; 5:1602–1607.

9 Hassan SW, Lawal M, Muhammad BY, Umar RA, Bilbis LS, Ebbo AA, Dabai YU Isolation and identification of bioactive antibacterial component(s) in root extracts of *Boscia angustifolia* (Capparidaceae). J Pharm Toxicol 2007; 2:366–372.

10 Chini C, Bilia AR, Keita A, Morelli I. Protoalkaloids from *Boscia angustifolia*. Planta Med 1992; 58(5):476.

11 Kim Y, Narayanan S, Chang KO. Inhibition of influenza virus replication by plant-derived isoquercetin. Antiviral Res 2010; 88(2):227–235.

12 Ibrahim AK, Youssef AI, Arafa AS, Ahmed SA. Anti-H5N1 virus flavonoids from *Capparis sinaica* Veill. Nat Prod Res 2013; 27(22):2149–2153.

13 Mitova M, Taskova R, Popov S, Berger RG, Krings U, Handjieva N. GC/MS analysis of some bioactive constituents from *Carthamus lanatus* L. Z Naturforsch C 2003; 58:697–703.

14 Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Food Sci Technol 1995; 28:25–30.

15 Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65(1-2):55–63.

16 Hayden FG, Cote KM, Douglas RG Jr. Plaque inhibition assay for drug susceptibility testing of influenza viruses. Antimicrob Agents Chemother 1980; 17(5):865–870.

17 Rao KV, Owoyale JA. Partial methylation of quercetin: direct synthesis of tamarixetin, ombuin and ayanin. J Heterocycl Chem 1976; 13:1293–1295.

18 In YW, Kim JJ, Kim HJ, Oh SW. Antimicrobial activities of acetic acid, citric acid and lactic acid against *Shigella* species. J Food Saf 2013; 33:79–85.

19 Wang GF, Shi LP, Ren YD, Liu QF, Liu HF, Zhang RJ *et al*. Anti-hepatitis B virus activity of chlorogenic acid, quinic acid and caffeic acid *in vivo* and *in vitro*. Antiviral Res 2009; 83(2):186–190.

20 Rachaman ES, Ashani Y, Leader H, Granoth I, Edery H, Porath G. Sugar-oximes, new potential antidotes against organophosphorus poisoning. Arzneimittelforschung 1979; 29(6):875–876.

21 Letan A. The relation of structure to antioxidant activity of quercetin and some of its derivatives I. Primary activity. J Food Sci 1966; 31:518–523.