Anti-inflammatory effect and phenolic isolates of *Alhagi graecorum* Boiss (Family Fabaceae)

Magda T. Ibrahim

Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.
magda.tohamy_1963@windowslive.com

Abstract: Phytochemical investigation of 80 % methanol extract of the aerial part *of Alhagi graecorum Boiss* (Family Fabaceae (resulted in the isolation of five phenolic compounds were identified as one hydrolysable tannin 6-*O*-galloyl-(α/β)-D-glucopyranose (1), and four flavonol glycosides kaempferol 3-*O*-β-D-glucopyranoside (2), kaempferol 3-*O*-(6"-*O*-galloyl)-β-D-glucopyranoside,(3) Myricetin-4`-*O*-α-L-$^{1}C_4$ rhamnopyranoside (4) as well as Myricetin aglycone (5) All known metabolites have been identified in this genus for the first time. The structures were determined by spectroscopic methods (UV, ESI/MS, UV, ^{1}H- and ^{13}C NMR). *In vivo,* the anti-inflammatory activity of aqueous ethanol extract (AGEE) was evaluated using two animal models: the carrageenan induced rat paw edema and cotton pellets induced granuloma formation at dose -dependent manner. These bioactivities compared favorably with diclofenac sodium, which was used as positive control, and confirms the traditional usefulness of this plant for the treatment of inflammatory conditions and arthritis.
[Magda T. Ibrahim. **Anti-inflammatory effect and phenolic isolates of *Alhagi graecorum* Boiss (Family Fabaceae).** *J Am Sci* 2015;11(5):1-7]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 1

Keywords: *Alhagi graecorum*, 6-*O*-galloyl-(α/β)-D-glucopyranose, flavonol glycosides, anti-inflammatory

1. Introduction

Fabaceae is one of the largest families in plant kingdom comprising about 550 genera and more than 13,000 species [(1)].

Alhagi graecorum Bioss. is commonly known as Al-Agool, Shouk Aljemal) Camel thorn) is a shrubby evergreen perennial suffruticose herb, erect to ascending up to 60-100 cm high, very much branched with rigid spiny twigs about 1 inch long. It is native to North Africa the Middle East and Southeast Europe [(2)]. In Egypt *A. graecorum* is widely distributed and seems to have wide ecological amplitude it recorded from Nile region, Oasis, Mediterranean region, Eastern and Western Desert, Red Sea cost and Sinai [(1)]**.** It grows naturally in xeric, halic and mesic habitats [(3)]**.** The species is sometimes confused with *A. maurorum* and the two may be distinct ecotypes or even subspecies [(2)].

Alhagi graecorum Bioss species are being traditionally used as a general tonic, blood purifier, anthelmintic and to treat constipation, jaundice, arthritis, with antimicrobial activity, used for dysentery, upper respiratory system problems, wounds, hemorrhoids, uterus problems and roots are used as aphrodisiac as well as it is a good fodder for camels. *Alhagi graecorum* is now being recognized as *used* for rheumatic pains, liver disorders, urinary tract infection hemorrhoids [(4-7)] and Jaundice [(8)].

A huge number of different chemical constituents were reported in *Alhagi* species such as fatty acids and sterols [(9-11)]. flavonoids [(12-14)]. coumarins[(15)], alkaloids[(15)].This work aims at the isolation and identification of some phenolic compounds from 80 % aqueous methanol extract of the aerial part *of A. graecorum,* and establishing scientific evidence for its anti-inflammatory activity since the acclaimed potency of this plant in inflammatory conditions stimulated our interest to screen the aqueous ethanol extract for effect on inflammation of paw edema induced by the carrageenan and cotton pellets induced granuloma *in vivo.*

2. Experimental

Plant Material

The aerial parts of *Alhagi graecorum* Bioss were collected from the Sewa Oasis during summer 2012. Identification of the plant was verified by the Prof. M. M. Mourad, Professor of Taxonomy, Botany Department, Faculty of Science, Ain- Shams University, and by comparison with plant description in Flora of Egypt [(1, 16)]. A voucher specimen was kept in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Extraction and isolation

The air-dried powder aerial parts of *Alhagi graecorum* (1 kg) were extracted with hot 80% MeOH (5 X 6L) under reflux (60 ˚C). After evaporation of the solvent under reduced pressure, the residue (140 g) was extracted with $CHCl_3$ under reflux (1L X 5L). After evaporation of $CHCl_3$ under vacuum afford 5 g dry $CHCl_3$. The MeOH extract was desalted through precipitation with excess EtOH followed by drying of the filtrate in *vacuo* to give 100 g of brownish residue. It was suspended in water and chromatographed on a polyamide S (Fluka Hannover, Germany) column (5 X 120 cm) and eluted with H_2O followed by increased portions of MeOH to yield 40 fractions of 1L each. The fractions were concentrated under reduced pressure and monitored by paper chromatography (PC) using solvent

systems S_1 and S_2 [S_1: 15% aqueous acetic acid and S_2: *n*-butanol-acetic acid-water (4:1:5, top layer)] and UV-light to be combined into six major collective fractions (I-VI) Fraction I was phenolic free. The dry material of fraction II (10-20% MeOH, 20 g) was redissolved in H_2O and precipitated with excess EtOH to remove undesirable polar non-phenolic substances. Filtrate was subjected to repeated column chromatography (CC) on Sephadex LH-20 (Pharmacia, Uppsala, Sweden) and eluted with methanol to give pure **1** (40 mg). Fraction III (30 % MeOH, 3.5 g) was chromatographed on a microcrystalline cellulose (Merck, Darmstadt, Germany) column employing 30% EtOH in water as eluent, yielding two major subfractions with many other minors. The 1st subfraction was twice fractionated on Sephadex LH-20 using 80 % MeOH for elution to yield crude sample which was purified on Sephadex LH-20 with EtOH to afford **3** (20 mg). The 2nd subfraction was chromatographed on Sephadex LH-20 with *n*-BuOH saturated with water and EtOH yielding mixture of two minor compounds in a small amount to be separated. Fraction IV (40 % EtOH, 3 g) was chromatographed on a microcrystalline cellulose column using 20-80 % methanol as eluent to afford two main subfractions, which were then separately fractionated on Sephadex LH-20 employing the organic layer of *n*-butanol-isopropanol-water (BIW), 4: 1: 5 as eluent. Pure **4** (40 mg) was obtained by being separated on Sephadex LH-20 and eluted with methanol. Fraction V (50-60 % EtOH, 2.4 g) was fractionated on cellulose column using BIW, followed by repeated purification on a Sephadex column with EtOH, yielding pure sample of **2** (32 mg). Fraction VI (70-80%, 5 g) was subjected to fractionation on Sephadex with BIW for elution; the major subfraction obtained was then subjected to repeated column chromatography on Sephadex using *n*-BuOH saturated with water to give **5** (40 mg).The fractions were monitored by TLC (Merck F_{254} plates 20 x 20 cm using S_1 and methanol-ethylacetate-formic acid, 3 : 7 : 0.5), 2D-PC and Comp-PC using Whatmann No. 1 paper (Whatmann, Maidstone, UK systems S_1 and S_2). The compounds were visualized by spraying with Naturstoff (diphenyl borinic acid ethanol amine complex 1% in methanol), followed by ethylene glycol 400 (5% in ethanol v/v) for colour fixation as flavonoids revealing reagent.

General equipments and chemicals

The NMR spectra were recorded at 300 and 500 (^{1}H) and 125 (^{13}C) MHz, on a Varian Mercury 300 and JEOL GX-500 NMR spectrometers. The δ-values are reported as ppm relative to TMS in DMSO. HRESI-MS analyses were run on LTQ-FT-MS spectrometer (Thermo Electron, 400, Germany). UV analyses for pure samples were recorded, separately, as MeOH solutions and with different diagnostic UV shift reagents [17] on a Shimadzu UV 240 (P/N 204-58000) (Columbia, OH, USA) spectrophotometer. All other solvents used for extraction and separation processes were of analytical grade (El-Nasr Chemicals Co., Abou Zaabal, Egypt).

6-*O*-Galloyl-(α/β)-D-glucopyranose [**1**] Off-white amorphous powder; Chromatographic properties, R_f 0.66(S_1), 0.016(S_2) on PC, shine violet fluorescence spot by short UV light turned to blue with $FeCl_3$. UV/Vis λ_{max} (MeOH) nm: 220, 276, - ESI-MS: 331 $[M–H]^-$, 169 $[gallate]^-$, 125 $[gallate–CO_2]^-$ ^{1}H NMR (DMSO-d_6, 500 MHz): Galloyl moiety: 6.97, 6.91 (2H in total, each s, H-2'/6'α,β); Glucose moiety: 5.25 (2/3H, br s, H-1α), 4.80 (1/3H, d, J = 7.8 Hz, H-1β), 4.40-4.25 (3H in total, m, H-5, H-6a/6b in α,β-isomers), 3.90-3.50 (3H, m, H-2, 3, 4). ^{13}C NMR (DMSO-d_6, 125 MHz): Galloyl moiety 165.39 (C-7'α,β), 146.19, 146.10 (C-3'/5' in α,β-isomers), 138.10, 137.90 (C- 4' in α,β-isomers), 119.18, 119.00 (C- 1' in α,β-isomers), 109.71, 109.64 (C-2'/6' in α,β-isomers); Glucose moiety: 95.70 (C-1 β), 91.90 (C-1 α), 76.58 (C-3α), 75.56 (C-5α), 74.81 (C-2α), 74.10 (C-3β), 73.44 (C-2β), 71.12 (C-4 α), 70.25 (C-4β), 67.83 (C-5β), 63.75 (C-6 α,β-isomers).

Kaempferol 3-*O*-β-D-glucopyranoside [**2**] Yellow amorphous powder; Chromatographic properties : R_f 0.41(S_1), 0.05(S_2) on PC, purple fluorescence spot by long UV light turned to yellow with Naturstoff and faint green with $FeCl_3$. UV/Vis λ_{max} (MeOH) nm: 265,301sh348; (+ NaOMe): 277,327, 408; (+ NaOAc): 274,305, 378; (+ NaOAc/H_3BO_3): 271, 305 sh, 360; (+ $AlCl_3$): 274, 305, 353; 398, 408 ;(+ $AlCl_3$/HCl):277, 308, 347, 397. ^{1}H and ^{13}C NMR (500 & 125 MHz, DMSO-d_6): Table **1**.

Kaempferol 3-*O*-(6"-*O*-galloyl)-β-D-glucopyranoside [**3**] Dark yellow amorphous powder; Chromatographic properties: R_f 0.24(S_1), 0.46(S_2) on PC, purple fluorescence spot by long UV light turned to yellow with Naturstoff and deep green with $FeCl_3$. UV/Vis λ_{max} (MeOH) nm: 221,266, 272sh, 301 sh., 350; (+ NaOMe): 278, 325, 405; (+ NaOAc): 274, 305, 378; (+ NaOAc/H_3BO_3): 271, 305 sh, 366; (+ $AlCl_3$): 276, 305, 355; 398 ;(+ $AlCl_3$/HCl): 275, 305, 345, 395. ; ^{1}H and ^{13}C NMR (500 & 125 MHz, DMSO-d_6): Table **1**.

Myricetin-4`-*O*-α-L-^{1}C$_4$ rhamnopyranoside [**4**]

Yellow amorphous powder, Chromatographic properties and UV-spectral data : R_f 0.20(S_1), 0.29(S_2) on PC,Yellow fluorescence spot by long UV light turned to yellow with Naturstoff and faint blue with $FeCl_3$. UV/Vis λ_{max} (MeOH) nm: 253, 302, 373; (+ NaOMe): 284 sh, 319, 421; (+ NaOAc): 325, 385; (+ NaOAc/H_3BO_3): 339, 390; (+ $AlCl_3$): 269, 309 sh, 447 ;(+ $AlCl_3$/HCl): 269, 314, 449. ^{1}H NMR (DMSO-d6, 300 MHz): δ ppm 7.18 (2H, s, H-2`/6`), 6.41 (1H, brs, H-8), 6.17 (1H, brs, H-6), 4.9 (1H, brs, H-1``), 1.09 (3H, brd, H-6",), 3.17- 4.1 (m, remaining sugar

protons). Negative ESI-MS/MS, *m/z* 463.09 $[M-H]^-$ for a MF: C_{21} $H_{19}O_{12}$, 316.04 [M-deoxyrhamnoside]$^-$.

Myricetin [**5**]

Yellow amorphous powder, Chromatographic properties**:** R_f 0.42(S_1), 0.01(S_2) on PC, Yellow fluorescence spot by long UV light turned to red with Naturstoff and faint blue with $FeCl_3$. UV/Vis λ_{max} (MeOH) nm: 254, 302,377; (+ NaOMe): 284 sh, 317.5, 416; (+ NaOAc): 300 sh, 332; (+ NaOAc/H_3BO_3): 265 376; (+ $AlCl_3$): 269, 309 sh, 447 ;(+ $AlCl_3$/HCl): 269, 314, 448.

***In Vivo* Anti-inflammatory Studies**

Carrageenan Induced Rat Paw Edema

The carrageenan induced rat paw edema was carried out as described by Winter *et al.* [18] to evaluate acute anti-inflammatory activity of aqueous ethanol extract. Paw edema was induced by injecting 0.1 ml of 1% (w/v) carrageenan suspension in 0.9% (w/v) sterile saline into the plantar tissue of the left hind paw of all animals, one hour following oral administration of either control vehicle, Diclofenac sodium or plant extract. The right paw served as reference to measure the degree of inflammation in the left one. Increase in paw volume was measured at four hourly intervals, following carrageenan injection, using a plethysmograph [19]. The percentage inhibition of inflammation, calculated as inhibition of edema volume, was calculated [20] as follows:

Percentage Inhibition of Inflammation= (*Vc* -*Vt* / *Vc*) X100

Vt is the average paw edema volume of each extract treated group, as well as Diclofenac sodium group; *Vc* is the paw volume of the negative control group that only received the vehicle.

Cotton Pellets Induced Granuloma in Rats

The granuloma in albino Wistar rats was induced by implanting cotton pellets [21]. All animals were anaesthetized with ether after shaving the fur, and 10 mg of sterile cotton pellets were inserted, one in each axilla. The extract, control vehicle and Diclofenac sodium were administered orally every day for 7 days. On the eighth day, the animals were anaesthetized and the cotton pellets surgically removed and cleaned from extraneous tissues. The moist pellets were weighed, dried at 60° C for 24 h and then re-weighed. Increment in dry weight of pellets was taken as measure of granuloma formation.

Statistical Analysis

Values were expressed as mean ± S.E.M. Statistical significance of weight or volume change was determined by ANOVA, followed by Dunnet's *t*-test; values with $P<0.05$ and $P<0.01$ were considered as statistically significant.

GraphPad Prism version 4.0, GraphPad Software Inc., was used for statistical analysis.

3. Results and Discussion

Compound **1** showed shine violet fluorescence under short UV-light. On complete acid hydrolysis, it yielded gallic acid and glucose (Comp-PC).

Methanol UV spectrum showed absorption band at about λ_{max} 276 nm. Negative ESI/MS that exhibited the corresponding pseudo molecular ion peaks as $[M-H]^-$, together with gallic acid fragments at *m/z* 169 $[gallate]^-$ and 125 $[gallate - CO_2]^-$ [22] so may expected to be of gallotannin nature. Duplication of all ^1H signals (in 1 : 2 ratio) of the ^1H NMR spectrum of **1** together with the two singlets at 6.97 and 6.91 (2H in total) for one galloyl moiety and down field location of CH_2-6 protons signals at the range of 4.40-4.25 were indicative evidences for 6-*O*-galloylglucose structure. Also duplication of C-1 signal at 95.7 (β-isomer) and 91.9 (α-isomer) in ^{13}C NMR spectrum of **1** and the down field shift of C-6 at 63.75 together with all other ^{13}C NMR were confirmative data for α/β-configuration of glucose moiety. J-values and splitting pattern of all sugar protons, especially those of H-1 at 5.25 (br s) and 4.80 (d, J = 7.8), and ^{13}C δ-values indicated $^4C_1$-conformation of glucose [23]**.** Hence, **1** was established as 6-*O*-galloyl-(α/β)-D-$^4C_1$-glucopyranose.

Compounds **2** and **3** showed chromatographic properties (R_f-values, fluorescence under long UV-light and their changes with ammonia vapors and spray reagents) of kaempferol glycosides [17]

Compound **3** gave intense green colour with $FeCl_3$ and indigo-red with KIO_3 spray reagent referring to the presence of a galloyl function. Additionally, **3** showed characteristic MeOH-UV absorption bands of 3-*O*-substituted flavonols (band I at 350 and II at 266 nm); intense absorption band at 266 indicated the probability for galloyl moiety in the structure of **3** [14,24]**.**

On complete acid hydrolysis of **3** released gallic acid in the organic phase together with the aglycone indicating that the structure is galloylglycoside esters.

The ^1H NMR spectra of **2** and **3** exhibited an AX spin coupling system of two *ortho* doublets, 2H each, at δ ~ 8 (H-2'/6') and ~ 6.8 (H-3'/5') indicative to 4'-hydroxy B-ring. A 3, 4-dihydroxy A-ring was deduced from the two *meta* coupled protons, each 1H as broad singlet, at ~ 6.4 (H-8) and 6.2 (H-6). The splitting pattern and J-values of the sugar moiety protons in the aliphatic region specially those of anomeric protons (H-1") and H-3"/4" referred to their $^4C_1$-β-pyranose stereo-structure and confirm glucose moiety. In the aromatic region, one singlet, (2H), was assigned at 6.92 for H-2/6 equivalent protons of one galloyl moiety in the structure of **3**. The attachment of the galloyl moiety to OH-6" was confirmed due to the strong down field shift of CH_2-6" diastereomeric protons at 4.26 (1H, br d, J = 12.5 Hz, H-6"$_a$) and 4.17 (1H, dd, J = 12.5, 5 Hz, H-6"$_b$). These documents were further confirmed by the characteristic down field shift of C-6" resonance at δppm 63.25 (Δ ~ + 3 ppm) in ^{13}C NMR spectrum and

the five ^{13}C-resonances of the galloyl moiety at 166.19 (C-7'''), 145.99 (C-3'''/5'''), 138.60 (C-4'''), 119.82 (C-1'''), and,109.02 (C-2'''/6''')[24]. Accordingly, **2** and **3** were identified as: kaempferol 3-*O*-β-**D**-glucopyranoside and kaempferol 3-*O*-(6''-O-galloyl)-β-**D**-glucopyranoside respectively[25, 26].

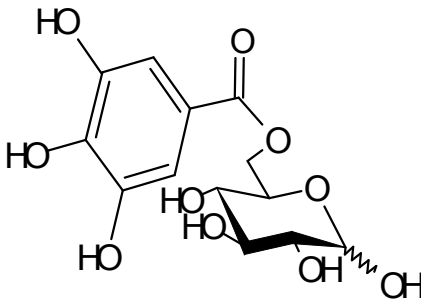
On the basis of its chromatographic properties and UV spectral data, compound 4 was expected to be myrcetin *O*- glycoside like structure. UV-spectrum in MeOH exhibited the two characteristic absorption maxima at λ_{max} 253 (band II) and 373 (band I) for a myrcetin aglycone. Bathochromic shift in band I (+ 29 nm) with decreasing intensity upon addition of NaOMe was indicative for a substituted 4`-OH and OH-3 due to the decomposition in band I [17]. Complete acid hydrolysis produced myrcetin and rhamnose in the organic and aqueous phase respectively (COPC). Negative ESI-MS spectrum exhibits a molecular ion peak at *m/z* 463 corresponding to M. wt 463 and MF $C_{21}H_{19}O_{12}$ and fragment ion at 316 attributable to loss of rhamnosyl moiety.

The ^{1}H NMR **4** showed in the aromatic region a three proton singlet resonances of H-2'/6',, H-8, and H-6 for 3`, 4`, 5`-triihydroxy B- and 5, 7-dihydroxy A-ring protons. The location of sugar moiety at 4' OH and its identification as rhamnose was concluded through the intrinsic upfield location of H-1'' at 4.9 (*brs*) and 1. 08 (*J*= 6). These results was further confirmed by ^{13}C NMR which showed 13 carbon resonances among which the key signals at δ 175.61 (C-4), 107.16 (C2'/ 6'), 145.68 (C-3'/4`) characteristic for myrcetin aglycone. The location of L-rhamnoside moiety on OH-4' was confirmed from the alternative α-up / β down field effect on the resonances of B-ring [17]. This followed from upfield of C- 4' (135.87, Δ 2 ppm), slight downfield of C-3'/ 5' (145.68, Δ 2 ppm), upfield of C-2'/ 6' (107.16, ~Δ 2ppm) and downfield of C-1' at (120.71, Δ 1.5 ppm). The upfield shift of C-2 (- ~10 ppm) and downfield shift of C-3 (+ ~ 3ppm) [26] give another evidence that **4** has free **3** OH in addition to UV data. Accordingly, **4** was identified as myricetin 4`-*O*-α-L-^{1}C$_4$ rhamnopyranoside.

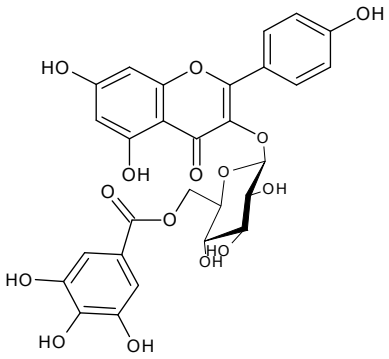
Compound **5** was identified as myricetin by comparisons of its physical data with those reported in the literature [27] and direct CoPC with authentic sample. Also, ^{1}H NMR **5** showed in the aromatic region three proton singlet resonances of H-2'/6',, H-8, and H-6 for 3`, 4`, 5`-triihydroxy B- and 5, 7-dihydroxy A- ring protons that confirm the structure.

Alhagi graecorum was used in folk medicine as a remedy for rheumatic pains, liver disorders, urinary tract infection and for various types of gastrointestinal discomfort.

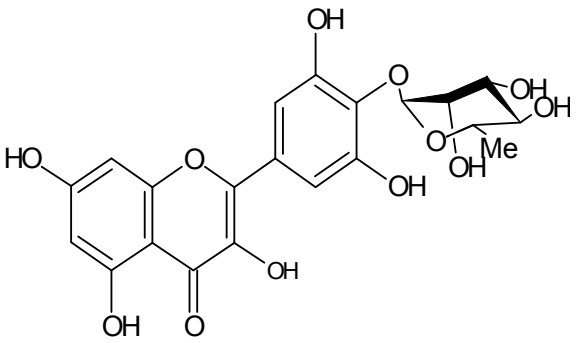
In anti-inflammatory studies, Carrageenan-induced rat paw edema model has been in use to evaluate anti-inflammatory activity of drugs. The carrageenan induced edema develops by mediators in three phases. The initial phase is caused by histamine release, whereas the second phase is mediated by kinin and bradykinin, and the late phase by prostaglandins [28-29]. Most anti-inflammatory drugs are effective at the late phase of edema formation [30]. As shown in Table **1**, Diclofenac sodium 13.5 mg/kg showed significant inhibition of rat paw edema at 4 h (82.0%). At 500 mg/kg, the paw edema inhibition following treatment with aqueous ethanol extract was 79.8%. At this dose, the anti-inflammatory activity of the extract is comparable to Diclofenac sodium, and a significant reduction can be observed even 2 h post induction.



1



3



4

Table 1. ^{1}H and ^{13}C NMR (500, 300 and 125, 75 MHz, DMSO-d_6) of flavonoids 2 -5

No.	2		3		4		5
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_H
2	156.87		156.94		146.74		
3	133,12		133.70		135.87		
4	178.22		177.83		175.61		
5, OH	160.96	12.63 br s	161.72	12.5 br s	160.52	12.5 br s	
6	99.90	6.21 br s	101.31	6.20 br s		6.17 br s	6.18 br s
7	165.03		165.77		164.11		
8	94.45	6.44 br s	94.10	6.44 br s		6.41 br s	6.38 br s
9	156.99		156.84		156.02		
10	103.97		104.44		102.76		
1'	120.11		121.16		120.73		
2'	132.28		131.36				
2'/6'		8.03 d (9.2)		8.06 m	107.16	7.18 br s	7.23 br s
3'	116.85		115.65				
3'/5'		6.87 d (9.2)		6.80 d (9.2)	145.68		
4'	160.37		160.45		135.87		
5'	116.85	6.87 d (9.2)	115.65	6.80 d (9.2)			
6'	129.42	8.03 d (9.2)	131.32				
1"	102.02	5.46 d (7)	101.90	5.46 d (7.6)	98.94	4.9 br s	
2"	73.69	3.70-3.08 m	74.56	3.34-3.08 m	71.47	3.17-4.1 m	
3"	77.05				70.35		
4"	70.74		69.76		72.34		
5"	78.46		76.56		67.61		
6" **$6"_a$** **$6"_b$**	- 60.77		- 63.25	4.26 br d(12.5) 4.17dd(12.5, 5)	17.94	1.08 d(6)	
2/6 galloyl			109.02	6.92 s			

J-values were reported in Hz between parentheses

The anti-inflammatory effect of the extract was further investigated by the cotton pellets induced granuloma formation in rats, which is a model for chronic inflammation. In this model granuloma formation is due to proliferation of inflammatory cells like macrophages, fibroblasts and neutophils [31-32]. Diclofenac sodium and the plant extract reduced the wet cotton pellet weight (Table **2**), an indication of reduction in accumulation of exudates at the inflammation site [32]. Administration of Diclofenac sodium at 13.5 mg/kg resulted in 59.0% weight reduction, whereas, AGEE at 300 and 500 mg/kg reduced the weight by 36.9 and 54.56% respectively suggesting an anti-proliferative activity [31, 33]. Pharmacological screening of extract of *Alhaji* has revealed that it possesses anti-inflammatory effect. These results in agreement with those reported in the literature [34].

Table 2. Anti-inflammatory Activity of AGEE in Carrageenan Induced Rat Paw Edema Model.

Group / Dose (mg/kg)	Increase in Paw Volume (ml)			
	1h	2h	3h	4h
Control	0.59±0.09	0.13±0.71	0.88±0.17	0.11±0.89
Diclofenac sodium 13.5	0.03±0.23 (51.1)	0.23±0.12* (67.6)	0.18±0.12* (79.6)	0.16±0.07** (82.0)
AGEE 200 mg/kg	0.22±38.0 (19.2)	0.34±0.11 (52.1)	0.28±0.11 (68.2)	0.26±0.15* (70.8)
AGEE 300 mg/kg	0.16±0.32 (45.16)	0.31±0.09 (56.3)	0.24±0.14* (72.7)	0.21±0.19* (76.4)
AGEE 500 mg/kg	0.09±0.25 (59.63)	0.22±0.12* (69.0)	0.20±013* (77.3)	0.18±0.04** (79.8)

Notes: All the result are expressed in term of Mean ± S.E.M., n=6 animals in each group; number in parenthesis indicates percentage inhibition in increase in paw volume. Statistical significance was determined by ANOVA, followed by Dunnet's *t*-test. *P <0.05, ** P <0.01, statistically significant.

Table 3. Effects of AGEE on Cotton Pellets Induced Granuloma Formation in Rats

Group / Dose (mg/kg)	Moist Cotton Pellet		Dried Cotton Pellet	
	Weight (mg(	% inhibition	Weight (mg(	% inhibition
Control	210.80±11.6	-	50.5±2.4	-
Diclofenac sodium 13.5	93.26±5.8**	55.76	20.5±0.5**	59.41
AGEE 200 mg/kg	170.48±10.4	19.13	40.22 ±1.3	20.356
AGEE 300 mg/kg	130.80±8.7*	38.09	31.93±1.4 *	36.77
AGEE 500 mg/kg	114.60±7.8**	45.64	22. 9±0.7**	54.65

Notes: All the result are expressed in term of Mean ± S.E.M. n=6 animals in each group; Statistical significance was determined by ANOVA, followed by Dunnet's *t*-test. * P<0.05, ** P<0.01, statistically significant.

Acknowledgment

The authors would like to thank Prof. Dr. Laila. Ghazy Dept. of Biology-Egyptian Atomic Energy Authority for her assistance concerning the anti-inflammatory studies.

References

1. Boulos L. Flora of Egypt Checklist Al Hadara Publishing Cairo Egypt pages 2009 ; 410
2. Awmack C S and Lock J M. The genus *Alhagi* (Leguminosae. Papilionoideae) in the Middle East. Kew Bull. 2002; 57: 435-445.
3. Hassanein A M and Mazen M A Adventitious bud formation in *Alhagi graecorum* plant cell Tissue Org. Cult. 2001; 65: 31- 35.
4. Nedhal A Al-Douri L and Al-Essa Y A. Survey of Plants Used in Iraqi Traditional Medicine Jordan Journal of Pharmaceutical Medicine Sciences 2010 ; 3(2): 100-108.
5. Mohamed Eldesouky Z, Amani S A, Mounerah R Al-Outhman and Reham M El-Meligy. Antimicrobial activities of Saudi Arabian desert plants. Phytopharmacology. 2012; 2(1): 106-113.
6. Harvey A L. Medicines from nature: are natural products still relevant to drug discovery?. Trends in Pharmacological Sciences, 1999; 20: 196-198.
7. Awaad A S, Maitland D J and Soliman G A. Antiulcerogenic Activity of *Alhagi maurorum*, Pharmaceutical Biology. 2006; 44(4): 292-296.
8. Mohammad S A, Mohammad R J and Mohammad E T Y. Ethno-Medicinal Plants Used to Cure Jaundice by Traditional Healers of Mashhad, Iran. Iranian Journal of Pharmaceutical Research. 2014; 13 (1): 157-162
9. Ghosal S, Srivastara R S, Battacharya S Kand Debanath P K. The active principles of *Alhagi pseuddalhagi* phenethylamine and tetrahydroisoquinoline bases. Planta Med. 1974; 26: 318–326.
10. Kudliki W P, William D, Kramer S K, Makhamed B G and Iskakov B K.: Eukaryotic protein synthesis initiation factor 2. Eur J Biochem. 1991; 197: 623–629.
11. Kalhoro M A, Kapadia Z and Badar Y.: Physicochemical studies of indigenous medicinal plants. Bangladesh J. Sci. Ind. Res. 1997; 32: 418–421.
12. Al-Yahya M A, Mossa J S, Al-Badr A A, Tariq M S and Al- Meshal I A. Phytochemical and biological studies on Saudi medicinal plants Part 12. A study on Saudi plants of family Leguminosae. Int. J. Crud Drug Res. 1987; 25: 65–71.
13. El-Saayed N H, Inshak M S, Kandil F I and Mabry T J. Flavonoids of *Alhagi graecorum*. Pharmazie. 1993; 48: 68–89.
14. Singh V P, Bineeta Y and Pandey V B. Flavanone glycosides from *Alhagi pseudalhagi*. Phytochemistry 1999; 51: 587–590.
15. Behari M and Gupt S C. The isolation and biogenesis of 24-alkylsterols in *Alhagi pseudoalhagi*. Acta. Cienc. Indica Chem. 1980; 6: 207–208.
16. Tackholm V. Students Flora of Egypt, 2nd ed. Cairo, Egypt, The Cooperative Printing Company, 1974; 657.
17. Mabry T J, Markham K R, Thomas M B. The systematic identification of flavonoids. In: *The ultraviolet spectra of flavones and flavonols*, Part II, Chapter V, Berlin: Springer Verlag 1970; 41-164.
18. Winter C A, Risley E A, Nuss G W. Carrageenan induced edema in hind paw of the rat as an assay for anti inflammatory drugs. *Proc. Soc. Exp. Bio. Med.* 1962; 111, 544–547.
19. Harris J M, Spencer P S J. A modified plythesmographic apparatus for recording

volume changes in rat paw. *J. Pharma. Pharmacol*. 1962; 14, 464-466.
20. Suleyman H, Demirezer L O, Kuruuzum A, Banoglu, Z N, Gocer F, Ozabakir G, Gepdiremen A. Anti-inflammatory effect of the aqueous extract from *Rumex patientia* L. roots. *J. Ethnopharmacol.* 1999; *65*, 141-148.
21. D'Arcy P F, Howard, E M, Muggleton P W, Townsend S B. The antiinflammatory action of griseofulvin in experimental animals. *J. Pharm. Pharmacol*. 1960; 12, 659-665.
22. Okuda T, Yoshida T and Hatano T. New Methods of Analyzing Tannins. *J. Nat. Prod.* 1989; 52, 1-31.
23. Gupta R K, Al-Shafi S M, Layden K and Haslam E. The Metabolism of Gallic Acid and Hexahydroxydiphenic Acid in Plants. Part 2. Esters of (S)-Hexahydroxydiphenic Acid with D-glucopyranose ($^4C_1$). *J. Chem. Soc. Perkin. Trans I*. 1982; 2525-2534.
24. Moharram F A, Marzouk M S A, Ibrahim M T and Mabry T J. Antioxidant Galloylated Flavonol Glycosides from *Calliandra haematocephola*. *Nat. Prod. Res*. 2006; 20, 927- 34.
25. Kim Y, Jang D-S, Park S-H., Yun J, Min B-K, Min K R and Lee H-K,. Flavonol glycoside Gallate and Ferulate Esters from *Persicaria Lapathifolia* Inhibitors of Superoxide Production in Human Monocytes Stimulated by Unopsonized Zynosan. *Planta Med.* 2000; 66, 72- 74.
26. Agrawal P K and Bansal M C. Flavonoid Glycosides. In *Studies in Organic Chemistry* 39*, ^{13}C NMR of Flavonoids*. Agrawal PK (ed). Elsevier: New York. 1989; 283-364.
27. Marzouk M S, El Dib R A, Tawfike A F, Moharram F A, El-Shenawy S M. Polyphenolic profile and biological study of *Oenothera speciosa Natural Product Communications*. 2009; 14(4), 1456-1467.
28. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan oedema in rats. *J. Pharmacol. Exp. Therap.* 1969; *166*, 96–103.
29. DiRosa M, Giroud J P, Willoughby D A. Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *J. Pathol.* 1971; 104, 15–29.
30. Niemegeers C J E, Verbruggen F J, Janssen P A J. Effect of various drugs on carrageenan-induced oedema in the rat hind paw. *J. Pharmacol*. 1964; 16, 810–816.
31. Swingle K F, Shideman F E. Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain anti-inflammatory agents. *J. Pharmacol. Exp. Ther*. 1972; 183, 226–234.
32. Snedegard G. Mediators of vascular permeability in inflammation. *Prog. Appl. Microcir.*, 1985; *7*, 96-112.
33. Spector W G. The granulomatous inflammatory exudates. *Int. Rev. Exp. Pathol*. 1969; *8*, 1–55.
34. Nadheerah F N. A Pharmacological Evaluation of Aqueous Extract of *Alhagi Maurorum* Global Journal of Pharmacology 2012; 6 (1): 41-46.

3/31/2015