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Phytochemical and Pharmacological Evaluations of Ethanolic Extract of *Bassia eriophora*

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ABSTRACT

*Bassia eriophora (Schrad.) Asch. is one of the major and widely distributed wild plants belonging to family Chenopodiaceae and growing in northern Saudi Arabia. In this study, Phytochemical and biological evaluations of the ethanolic extract of B. eriophora have been studied. Six known compounds were isolated; Quercetin (**1**), Quercetin 7-O-β-D-glucoside (**2**), Isorhamnetin 3-O-β-D-glucoside (**3**), Tetradecane (**4**), Pentacosanol (**5**) and Pheophytin a (**6**). Structure elucidation of the isolated compounds was carried out based on different spectroscopic techniques (^{1}H and DEPTQ- NMR, UV and Mass spectrometry) as well as by comparison with authentic samples whenever possible. Acute toxicity, analgesic, anti-inflammatory and skeletal muscle relaxant effects were tested for the ethanolic extract of the plant. The results indicated that the ethanolic extract of B. eriophora has a very low toxicity (LD_{50} of 33.4 g/kg). Additionally, the ethanolic extract in doses of 500 and 750 mg/kg showed significant analgesic effect using hot plate method. While doses of 250, 500 and 750 mg/kg exerted significant anti-inflammatory activities as indicated by carrageenan-induced paw edema in rats and significant relaxing effect as indicated by motor coordination test. Furthermore, cytotoxic and antioxidant activities were performed on the pure isolated compounds (1-6). Compounds 1, 2 and 3 showed similar cytotoxic activity, ranging from mild to moderate effects against MCF-7, HepG-2 and HCT-116 cell lines. Compounds 1, 2, 3 and 6 showed significant antioxidant effect on DPPH free radical.*

Key words: *Bassia eriophora*, flavonoids, LD50, anti-inflammatory, analgesic, cytotoxic, antioxidant.

INTRODUCTION

Chenopodiaceae (Amaranthaceae) is a large family including 174 genera and 2100-2500 species distributed all over the world with high nutritional and medicinal values [1]. Plants of this family are commonly grown in xerophytic, halophytic, tropical and subtropical regions [2]**.** Those plants are characterized by being succulent with well-developed or much reduced leaves and considered a major source for beet sugar [3]. *Bassia* is a genus of flowering plants belonging to family Chenopodiaceae with high nutritional and medicinal values. For instance, *B. muricata* is used in folk medicine as remedy for renal and rheumatic diseases and possess different degrees of anti-inflammatory, analgesic, antipyretic as well as antispasmodic effects [4]. Additionally, *B. latifolia* has antipyretic,

antiprogestational, hepatoprotective, antitumour, anti-inflammatory, analgesic, antiestrogenic and wound healing activities and also used traditionally in rheumatism, ulcers, bleedings and tonsillitis [5]. An infusion of *B. eriophora* has been used traditionally in Darab region of Iran in treatment of Alzheimer's, gingivitis and hair loss [6]. Although it is growing everywhere including northern KSA, the available literature revealed such a little data regarding *B. eriophora.* Therefore, the present study was conducted to investigate such a plant both phytochemically and biologically.

MATERIALS AND METHODS

General experimental procedures:

UV data was recorded on a Hewlett Packard Array 8452A spectrophotometer. NMR spectra were recorded on a Bruker 400 NMR spectrometer operating at 400 MHz (for ^{1}H) and 100 MHz (for ^{13}C) in CD_3OD or $CDCl_3$ solvents and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. ^{13}C multiplicities were determined by the DEPTQ experiment. The EIMS spectra were measured using El/MS 502 mass spectrometer having a direct inlet system and operating at 70eV. Column chromatography for isolation and purification was performed using silica gel 60 (Si gel 60, Merck) and Sephadex LH-20 (Sigma-Aldrich, Germany). Planar chromatography was performed on pre-coated TLC plates with silica gel 60 F_{254} (0.2 mm, Merck). Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4 as a general detecting agent followed by heating at 100°C or 1% ethanolic solution of $AlCl_3$ for flavonoids.

Plant material

B. eriophora aerial parts were collected in April, 2015 from the desert of Aljouf region, northern to Kingdom Saudi Arabia. It was kindly identified by Mr. Hamdan Ogereef Al-Hassan, M.Sc. (Camel and Range Research Center), Aljouf, KSA. A voucher specimen was kept and deposited at the herbarium of Pharmacognosy Department, college of Pharmacy, Aljouf University.

Extraction and isolation

The shade dried aerial parts of *B. eriophora* were finely powdered **(2 kg)** and exhaustively extracted with 70% ethanol (15 L X 3) after defatting for three times with *n*-hexane **(50 g)**. The combined ethanolic extracts were evaporated under reduced pressure to afford **45 g** of dark green residue. The residue **(45 g)** was suspended in 500 ml distilled water and shaken with n-butanol several times until no more yield was obtained to give **(25 g)** residue after combination and evaporation. A part of the n-butanol residue was subjected to Si gel CC and was eluted with CH_2Cl_2-MeOH (100:0→70:30). The collected fractions (each 100 ml) were monitored by TLC using CH_2Cl_2-MeOH of different ratios ranging from 99:1 till 25:75 as developers, and those showing similar TLC profiles were combined to give five major fractions (I-V). Fractions I and III were selected for further purification by repeated flash chromatography with eluent CH_2Cl_2-MeOH mixture.

Fr. I gave three sub-fractions (I_1, I_2 and I_3), the sub-fraction I_2 contained two major spots and was subjected to successive CC and resulted in isolation of compound 1 **(25 mg)** and compound 2 **(15 mg)**. Fr. III was subjected to Si gel CC to give four sub-fractions, from which fr. III_3 was chromatographed on Si gel CC to give compound 3 **(20 mg)** upon elution with CH_2Cl_2-MeOH (80:20).

A part of n-hexane fraction was chromatographed on silica gel column and was eluted with n-hexane: ethyl acetate mixture to give six different fractions (I-IV), fr. II was repeatedly chromatographed to yield five sub-fractions (II_1-II_5). The sub-fraction II_2 afforded compounds 4 **(15 mg)** and 5 **(17 mg)**. The sub-fraction II_3 afforded compound 6 **(17 mg)**.

All isolated secondary metabolites were chromatographed on sephadex LH-20 for further purification (Fig. 1).

Drugs and Chemicals

Carrageenan was purchased from Sigma-Aldrich (Taufkirchen, Germany). Diclofenac sodium and chlorpromazine (CPZ) were kindly obtained from Prince Mutaib Hospital (Sakaka, KSA), all other chemicals and solvents were purchased from Scharlau, Barcelona, Spain. RPMI1640, streptomycin, penicillin and fetal bovine serum were purchased from Invitrogen (Karlsruhe, Germany).

Animals

Male albino Wistar rats weighing 200–250 g and male Swiss albino mice weighing 23–25 g were obtained from the breeding colony of our animal house (College of Pharmacy, Aljouf University, KSA). The animals were maintained at 25 ± 1 °C and 55% relative humidity with 12:12-h light: dark cycle. Animals were supplied with standard rodent chow and water *ad libitum*. Twelve hrs before the experiments, animals were transferred to the laboratory and were maintained only with water *ad libitum*. Animals used in the present study were housed and cared in accordance with the protocols of Aljouf University and experiments were authorized by the Ethical Committee for Animal Care of the University.

Determination of LD_{50}

A preliminary experiments were conducted to find out the lowest dose that kills all mice (lethal dose 100, LD_{100}), the highest dose which fail to kill any of the animals (lethal dose 0, LD_0). Sixty four mice were randomly divided into 8 groups/eight in each group. Eight doses from ethanolic extract of *B. eriophora* with equal logarithmic intervals were orally administered. After treatment, mortality observed in each group within 24 hrs was recorded and calculated according to the Spearman-Karber method [7]**.**

Determination of analgesic activity of the ethanolic extract of *B. eriophora*

Hot plate test:

The analgesic activity of alcoholic extract of *B. eriophora* was evaluated in male Swiss albino mice according to Eddy and Leimbach method [8]. Thirty mice were divided into five groups of six mice each. Mice were pre-selected on the hot plate which maintained at 53 ± 0.5°C. The end point was considered as the time elapsed between placing the mouse on the hot plate and appearance of signs of acute discomfort, characterized by flicking or licking of the hind paw, forepaw or jumping in an attempt to escape from the pain. The maximum reaction time was fixed at 45 sec to prevent any injury to the tissues of the paws. The animals were treated with vehicle (saline, 0.1 ml/10 g, i.p), diclofenac sodium (10 mg/kg, i.p) or plant extract (250, 500 and 750 mg/kg, i.p) and left for 1 hr.

Determination of anti-inflammatory activity of the ethanolic extract of *B. eriophora*

Carrageenan-induced rat paw edema method:

Male Wistar rats weighing 200-250 g were divided into 6 groups, 6 rats each and 3 animals per cage. Each group of the animals were received subplantar administration of 100 µl of saline (control) or 100 µl of carragœnan 1% **(w/v)** in saline with or without 1 hr pre-treatment with i.p injection of the ethanolic extract of *B. eriophora* [9, 10]. Diclofenac sodium was used as a standard anti-inflammatory drug and administered i.p in a dose of 10 mg/kg, 30 minutes prior to carrageenan injection. The thickness of edema was measured by using a dial caliper immediately before subplantar injection and 4 hrs after injection of carrageenan with or without extracts or standard. The assessment of paw thickness was performed always in double blind and by the same operator [11].

The inhibition of edema thickness was calculated using the following formula;

Edema (%) inhibition = (Control value – Sample value / Control value) X 100

Determination of skeletal muscle relaxant effect of the ethanolic extract of *B. eriophora*

Motor coordination test (Rota-rod test):

A rota-rod tread mill device (Insight, Brazil) was used for the evaluation of motor coordination. Initially, 24 h before the test, mice capable of remaining on the rota-rod apparatus longer than 180 s (7 rpm) were selected. One hour after the administration of either *B. eriophora* ethanolic extract (250, 500 and 750 mg/kg, i.p), vehicle (saline/Tween 80, 0.2% i.p; control group) or chlorpromazine (CPZ; 2.5 mg/kg, i.p), each animal was tested on the rota-rod apparatus. The time (s) that the mice were able to remain on top of the bar was recorded for up to 180 s [12]**.**

Determination of cytotoxic activity of the isolated compounds

Cell culture:

Breast cancer (MCF-7), liver cancer (HepG2) and colon cancer (HCT-116) cell lines were obtained from the National Cancer Institute (Cairo, Egypt) and maintained in Roswell Park Memorial Institute medium (RPMI1640) supplemented with 100 mg/ml of streptomycin, 100 units/ml of penicillin and 10% of heat-inactivated fetal bovine serum in a humidified 5% (v/v) CO_2 atmosphere at 37°C.

Cytotoxicity assay:
The cytotoxic activity of the isolated compounds (1-6) was evaluated against breast cancer (MCF-7), liver cancer (HepG-2) and colon cancer (HCT-116) cell lines, using sulphorhodamine-B (SRB) assay method as described by Skehan et al, 1990. Briefly, MCF-7, HepG2 and HCT-116 cells were trypsinized, seeded with appropriate densities (5000 cells/100 µL/well) in 96-well plates. Cells were incubated in a humidified atmosphere at 37°C for 24 h. The cells were incubated with different concentrations from the tested compounds (0.01, 0.1, 1, 10, and 100 µM) or to 1% dimethyl sulfoxide (DMSO) for 72 h. At the end of the incubation time, the cells were fixed with 10% trichloroacetic acid at 4ºC for 1 h. After washing with tap water four times, the cells were incubated with 0.4% SRB for 30 min. Excess dye was removed by washing repeatedly with 1% (v/v) acetic acid. The protein-bound dye was dissolved in 10 mM Tris base solution for OD determination at 510 nm using a SpectraMax plus Microplate Reader (Molecular Devices, CA, USA). Cell viability was expressed relative to the untreated control cells [13]. Negative control cells were incubated without test samples and with or without DMSO. Cells treated with Doxorubicin served as a positive control.

Determination of antioxidant activity of the isolated compounds
Free radical scavenging activity of the pure isolated compounds (1-6) of *B. eriophora* was measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Serial dilutions were prepared using stock solutions (1 mg/mL) of pure compounds to obtain concentrations of 0.5, 0.25, 0.175, 0.087, 0.043, 0.021, 0.010, 0.005, 0.002, and 0.001 mg/mL. Equal volumes of ethanolic solution of 0.1 mM of DPPH and diluted solutions of each pure compound were mixed well by vigorous shaking then allowed to stand at room temp in a dark place for 30 min. The UV absorbance was measured at 517 nm (Vit. E was used as a reference standard) and the experiment was done in triplicate [14]. The average absorption was recorded for all concentrations and the IC_{50} values of each sample, which is the concentration of that sample required to reduce the absorbance at 517 nm by 50% of the DPPH free radical, was calculated according to the following equation:

% inhibition = A (control) - A (test or standard) / A (control) × 100.
Where A = Absorbance

Statistical analysis
Data obtained from the injection of ethanolic extract or the isolated compounds of *Bassia eriophora* for different activities were analyzed using ANOVA followed by Tukey test for multiple comparisons and expressed as mean ± SEM. Differences between the mean of treated animals and control groups were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Column chromatography of the n-butanol and n-hexane fractions of *B. eriophora* afforded six compounds (1-6). Structure elucidation of the isolated secondary metabolites was done on the basis of different spectroscopic measurements (UV, Mass and NMR spectroscopy) and by co-TLC plates against authentic standards whenever possible. The isolated compounds were characterized and identified as follows:

Compound **1 (25 mg)** Was isolated as a deep yellow amorphous powder soluble in MeOH; EI-MS *m/z*: 302.97 $[M+H]^+$; $C_{15}H_{10}O_7$. The ^{1}H-NMR (400 MHz, CD_3OD) δ (ppm): 6.19 (1H, d, *J* = 2.0 Hz, H-6), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 7.75 (1H, d, *J* = 2.0 Hz, H-2'), 6.91 (1H, d, *J* = 8.5 Hz, H-5'), 7.65 (1H, dd, *J*=2.0, 8.5 Hz, H-6'). The ^{13}C-NMR (CD_3OD, 100 MHz) δ (ppm), 146.59 (C-2), 135.81 (C-3), 175.71 (C-4), 161.8 (C-5), 97.83(C-6), 164.13 (C-7), 93.01 (C-8), 156.81 (C-9), 103.11 (C-10), 122.74 (C-1'), 114.59 (C-2'), 144.80 (C-3'), 147.35 (C-4'), 114.82 (C-5'), 122.74 (C-6'). Compound **1** was considered quercetin (Figure 1) and the structure elucidation was confirmed by comparison of the given spectral data with those reported literature for similar compounds [15].

Compound **2 (15 mg)**, was obtained as a pale yellowish amorphous powder freely soluble in MeOH and H_2O; EI-MS *m/z*: 465 $[M+H]^+$; $C_{21}H_{20}O_{12}$. The ^{1}H-NMR (400 MHz, CD_3OD) δ (ppm): 6.22 (1H, d, *J* = 2.0 Hz, H-6), 6.46 (1H, d, *J* = 2.0 Hz, H-8), 7.71 (1H, d, *J* = 2.0 Hz, H-2`), 6.86 (1H, d, *J* =8.5 Hz, H- 5`), 7.66 (1H, dd, *J* = 8.5, 2.0 Hz, H-6`), 5.40 (1H, *d*, *J* = 7.5 Hz, H-1``anomeric proton of glucose), 3.35 – 3.47 (4H, m, H-2``− 5``), 3.52 (1H, *dd*, *J* = 12.0, 5.0 Hz, Ha-6``), 3.75 (1H, *dd*, *J* = 12.0, 2.5 Hz, Hb-6``). The $^{13}$C-NMR (100 MHz, $CD_3OD$) □: 148.52 (C-2), 135.40 (C- 3), 179.77 (C-4), 158.23 (C-5), 96.55 (C-6), 165.31 (C-7), 96.05 (C-8), 160.44 (C-9), 105.24 (C-10), 122.11 (C-1`), 117.85 (C- 2`), 148.53 (C-3`), 146.13 (C-4`), 116.45 (C-5`), 122.82 (C-6`), 104.67 (anomeric carbon of glucose, C-1``), 75.32 (C-2``), 78.51 (C-3``), 75.45 (C-4``), 74.31 (C- 5``), 62.15 (C-6``). Compound **2** was

considered quercetin 7-*O*-β-D-glucoside (Figure 1) and the structure elucidation was confirmed by comparison of the given spectral data with those reported literature for similar compounds [16].

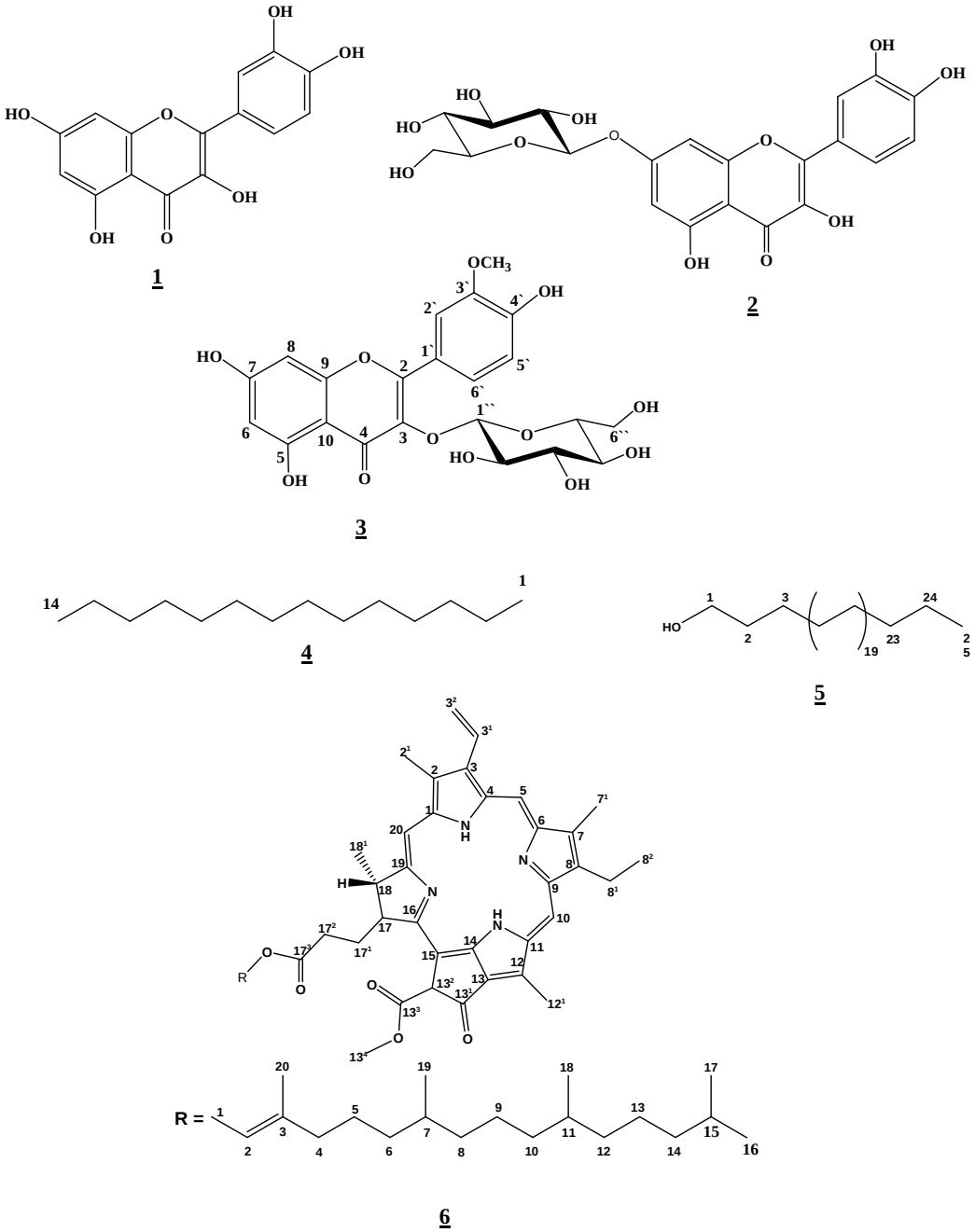

Figure 1. Chemical structures of compounds (1-6) isolated from *B. eriophora*

Compound **3 (20 mg)** was obtained as a pale yellow amorphous powder and was soluble in both MeOH and H_2O; EI-MS *m/z*: 479 $[M+H]^+$; $C_{22}H_{22}O_{12}$. The ^{1}H-NMR (400 MHz, CD_3OD) δ (ppm):
6.24 (1H, *d*, *J* = 2 Hz, H-6), 6.30 (1H, *d*, *J* = 2 Hz, H-8), 7.88 (1H, *d*, *J* = 2 Hz, H-2`), 3.91 (3H, *s*, $OCH_3$ at C-3`), 6.92 (1H, *d*, *J* = 8 Hz, H-5`), 7.58 (1H, *dd*, *J* = 8, 2 Hz, H-6`),5.23 (1H, *d*, *J* = 7.5 Hz, H-1``), 3.17 – 3.31 (1H, m, H-2``), 3.39 (1H, m, H-3``), 3.42 (1H, m, H-4``), 3.45 (1H, m, H-5``), 3.50 (1H, *dd*, *J* = 11.5, 5.5 Hz, Ha-6``), 3.70 (1H, *dd*, *J* = 11.5, 2.0 Hz, HB-6``). The ^{13}C-NMR (100 MHz, CD3OD) δ (ppm): 158.22 (C-2), 134.95 (C-3), 181.34

(C-4), 164.58 (C-5), 99.27 (C-6), 166.32 (C-7), 94.62 (C-8), 158.77 (C-9), 105.33 (C-10), 122.66 (C-1`), 112.93 (C-2`), 149.87 (C-3`), 148.52 (C-4`), 114.47 (C-5`), 123.11 (C-6`), 103.89 (anomeric, C-1``), 74.88 (C-2``), 77.05 (C-3``), 72.02 (C-4``), 76.34 (C-5``), 61.24 (C-6``), 55.51 (OCH_3, C-3`). Compound **3** was considered isorhamnetin-3-O-β-D-glucoside (Figure 1) and the structure elucidation was confirmed by comparison of the given spectral data with those reported literature for similar compounds [17]**.**

Compound **4 (15 mg)** was isolated as a white amorphous powder soluble in $CHCl_3$; EI-MS *m/z*: 199 $[M+H]^+$; $C_{14}H_{30}$. The ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 0.82 (6H, t, J=7.20 Hz, H-1, 14), 1.18 (24H, m, H-2−13). The ^{13}C NMR (100 MHz) δ: 14.12 (C-1,14), 22.70 (C-2,13), 31.94 (C-3, 12), 29.38-29.88 (C-4−11). Compound **4** was considered tetradecane (Figure 1) and the structure elucidation was confirmed by comparison of the given spectral data with those reported literature for similar compounds [18, 19].

Compound **5 (17 mg)** was isolated as white amorphous powder soluble in $CHCl_3$, EI–MS m/z: 369.29 $[M+H]^+$, $C_{25}H_{52}O$. The ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.57 (2H, t, J=6.40 Hz, H-1), 1.49 (2H, m, H-2), 1.18 (44H, m, H-3−24), 0.81 (3H, t, J=7.20 Hz, H-25). The ^{13}C NMR (100 MHz, $CDCl_3$), δ: 63.12 (C-1), 32.83 (C-2), 25.74 (C-3), 29.37-29.71 (C-4−22), 31.93 (C- 23), 22.70 (C- 24), 14.12 (C- 25). Compound **5** was considered Pentacosanol (Figure 1) and the structure elucidation was confirmed by comparison of the given spectral data with those reported literature for similar compounds [19]**.**

Compound **6 (17 mg)** was obtained as a dark green amorphous powder soluble in $CHCl_3$, EI-MS *m/z*: 871 $[M+H]^+$; $C_{55}H_{74}N_4O_5$. The ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 3.31 (3H, s, H-2^1), 7.95 (1H, dd, J= 18.0, 11.6 Hz, H-3^1), 6.10 (1H, d, J= 11.6 Hz, H-3^2), 6.21 (1H, d, J= 18.0 Hz, H-3^2), 9.26 (1H, s, H-5), 3.12 (3H, s, H-7^1), 3.54 (2H, m, H-8^1), 1.60 (3H, t, J=7.2 Hz, H-8^2), 9.41 (1H, s, H-10), 3.60 (3H, s, H-12^1), 6.18 (1H, s, H-13^2), 3.80, (3H, s, H-13^4, OCH_3), 4.14 (1H, d, J=7.6 Hz, H-17), 1.98, 2.25 (each 1H, m, H-17^1), 2.68, 2.73 (each 1H, m, H-17^2), 4.37 (1H, q, J=6.4 Hz, H-18), 1.74 (3H, d, J=6.8 Hz, H-18^1), 8.47 (1H, s, H-20). The phytyl group side chain is characterized by δ: 4.43 (2H, m, H-1), 5.28 (1H, m, H-2), 0.77 characteristic for 4 methyl groups (12 H, d, J=6.4 Hz, H-16, 17, 18, 19), 1.53 (3H, s, H-20). Compound **6** was considered Pheophytin a (Figure 1) and the structure elucidation was confirmed by comparison of the given spectral data with those reported literature for similar compounds [20].

To our best knowledge, this is the first report on the isolation of compounds 1-6 from *B. eriophora*.

Acute toxicity of ethanolic extract of *B. eriophora*

The current study examined the supposed CNS effect of an ethanolic extract of *B. eriophora*. The results showed that *B. eriophora* exerts sedative and hypnotic effect on the CNS. Moreover, it was also found that acute oral administration of *B. eriophora* produced LD_{50} of 33.4 g/kg Therefore, it is possible that ethanolic extract of *B. eriophora* may not be toxic at our experimental doses up to 5000 mg/kg.

Analgesic effect of ethanolic extract of *B. eriophora*

The results of the analgesic effect of ethanolic extract of *B. eriophora* 1 hr after oral administration as indicated by hot plate method showed that there was no significant difference on the thermal stimulus in mice treated with normal saline (negative control). In addition, mice treated with 250 mg/kg plant extract showed no significant changes in reaction time compared to vehicle treated mice. On the other hand, mice treated with 500 mg/kg and 750 mg/kg of ethanolic plant extract showed significant increase in the reaction time compared to negative control group. Furthermore, animals treated with standard diclofenac sodium10 mg/kg showed significant increase in the reaction time compared to vehicle treated mice (Figure 2).

The ethanolic extract of *B. eriophora* failed to increase the reaction time of the mice on hot plate method in 250 mg/kg in this study. Hot plate method produces two measureable behavioral components in response to thermal pain. Responses such as paw licking and jumping in mice are considered to be supraspinally integrated [21]. Thus, the failure of the extract (in a relative low dose) to inhibit these behaviors on hot plate method indicates that it might not be acting at supraspinal level in low doses. In high doses (500 and 750 mg/kg), ethanolic extract *B. eriophora* might be acting on supraspinal level.

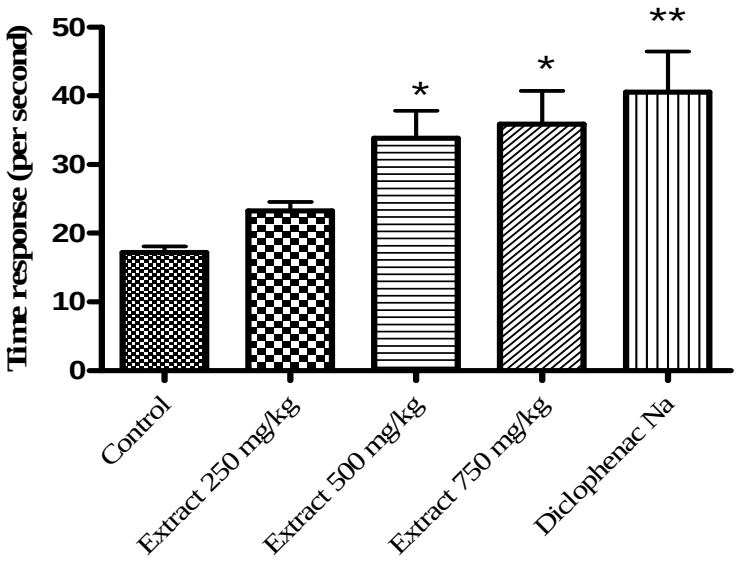

Figure 2: Analgesic activity of EtOH extracts of *B. eriophora* and diclofenac sodium 1 hr after extract administration
*Bars indicate mean ±SEM. *P < 0.05 and **P < 0.01 considered statistically significant compared to the vehicle treated mice control using one way analysis of variance followed by Tukey as multiple comparison. Asterisk; significant different from control.*

Anti-inflammatory activity of ethanolic extract of *B. eriophora*

Acute anti-inflammatory effect of ethanolic extract of *B. eriophora* was evaluated by carrageenan-induced hind paw edema in rats. *B. eriophora* in doses of 250, 500 and 750 mg/kg exerted significant reduction of the edema thickness (1.92±0.067, 1.69±0.112 and 1.58±0.096, respectively) compared to carrageenan injected group with value of 2.404±0.14 (Figure 3). It is known that the inflammatory edema caused by carrageenan in general is reaching its maximum level at the first hour of injection and subsequently starts to diminish. Carrageenan-induced edema is mediated by cell migration and fluid exudation with rapid release of inflammatory mediators including histamine, bradykinin and serotonin [22].

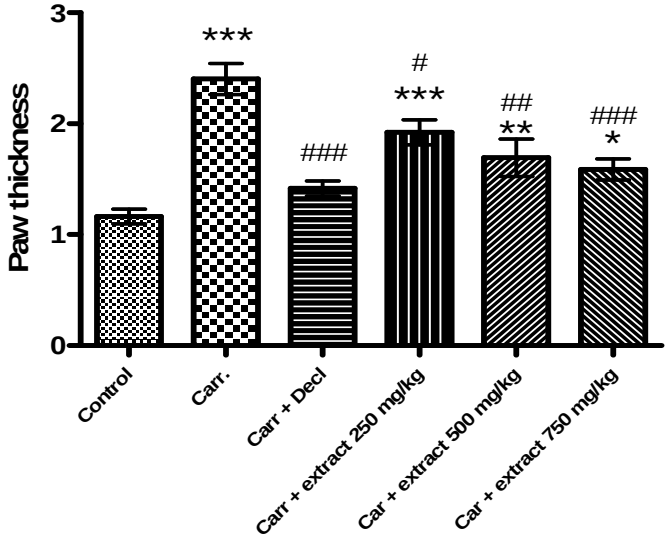

Figure 3: Anti-inflammatory activity of EtOH extract of *B. eriophora* and diclofenac sodium 4 hrs after carrageenan injection
*Bars indicate mean ±SEM. *P < 0.05, **P < 0.01 and ***P < 0.001 considered statistically significant compared to the vehicle treated mice. #P < 0.05, ##P < 0.01 and ###P < 0.001 considered statistically significant compared to the carrageenan-injected mice using one way analysis of variance followed by Tukey as multiple comparison. Asterisk; significant different from control.*

This acute response is maintained by the production of prostaglandin (PGs) and nitric oxide (late phase) which is activated by inducible COX and nitric oxide synthase (iNOS) [23]. In our study, the inhibition of ethanolic extract of *B. eriophora* to carrageenan-induced edema may be mediated by the blocking of the production of histamine, serotonin and bradykinin and consequently the production of inducible PGs and iNOS. This effect is similar to that of all non-steroidal anti-inflammatory drugs such as diclophenac sodium.

Skeletal muscle relaxant effect of *B. eriophora*

The skeletal muscle relaxant effect of ethanolic extract of *B. eriophora* has been shown in figure 4. Mice treated with ethanolic extract of *B. eriophora* at doses of 250, 500 and 750 mg/kg body weight and CPZ at a dose of 2 mg/kg decreased fall-off time (motor coordination). The results obtained from both standard and extract treated groups were compared with the control group. No significant difference between all doses of the plant extract in the motor coordination was observed at 60 min of duration. Mice treated with the standard CPZ (2 mg/kg) showed significant reduction in the falling time (P<0.001) compared to vehicle treated animals (Figure 3).

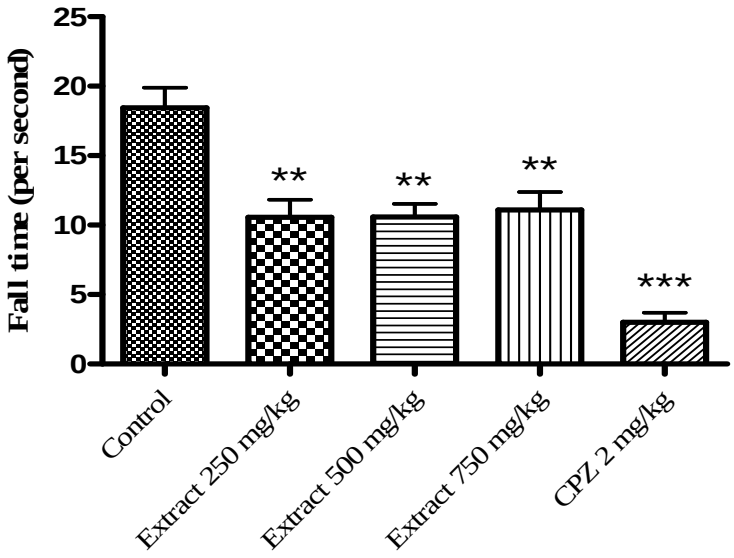

Figure (4): Effect of *Bassia eriophor*a on the reaction time using rota-rod method.

*Data represent mean + SEM. **P < 0.01 and ***P< 0.001 considered statistically significant compared to control using one way analysis of variance followed by Tukey as multiple comparison.*

Cytotoxic activity of the isolated compounds

In this work, cytotoxic activity of the isolated compounds (1-6) was evaluated against breast cancer (MCF-7), liver cancer (HepG-2) and colon cancer (HCT-116) cell lines, using sulphorhodamine-B (SRB) assay method. IC_{50} for all compounds was calculated and represented in µg/ml (Table 1). The results showed that compound 1 has relative strong activity on all cell lines. Compound 2 displayed moderate activity, while compound 3 represented mild activity.

Table 1. Cytotoxic activity of isolated compounds against MCF-7, HepG-2 and HCT-116

Compounds	Cytotoxic activity (IC_{50} µg/ml)		
	MCF-7	HepG-2	HCT-116
1	21.4	25.65	18.45
2	42.56	34.95	38.5
3	77.4	72	67
4	>500	>500	>500
5	>500	>500	>500
6	>500	>500	>500
Doxorubicin	0.1	1.00	1.24

Antioxidant activity of isolated compounds

Determination of the antioxidant activity of pure isolated compounds (1-6) was performed by DPPH scavenging assay. Compounds 1, 2, 3 and 6 showed strong scavenging activity of DPPH with IC_{50} of 36.09±1.71, 53.83±2.1, 88.32±1.04 and 29.67± 3.12, respectively compared to Vit. E (with IC_{50} of 91.6±1.4). On the other hand, compounds 4, 5 and 6 showed no significant scavenging effect against DPPH in comparison to the reference standard (Table 2). Compounds 1, 2, 3 and 6 were able to reduce the stable free radical of DPPH to the yellow colored diphenylpicrylhydrazine. This effect implies that these compounds are capable of donating hydrogen to a free radical in order to remove abnormal electron which is responsible for radical's reactivity.

Table 2: Effect of isolated compounds on the *in vitro* free radical generation

Compounds	DPPH scavenging activity (IC_{50} in µM)[1]
1	36.09±1.71
2	53.83±2.1
3	88.32±1.04
4	-ve
5	-ve
6	29.67± 3.12
Vitamin E[2]	61.6±1.4

[1]IC_{50} denotes the concentration of sample required to scavenge 50% of the DPPH free radicals.
[2]Vitamin E served as a positive control.
Data in table represent mean ± SEM of at least two independent measurements.
-ve = IC_{50} larger than 300 µM.

CONCLUSION

In conclusion, six known metabolites have been isolated for the first time from *B. eriophora* extract. The results indicated that up to 33.4 g/kg dose of *B. eriophora* extract did not produce any symptoms of acute toxicity. Ethanolic extract of *B. eriophora* displayed analgesic and anti-inflammatory activities and supported a future traditional use of this plant in inflammation and pain relief. In addition, the presence of quercetin and its derivatives explained cytotoxic and anti-oxidant activities.

Conflict of Interests

The authors declare that there is no conflict of interests concerning this paper.

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