Research Journal of

Medicinal Plant

ISSN 1819-3455

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 5 (5): 531-546, 2011
ISSN 1819-3455 / DOI: 10.3923/rjmp.2011.531.546
© 2011 Academic Journals Inc.

Chemical Constituents and Biological Activities of *Fagonia indica* Burm F.

[1]Naglaa G. Shehab, [2]Amina Mahdy, [3]Saeed A. Khan and [4]Salma M. Noureddin
[1]Department of Pharmacognosy,
[2]Department of Pharmacology and Therapeutics,
[3]Department of Pharmaceutical and Medicinal Chemistry, Dubai Pharmacy College, UAE
[4]Dubai Pharmacy College, UAE

Corresponding Author: Naglaa G. Shehab, Department of Pharmacognosy, Dubai Pharmacy College, UAE Tel: 00971 503212716 Fax: 0097142646740

ABSTRACT

Four flavonoidal compounds identified as quercetin, isorhamnetin-α-3-O rhamnoside, quercetin 3-O-β-D-glucopyranosyl -(1''-6''')-β-D-glucopyranoside and quercetin 3-O-β-D-galactopyranosyl -(6''-1''')- α-L-2''' acetyl rhamnose-(3'''-1'''') β-D-glucopyranoside were for the first time isolated from the ethyl acetate and n-butanol soluble fractions of the alcoholic extract of *Fagonia indica* Burm F. in addition to oleanolic acid, β-sitosterol-3-O-β-D-glucoside and stigmasterol 3-O-β-D-glucoside. Flavonoids amounted to 3% as estimated colorimetrically by using aluminum chloride. The analgesic activity of the alcoholic extract of *Fagonia indica* Burm F. was tested by the writhing and the hot-plate tests using acetyl salicylic acid (200 mg kg^{-1}, i.p.,) and morphine (10 mg kg^{-1}, i.p.,) as reference drugs. The cytotoxic and the antimicrobial activities of the alcoholic extract as well as its fractions were also determined. The alcoholic extract elicited activity on both acetic acid-induced writhing response as well as hot plate test in mice showing its central and peripheral mediated antinociceptive action. The alcoholic extract as well as its fractions exhibited marked antitumor activity against the two cell lines tested with the ethyl acetate fraction showing the least IC_{50} against both carcinoma cells MCF7 and HCT cells while the chloroform fraction showed highest antimicrobial activity against *Pseudomonas aeruginosa*.

Key words: *Fagonia indica*, analgesic, antimicrobial, anti-tumor

INTRODUCTION

Genus *Fagonia* includes about 35 species that are distributed in the deserts and dry areas in India, tropical Africa, Chile and South West USA. Many of the species are spiny herbs or sub-shrubs (Chopra *et al*., 1982). *Fagonia* species are reported to be medicinal in the scientific literature as well as in folk medicine. It was reported that some species have cytotoxic (Ibrahim *et al*., 2007) anti-microbial (Gehlot and Bohra, 2000) and antihypertensive (Gibbona and Oriowo, 2001) activities. These activities were attributed to the presence of a variety of active ingredients including triterpenoidal saponins (Abdel Khalik *et al*., 2000), flavonol glycosides (Lamyaa *et al*., 2008) as well as acids like ursolic and oleanolic acids either alone or with their derivatives (El-Wakil, 2007).

Fagonia indica Burm F. (Mushikka or white spine) is a plant distributed in the deserts of Asia and Africa (Beier *et al*., 2004). It is used in folk medicine for cancer as well as most of the disorders

considered to be due to poisons. Survey of literature revealed few reports indicating the presence of saponins and oleanolic acid in *Fagonia indica* Burm F. (Shaker *et al*., 1999; Yeung and Che, 2009) and almost no reports about isolation of the flavonoidal constituents. Moreover, only few studies investigated the pharmacological effects of the plant extract (Soomro and Jafarey, 2003; Mandeel and Taha, 2005) with no reports about its possible analgesic effect or antitumor activity using two cell lines (breast and colon).

The aim of the present study therefore is to determine the constituents of the alcoholic extract of *Fagonia indica* Burm F. to evaluate the acute toxicity of the plant extract as a measure of its safety and to investigate the possible antinociceptive as well as the anti-tumor activities of the total alcohol and the fractions prepared. This is considered as a new challenge since no published data explored this area of research.

MATERIALS AND METHODS

Plant material: The whole plants of *Fagonia indica* Burm F. were collected during July till August (2008) from desert plants growing in Dubai, United Arab Emirates, while the whole research study has been performed during the period from July to the end of November (2008). Identification was kindly verified by Dr. Hassnaa Ahmed Hosny, Professor of Plant Taxonomy, Department of Botany, Faculty of Science, Cairo University. Vouchers specimens were kept at the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt.

Equipments for phytochemical investigation: TLC was carried out on pre-coated silica gel 60 F_{254} plates (E.Merck). Stationary phases used for column chromatographic fractionation viz., Silica gel G 60, RP-18 (E. Merck) and Sephadex LH-20 (Sigma- Aldrich). M.P. was measured on a Digital melting point apparatus (Electrothermal IA 9000 series) and was uncorrected. UV absorption spectra were run on a Shimadzu 1700 spectrophotometer. ^{1}H-NMR spectra were obtained at 500 MHz and ^{13}C -NMR spectra at 125 MHz on JEOL GX-500 spectrometer with the chemical shifts (δ ppm) expressed relative to TMS as internal standard.

Animals: Male albino mice, weighing 20-25 g were provided by the Pharmacology Department, Dubai Pharmacy College. They were located in a special well-equipped atmosphere and had free access to food and water. They are left for a period of one week for accommodation before performing the experiments.

Drugs, chemicals and solvents: Acetyl salicylic acid and naloxone hydrochloride were purchased from Sigma, USA; morphine hydrochloride from E. Merck, glacial acetic acid from Fisher Scientific, USA; sodium bicarbonate and all organic solvents used were obtained from El-Nasr Chem. Co., Cairo, Egypt.

Microorganisms:

- Gram positive bacteria: *Staphylococcus aureus* and *streptococcus beta hemolytica*
- Gram negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and *proteus*
- Fungi*; Candida albicans:* All were obtained from Microbiology Department, Dubai Medical College

Anti-tumor cell lines: Mammary carcinoma F7cells (MCF7) and Human colon tumor cells (HCT) were purchased from National Cancer Institute, Cairo University, Egypt.

Preparation of the different plant extracts: Alcoholic extract of the plant under investigation was obtained by exhaustive cold maceration of air-dried powdered plant (1.370 kg) in ethanol (70%). The alcoholic extract was then evaporated to dryness, under vacuum, at a temperature not exceeding 50°C. The solvent-free residue (40.0 g) was suspended in water and fractionated by successive extraction with n-hexane, chloroform, ethyl acetate and n-butanol saturated with water. The extracting solvent, in each case, was removed by vacuum distillation. For biological study, the different extracts were reconstituted with distilled water or with tween 80.

Phytochemical study

Colorimetric estimation of the flavonoid content: One gram of air-dried defatted powdered plant was subjected to quantization of their flavonoid content. The method adopted is based on measuring the intensity of the yellow color developed when flavonoids are mixed with aluminum chloride (Mabry *et al.*, 1970). The percentage of flavonoids was calculated as quercetin with reference to a pre-established standard calibration curve using different concentrations (ranging from 8-200 μg 5 mL^{-1}) of 0.04% w/v ethanolic solution of quercetin. Results obtained were the average of triplicate experiments.

Investigation of the chloroform extract: A portion (7.1 g) of the chloroform fraction was applied on VLC column (25×5 cm). Gradient elution was performed with hexane-chloroform, chloroform-ethyl acetate mixtures. Fractions, 100 mL each, were collected and monitored by TLC (solvent systems, chloroform methanol 9.7:0.3 and 9:1, spots visualization, by examination under UV before and after exposure to NH_3 vapor and by spraying with P-anisaldehyde). Those shows the same chromatographic patterns were pooled yielding five collective fractions (Fc1-Fc5).

Fc4 (Column system, chloroform-ethyl acetate, 85:15; 0.5 g and showed 5 spots with R_F values 0.77, 0.70, 0.64, 0.60 and 0.50) and Fc5 (Column system, chloroform-ethyl acetate, 70:30; 0.61 g and showed 6 spots with R_F values 0.60, 0.41, 0.40, 0.23, 0.15 and 0.09) were subjected to refractionation on Reversed Phase Silica (RP-18) columns by using methanol-water mixtures, afforded 3 compounds S_1-S_3, respectively.

Investigation of the ethyl acetate and n-butanol extracts: Portions (3.99 and 6.3 g) of the ethyl acetate and butanol extracts respectively were fractionated on Sephadex LH-20 column (52.5×2.5 cm). Gradient elution was performed with water-methanol mixtures followed by methanol. Fractions (50 mL each) were collected and monitored by TLC using solvent system chloroform-methanol 8.5:1.5 and 8:2; spots were visualized by examination under UV before and after exposure to NH_3 vapor and by spraying with p-anisaldehyde. Similar fractions were pooled to yield collective fractions (Fe1-Fe4 from ethyl acetate and Fb1-Fb4 from butanol extracts). Fe2 (coming f rom Sephadex column system, methanol-water 50:50; 0.56 g, showed 2 spots with R_F: 0.37 and 0.11) and Fe3 (coming from Sephadex column system, methanol- water 60:40; 0.27 g, showed 3 spots with R_F: 0.60, 0.50 and 0.37) and Fb2 (coming from sephadex column system, methanol-water 8:2; 0.6 g; and showed 3 spots with R_F : 0.83, 0.45 and 0.18) were subjected to refractionation, on Sephadex LH- 20 columns by isocratic elution using methanol -water 8:2, afforded 4 compounds S_4-S_7, respectively which gave positive tests for flavonoids.

The identification of all isolated compounds was based on physicochemical and spectral analysis as well as on comparison with reference samples and published data.

Biological study

Acute toxicity testing of the total plant alcoholic extract: The acute toxicity of the extract was tested in mice by both oral and intraperitoneal routes. Several doses of the extract were chosen in a manner allowing for equal logarithmic intervals between the different doses. Accordingly, the chosen doses were 32, 64, 128, 256, 512, 1024, 2048 and 4096 mg kg^{-1} body weight to different groups of mice, each consisting of 10 animals. For oral toxicity testing, food was withheld from the animals overnight before administration of the extract. The extract was freshly prepared by reconstitution in distilled water. Mortality was assessed over two consecutive days and animals were observed for any illness or abnormal behavior.

Antinociceptive activity of the total plant alcoholic extract

Writhing test: Thirty five mice have been selected and divided into 5 groups consisting of 7 animals each. Three groups were treated with the alcoholic extract of *Fagonia indica* Burm F. at doses (2, 5 and 10 mg kg^{-1}). Aspirin at a dose of 200 mg kg^{-1} (Sarra *et al.*, 2005) or normal saline for control were injected i.p. to the rest 2 groups of mice. Injection was carried out 30 min before induction of writhing. Trial of higher doses of the extract (25, 50 and 100 mg kg^{-1}) has been also carried out (data not shown). The test was conducted according to that described by Koster *et al.* (1959). The writhing response was elicited by an i.p. injection of 0.1 mL 0.6% acetic acid. Animals were then placed individually into glass beakers and 5 min were allowed to elapse before they were observed for 10 min. The number of writhes during 10 min was recorded for each animal. Significant reduction in the number of writhes compared to the control was considered analgesic response.

Hot-plate test: Two sets of experiments have been carried out. Each set consisted of five groups of mice of 8 animals each. In the first set, the first group was given normal saline and served as control group while the other four groups were i.p., injected with either morphine at a dose of 10 mg kg^{-1} (Natorska and Plytycz, 2005) or increasing doses of the plant alcoholic extract (2, 5 and 10 mg kg^{-1}). In the second set, animals were subjected to the same treatment regimens as in the first one except that the mice in groups 2-5 were injected with naloxone at a dose of 2 mg kg^{-1}, i.p., (Wibool *et al.*, 2008) 15 min before administration of the stated agent. All mice were subjected to the hot-plate test before any treatment, then, the stated agents were given in a total volume of 0.2 mL. The hot-plate test was performed at 30 and 60 min thereafter. The test was conducted according to that described by Eddy and Leimbach (1953). Mice are brought to the testing room and allowed to acclimatize for 10 min before the test begins. Pain reflexes in response to the thermal stimulus are measured using a Hot-Plate Analgesia Meter (Ugo Basile, Varese, Italy). The surface of the hot plate is heated to a constant temperature of 55±0.5°C, as measured by a built-in digital thermometer. Mice are placed on the hot plate which is surrounded by a clear acrylic cage and the latency to respond with either a hindpaw lick, or jump is measured to the nearest 0.1 second. The mouse is then immediately removed from the hot plate. A maximum cut-off period of 30 seconds was applied to avoid nerve damage. Animals are tested once at a time and are not habituated to the apparatus prior to testing.

Statistical analysis: For results of the writhing experiment, data were analyzed by Kruskal-Wallis non parametric one-way ANOVA followed by Dunn's multiple comparison test. For results of the hot-plate experiment, data were analyzed by one-way ANOVA followed by

Tukey-Kramer multiple comparison test (Armitage and Berry, 1987). For both statistical procedures, differences at $p<0.05$ were considered significant.

Screening for anti-microbial activity towards selected microorganisms: The alcohol and the extracts prepared from it were subjected to in-vitro screening for antimicrobial activity. The samples were tested at dose of 200 mg mL^{-1} using Tween 80 to assist solubility. Selected strains of bacteria and fungi were used adopting the agar diffusion technique cup method for bacteria and fungi (Lorian, 1991). The effect of the different samples were compared with broad spectrum antibiotics; Penicillin 10 mg and Doxycycline HCl 30 mg. Evaluation of the antimicrobial activity was based on measuring the diameters of the observed zones of the inhibition in mm.

Anti-tumor activities of the total plant alcoholic extract, the ethyl acetate and the butanol fractions: Potential cytotoxicity of the total alcoholic extract and the ethyl acetate and butanol fractions prepared from the parent alcoholic extract, were tested using the method of Skehan *et al.* (1990). Intensity of the color was measured at λ570 nm in an ELISA reader. The percentage of surviving fraction of each tumor cell line was recorded for each concentration of the different extracts tested.

RESULTS AND DISCUSSION

Phytochemical study

Colorimetric estimation of the flavonoid content: The percentage of flavonoids as determined by $AlCl_3$ method for the whole plant was found to be 3.5%.

Constituents of the chloroform extract

Compound S_1: white needles crystals, 20 mg, R_F value 0.5, m.p. 310-312°C (methanol) was soluble in chloroform-methanol mixture. It exhibited the following spectra data: ^{1}H-NMR, δ(500 MHz, DMSO): 0.77, 0.79, 0.81 0.93, 0.94, 1.0, 1.20, (7x CH_3) 3.94 (1H, m, H-3), 5.3 (1H, t, olefinic H-12).

Compound S_2: white needle crystals, 50 mg, R_F value 0.41; m.p. 140-143 °C (methanol) was soluble in chloroform-methanol mixture. It exhibited the following spectra data: ^{1}H-NMR, δ(500 MHz, DMSO): 0.75 (3H, s, Me-18), 0.78 (3H, d, J = 7.2 Hz, H-26), 0.80, (3H, d, J = 7.2 Hz, H-27), 0 .87 (3H, t, J = 8.4 Hz, H-29), 0.94 (3H, d, J = 6 Hz, H-21), 1.20 (3H, s, H-19), 3.94 (1H, m, H-3), 4.66 (1H, d, J = 8Hz, H-1π, Glc.), 5.36 (1H, s, H-6).

Compound S_3: white needles crystals, 30 mg, R_F value 0.40, m.p. 170°C (methanol) was soluble in chloroform-methanol mixture. It exhibited the following spectra data: ^{1}H-NMR, δ(500 MHz, DMSO): 0.60 (3H, s, Me-18), 0.81 (3H, d, J = 7.2 Hz, H-26), 0.88, (3H, d, J = 7.2 Hz, H-27), 0.92 (3H, t, J = 8.4 Hz, H-29), 1.2 (3H, d, J = 6 Hz, H-21), 1.50 (3H, s, H-19), 3.94 (1H, m, H-3), 4.40 (1H, d, J = 8Hz, H-1π, Glc.), 5.21 (2H, m, H-23), 5.40 (1H, s, H-6).

^{13}C-NMR data for compounds S_1-S_3 was present in Table 1.

The ^{1}H-NMR spectrum of compound S_1 showed 7 methyl groups at δH 0.77 0.79, 0.81, 0.93, 0.94, 1.0 and 1.2. The signal at δH 5.3 was due to the olefinic proton at C-12 while the proton germinal to the hydroxyl group was observed at δH 3.94. From the ^{1}H-NMR and ^{13}C-NMR and in accordance with the published data (Adesina and Reisch, 1985), Compound S_1 is identified as oleanolic acid.

Table 1: ^{13}C-NMR data (125 MHz, δ ppm) of compounds S_1-S_3 isolated from chloroform extract of *Fagonia indica* Burm F.

Compound No.	Compound S_1	Compound S_2	Compound S_3
1	33.2	37.2	37.3
2	22.0	31.5	31.6
3	73.0	71.7	71.7
4	33.6	42.3	42.0
5	50.0	140.1	140.1
6	13.1	121.8	121.8
7	27.0	31.9	31.9
8	34.1	31.9	31.9
9	42.5	50.1	50.0
10	31.9	36.2	36.5
11	18.1	21.1	21.0
12	120.1	39.8	39.7
13	140.0	42.3	42.2
14	36.7	56.7	56.8
15	22.9	24.3	24.3
16	18.2	28.2	28.9
17	41.4	56.0	42.2
18	35.8	18.8	18.8
19	40.9	11.8	12.2
20	25.5	36.0	40.5
21	28.0	18.5	21.0
22	26.3	33.9	138.1
23	22.5	26.1	129.2
24	10.4	45.5	51.2
25	10.2	29.1	31.9
26	11.8	19.0	19.0
27	20.7	19.8	21.0
28	181.0 (CO)	23.0	25.0
29	27.0	11.6	12.0
30	17.8	-	-
1'	-	103.7	103.7
2'	-	073.7	73.7
3'	-	075.5	75.5
4'	-	070.3	70.3
5'	-	075.5	75.5
6'	-	061.7	61.7

The ^{1}H-NMR spectra of compounds S_2 and S_3 demonstrated the characteristic features of a steroidal nucleus, this is in accordance with the previous findings of Goad and Toshihiro, (1997). Both compounds showed presence of 6 methyl groups. The two methyl singlet at δH 0.6-0.75 and 1.20 were assigned for H-18 and H-19 respectively. The three doublets at δH 0.78-0.81, 0.80 -0.88 and 0.94-1.20 were attributed to H-26, H-27 and H-21, respectively, while the triplet at δH 0.87-0.92 assigned to H-29, which is characteristic for C-24 ethyl sterols. In addition, compound S_3 showed multiplet at δH 5.21 assigned to H-23. The anomeric proton that appeared at δH 4.40-4.66 with J = 8 Hz indicated a β-linkage. The ^{13}C-NMR revealed the presence of 35 signals, 29 of which were assignable for the C-24 ethyl sterols nucleus (Table 1) and the other 6 signals corresponded to the sugar moiety. The olefinic carbons at C-5 and C-6 were verified by the presence

of signals at δc 140.1 and 121.8 in both compounds S_2 and S_3. Addition olefinic carbons in compound S_3 at C-22 and C-23 were verified by the presence of signals at δc 138.1 and 129.2 (Stigmasterol nucleus). The ^{13}C-NMR data of the sugar moiety combined with the chromatographic examination of the hydrolytic products (TLC and PC), allowed the identification of **β**-D-glucose as the sugar component (co-chromatography) in both compounds S_2 and S_3.

From the above data compound S_2 was identified as **β**-sitosterol-3-O-**β**-D-glucoside while compound S_3 was identified as Stigmasterol 3-O-**β**-D-glucoside.

Constituents of the Ethyl acetate and n-Butanol Fractions

Compound S_4: Yellow powder, 50 mg, (fraction Fe3), m.p 313-314 °C, R_F 0.6, (System: chloroform-methanol 8.5:1.5; yellow in VL, yellow fluorescence in UV / NH_3 or $ALCl_3$).

Compound S_5: Yellow powder, 30 mg, (fraction Fe3), m.p 330 °C, (R_f 0.5 System: chloroform-methanol 8.5:1.5; faint brown in VL, brown fluorescence in UV turning to yellow on exposure to NH_3 and acquiring a yellow color when sprayed with $AlCl_3$ yielding a yellow fluorescence in UV).

Compound S_6: Yellow powder, 80 mg, (fraction Fe2),(R_f 0.37, System: chloroform-methanol 8.5:1.5; faint brown in VL, brown fluorescence in UV turning to yellow on exposure to NH_3 and acquiring a yellow color when sprayed with $AlCl_3$ yielding a yellow fluorescence in UV).

Compound S_7: Yellow powder, 100 mg, (fraction Fb2), (R_f0.45, chloroform-methanol-water 8:2:0.1; faint brown in VL, brown fluorescence in UV turning to yellow on exposure to NH_3 and acquiring a yellow color when sprayed with $AlCl_3$ yielding a yellow fluorescence in UV).

Compound S_4 was identified as the flavonol aglycone quercetin based on co-chromatography alongside with an available authentic sample, m.p., m.m.p. and UV spectral data in methanol with or without the addition of different shift reagents as well as by comparison with reported data (Danial and Carl, 1969).

Compounds S_5-S_7: The UV analysis of compounds S_5-S_7 (Table 2) showed that they have most probably flavonol substituted skeleton (band I 328-358 nm). Bathochromic shifts in band I (34-55 nm) with increased intensity upon addition of sodium methoxide revealed the presence of a 4' hydroxyl group and absence of free 3 or 7-hydroxyl group (no decomposition after 5 min). Which also proved by the bathochromic shift in band I upon addition of sodium acetate (13-24 nm).

In ^{1}H- NMR of compounds S_5-S_7 (table 3), the occurrence of two doublets signals at δH (6.40-6.63) and (6.67-6.76) (J = 2Hz), indicated the presence of two meta protons at C-6 and C-8 of ring A, respectively. Signals at δH (7.35-8.0) (1H, d, J = 2.0 Hz); δH (6.74-6.88) (1 H, d, J = 8.4 Hz) and at δH (7.00-7.46) (dd, J = 2.0 and 8.4 Hz) indicated a 1', 3', 4' tri-substituted system in ring B. In addition, compound S_5 showed signal at 3.75 (3H, s, OCH_3).

From the previous data and from the results of acid hydrolysis, isorhamnetin was identified as the aglycone moiety of compound S_5 while was identified as the aglycone moiety of compounds S_6 and S_7.

The sugar part of compound S_5 was identified as rhamnose by TLC after acid hydrolysis and by presence of an anomeric proton signal at δH 4.8 ppm (1 H br.s) and a methyl group at 1.2 ppm (3H,d, J = 2 Hz) .Comparison of ^{13}C-NMR spectral data of the sugar moiety (102.7, 72.2, 75.4, 73.8,

Table 2: Chromatographic and UV spectral data of compounds S4-S7 isolated from ethyl acetate and butanol extracts of *Fagonia indica* Burm F.

Chromato-graphic data	Compound				UV Spectral data (λ_{max} nm)				
	S_4	S_5	S_6	S_7	Reagent	S_4	S_5	S_6	S_7
R_F value	0.60*	0.5*	0.37*	0.45**	CH_3OH	250,269sh, 300sh, 368	254,279sh, 318,356	256,270sh, 353	257,269sh, 358
Colour in: Visible light	Y	f.br	f.br	f.br	CH_3ONa	240sh,320	284,330 ,390	277,320sh, 409	244,270, 396
NH_3	Y	Y	Y	Y	$AlCl_3$	270,304sh, 330,450	269,274sh, 318sh,390	210,304sh, 348,430	276,300sh, 343sh,430
$AlCl_3$	Y	Y	Y	Y	$AlCl_3$/HCl	260,301sh, 350,420	267,278sh, 300sh,359sh,399	270,301sh, 360,410	270,300sh, 366sh,404
Fluorescence in: UV	br.	br.	br.	br.	CH_3COONa	254sh,270, 325,390	278,316, 396	270,295sh, 370,409	270,294sh, 370,416sh
UV/NH_3	Y	Y	Y	Y	CH_3COONa/ H_3BO_3	260,303sh, 380	254,267sh, 314sh,360	270,370	261,294sh, 380
UV/ $AlCl_3$	Y	Y	Y	Y					

*Solvent system, Chloroform- methanol 8.5:1.5; ** Solvent system, Chloroform-methanol- water 8.0:2.0:0.1

Table 3: 1H-NMR data (DMSO, 500 MHz, δ ppm) of compounds S5- S7 isolated from ethyl acetate and n- butanol extracts of *Fagonia indica* Burm F.

Proton	Compound S5	Compound S6	Compound S7
6	6.63 (1 H,d, J = 2.0 MHz)	6.40(1 H,d, J=2.0 MHz)	6.60(1 H,d, J=2.0 MHz)
8	6.70 (1 H,d, J = 2.0 MHz)	6.76(1 H,d, J=2.0 MHz)	6.67(1 H,d, J=2.0 MHz)
2'	7.40(1 H,d, J = 2.0 MHz)	8.00(1 H,d, J=2.0 MHz)	7.35(1 H,d, J=2.0 MHz)
5'	6.80(1 H,d, J = 8.4MHz)	6.88(1 H,d, J=8.4MHz)	6.74(1 H,d, J=8.4MHz)
6'	7.00(1 H,dd, J = 2.0,8.4)	7.46(1 H,dd,J=2.0,8.4)	7.0(1 H,dd,J=2.0,8.4)
OCH3	3.75 (3H,s)	-	-
1"	4.8 (1H,d, J = 2)	5.40 (1H,d,J=8)	6.00(1H,d,J=8)
2"-5"	3.3-4.1 (m)	3.2-3.7(m)	3.2-3.7(m)
6"	1.20 (3H,d, J = 8)	4.00 (m)	4.10 (m)
1'''		5.53 (1H,d,J=8)	5.00(1H,d,J=2)
2''' acetyl			1.8 (3H)
3'''-5'''		3.2-3.7(m)	3.2-3.8(m)
6'''		4.10 (m)	1.20 (3H,d,J=8)
1''''			5.30(1H,d,J=8)
2''''-5''''			3.2-3.7(m)
6''''			4.10 (m)

73.5 and 18.6) with those published data, further confirmed the sugar moiety as rhamnose (Agrawal, 1989) (Table 4).

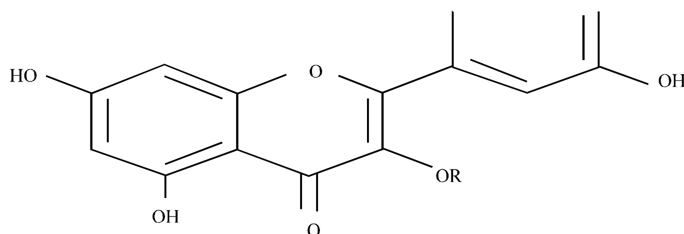
From the above data, compound S_5 could be identified as Isorhamnetin-α-3-O rhamnoside.

In compound S_6, the presence of 2 anomeric proton signals at δH 5.40 and 5.53 with J = 8, indicated the presence of 2 sugar units β-linked to the aglycone at C-3. The ^{13}C-NMR data of the 2 sugar units (100.8,74.2,76.4,69.9,76.4 and 60.6 ;101,74.2,76.4,69.9,76.4 and 60.8) were in good agreement with those of glucose (Agrawal,1989). From the above data, compound S_6 could be identified as Quercetin-3-O-β-D-glucopyranosyl-(1-6)-β-D glucopyranoside.

Table 4: 13C-NMR data (125 MHz, δ ppm, DMSO) of compounds S5- S7 isolated from ethyl acetate and n-butanol extracts of *Fagonia indica* Burm F.

C number	Compound S5	Compound S6	Compound S7
2	147.3	156.3	156.5
3	136.0	133.3	129.4
4	175.9	177.5	177.0
5	160.4	161.3	161.0
6	97.4	98.8	98.0
7	164.9	164.4	164.0
8	91.8	93.1	93.0
9	156	156.3	156.0
10	103.7	104.1	105.0
1'	121.9	122.1	120.0
2'	115.2	115.3	115.2
3'	145.8	146.9	144.0
4'	145.8	149.4	148.5
5'	115.6	115.2	119.9
6'	120.1	121.0	122.3
CH 3	56.0	-	-
1"	102.7	100.8	104.9
2"	72.2	74.2	73.0
3"	75.4	76.4	73.3
4"	73.8	69.9	71.1
5"	73.5	76.4	74.5
6"	18.6	60.6	69.6
1'''	-	101	101.8
2'''	-	74.2	73.3
3'''	-	76.4	79.3
4'''	-	69.9	75.7
5'''	-	76.4	69.6
6'''	-	60.8	18.9
COOCH 3	-	-	27.0
COOCH 3	-	-	169.7
1''''	-	-	105.0
2''''	-	-	74.8
3''''		-	78.2
4''''	-	-	72.4
5''''	-	-	77.6
6''''	-	-	60.5

Compound S_7 showed 3 anomeric proton signals indicated the presence of 3 sugar units. 2 of them appeared at δH 6.0 and 5.3 with J = 8 indicated the presence of 2 sugar units β-linked to the aglycone at C-3. The other sugar unit showed signals at δH 5.0 with J = 2, 1.2 (3H, d, J = 2 Hz) and at 1. 8 (3H,S) indicated the presence of 2" O-acetyl rhamnose unit (Danial and Carl, 1969; Agrawal,1989). The ^{13}C-NMR data of the 3 sugar units (104.9, 73.0, 73.3, 71.1, 74.5, 69.6; 101.8, 73.3, 79.3, 75.7, 69.6, 18.9, 27 and 105.0, 74.8, 78.2, 72.4, 77.6, 60.0) were in good agreement with those of galactopyranosyl -(6"-1''')- α-L-2''' acetyl rhamnose -(3'''-1'''') β-D-glucopyranoside (Agrawal,1989). From the above data, compound S_7 could be identified as quercetin 3-O-β-D-galactopyranosyl -(6"-1''')- α-L-2''' acetyl rhamnose-(3'''-1'''') β-D-glucopyranoside which is a new compound isolated from Genus *Fagonia*. Structures are shown in Fig. 1.



R	H	Quercetin
R	Glucpyransoy1-(1"-6") glucopyranoside	Quercetin3-O-β-D-glucopyranosy1-(1"-6")-β-D-glucopyranoside.
R	Glucpyransoy1-(1"-6")- 2''' acetyl rhamnose-(3'''-1'''') glucopyranoside	Quercetin3-O-β-D-glucopyranosy1-(1"-6")-α-L-2''' acety1 rhamnose-(3'''-1''')β-D-glucopyranoside.

Fig. 1: Structure of compounds S4, S6 and S7

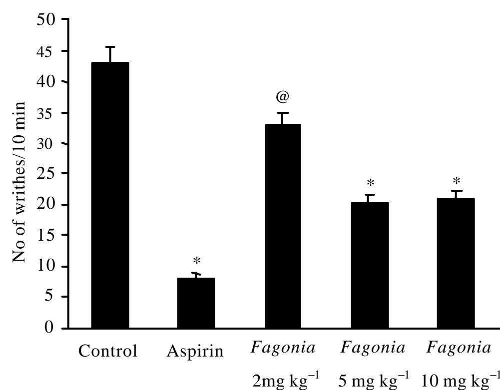


Fig. 2: Effect of i.p injection of either aspirin (200 mg kg^{-1}), or increasing doses of *Fagonia indica* Burm F. alcoholic extract on number of writhes per 10 min. Each bar represents the mean±SEM of 7 animals. *Significantly different from control group at $p<0.05$,@ significantly different from aspirin-treated group at $p<0.05$

Biological study

Acute toxicity testing of the total plant alcoholic extract: Oral and intraperitoneal administration of the extract in doses up to about 4 g kg^{-1} body weight produced no mortality and no signs of morbidity or behavioral changes in any of the animals of the treated groups during the period of observation. This result indicates wide safety margin of the standardized *Fagonia indica* Burm F. alcoholic extract and justifies its wide-spread use in folk medicine.

Antinociceptive activity of the total plant alcoholic extract

Writhing test: Results shown in Fig. 2 reveal that the number of writhes induced by 0.6% acetic acid in normal mice reached an average of about 42 writhes per 10 min. Aspirin produced about 80% inhibition in the number of writhes induced by acetic acid in mice. Compared to aspirin, 2 mg of the *Fagonia* extract did not show any significant inhibition of writhing response in the treated animals. On the other hand, 5 mg as well as 10 mg of the extract showed significant

inhibition of the acetic acid-induced writhing in mice. Both concentrations produced 52% inhibition of the writhing response as compared to the control non-treated animals. Higher doses of the extract (25, 50 and 100 mg kg^{-1}) showed almost complete inhibition of writhing indicating a maximum response.

Hot plate test: Results are demonstrated in Fig. 3 and 4. The latency period was found to be significantly increased upon treatment with morphine as compared to the normal control group

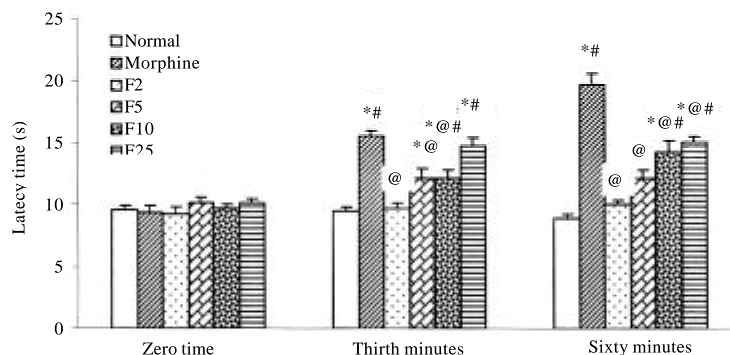


Fig. 3: Effect of i.p., injection of either morphine (10 mg kg^{-1}), or increasing doses of *Fagonia indica* Burm F. alcoholic extract on the latency period of the hot-plate test in seconds. Each bar represents the Mean±SEM of 8 animals. *Significantly different from normal at the corresponding time interval, @Significantly different from morphine at the corresponding time interval, #Significantly different from the corresponding group at zero time

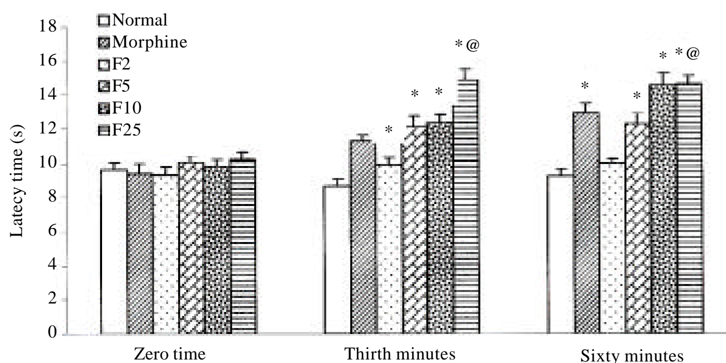


Fig. 4: Effect of i.p., injection in presence of naloxone of either morphine (10 mg kg^{-1}), or increasing doses of *Fagonia indica* Burm F. alcoholic extract on the latency period of the hot-plate test in seconds. Each bar represents the Mean±SEM of 8 animals. *Significantly different from normal at the corresponding time interval, @Significantly different from morphine at the corresponding time interval. Morphine-treated as well as *Fagonia*-treated groups at doses of 5, 10 and 25 mg kg^{-1} were significantly different from their corresponding values at zero time but marks of significance are omitted from the graph for simplicity

which is in accordance with previous findings (Kim *et al.*, 2005). *Fagonia indica* Burm F. alcoholic extract again showed antinociceptive effects with the higher doses tested at 30 as well as 60 min following administration. A dose of 25 mg kg^{-1} of the plant extract showed an analgesic effect which was non-significant from morphine at 30 min interval. In the present study, pretreatment of animals with naloxone produced similar analgesic effects with no significant differences in all groups except morphine group which shows significant attenuation of the hot-plate response of animals.

The previous results demonstrated that the alcoholic extract of *Fagonia indica* Burm F. was able to induce an analgesic/antinociceptive effect in mice in the writhing test. In this test, both peripheral and central analgesics could be evaluated and many investigators recommend it as a simple screening method (Vogel and Vogel, 1997; Talarek and Fidecka, 2002). In addition, the writhing t est possesses high sensitivity and it makes possible to detect analgesic activity of non-steroidal anti-inflammatory drugs. The peripheral analgesic response is thought to involve local peritoneal receptors and acetic acid causes algesia by liberating endogenous substances that excite pain endings (Raj, 1996). Aspirin, as is well established, inhibits cyclo-oxygenase in peripheral tissues, thus interferes with the mechanism of transduction in primary afferent nociceptors (Fields, 1987). At doses starting 25 mg kg^{-1}, the extract produced complete inhibition of writhing response which indicates maximum response and confirms a potent analgesic effect mediated peripherally. This effect is probably related to decreased sensitization of nociceptive receptors to prostaglandins since these substances are the main mediators of pain transmission through the peripheral neurons (Dickenson, 1995).

A dose-dependent antinociceptive effect in the acute thermal pain model (hot-plate test) was also verified in mice upon treatment with the alcoholic extract of *Fagonia indica* Burm F. A prolongation of the latency period was observed following pre-treatment with morphine as well as the higher two doses of the extract suggesting that the plant extract has possible central analgesic properties. Morphine, acts by combining to opioid receptors and this in turn acts by either increasing the membrane K^+ conductance or decreasing Ca^{++} conductance (Khanna and Sharma, 2001). Ca^{++} channel blockers (Verma, 1997) and K^+ channel openers (Malhotra *et al.*, 1997) both have been shown to have analgesic action of their own and they potentiate the analgesic effect of morphine. In the present study, 2 mg kg^{-1} of naloxone co-administration to animals treated with the different doses of the plant extract produced no significant differences in analgesic activity. These results could indicate that the central endogenous opioid system is probably not involved in the antinociceptive action achieved by the plant extract. Noteworthy, it was reported in other studies that naloxone was used at 5 and 10 mg kg^{-1} (Jayaram *et al.*, 1995; Sawynok *et al.*, 1995) which raises the possibility of obtaining different results upon using doses of naloxone higher than that used in the present investigation. Could the extract possess any of the abovementioned antinociceptive pathways involving K^+ or Ca^{++} conductance needs further studies to be investigated and confirmed.

The antinociceptive effect of *Fagonia indica* Burm F. demonstrated in this investigation could be attributed, at least in part, to the presence, among its constituents, of flavonoids especially quercetin and its glycosides. Indeed, other studies have demonstrated that various flavonoids such as rutin, quercetin, hesperidin and biflavonoids produced significant antinociceptive and/or anti-inflammatory activities (Galati *et al.*, 1994; Ramesh *et al.*, 1998).

Antimicrobial screening

Under the experimental conditions used and as shown in Table 5 the following could be concluded:

- All the extracts at dose of 200 mg mL^{-1} exhibit marked antimicrobial activity against gram negative bacteria (*pseudomonas aerugenosa* and *E. coli*) comparing with the standard antibiotics, except hexane extract showed no activity against *pseudomonas aerugenosa*
- Hexane and butanol extracts only showed activity at dose of 200 mg mL^{-1} against *candida albican*
- Almost all the extracts (except butanol extract) showed activity against proteus
- All the extracts in a dose of 200 mg mL^{-1} showed no activity against *Streptococcus beta heamolytica*

Anti-tumor activities of the total plant alcoholic extract, the ethyl acetate and the butanol fractions: In a continuation of our interest in the chemical composition and biological activities of the *Fagonia indica* Burm F. the antitumor activity of the alcoholic extract and their fractions (ethyl acetate and butanol) which contain mainly flavonoids constituents was reported.

Under the experimental conditions used and as shown in Table 6 the following could be concluded: all the tested extracts exhibited marked antitumor activity against Memory Carcinoma F7 (MCF7) and Human Colon tumor cells (HCT).

Table 5: Effect of the different extracts of *Fagonia indica* plant on selected bacteria and fungi

	Diameter of zone of inhibition (mm)					
Extracts/antibiotics	Escherichia coli	Staphylococcus aurous	Pseudomonas aurgenosa	Streptococcus beta hemolytica	Candida albican	Protus
Total alcohol	22+++	-	17+++	-	-	16+++
Hexan	18+++	18+++	-	-	10+	15++
Chloroform	23++++	-	28++++	-	-	22+++
Ethyl acetate	25++++	14++	20+++	-	-	18+++
Butanol	20+++	12++	20+++	-	10+	-
Penicillin 10 units	8+	30++++	-	21+++	12++	-
Doxycycline Hcl 30 mg	14++	31++++	10+	25+++	8+	-

- :No. inhibition zone; ++ :Diameter of inhibition zone 12-15 mm; +++ :Diameter of inhibition zone 16-22 mm; ++++ :Diameter of inhibition zone more than 23 mm

Table 6: Effects of the total alcoholic extract of *Fagonia indica* Burm F. and its prepared ethyl acetate and butanol fractions on MCF7 and HCT

	MCF7/ percentage of surviving fraction			HCT/ percentage of surviving fraction		
	Extract fraction			Extract fraction		
Conc. (mg mL^{-1})	Alcohol	Ethyl acetate	Butanol	Alcohol	Ethyl acetate	Butanol
1.00	100	99	100	97	94	99
2.50	99	97	98	93	89	97
5.00	97	97	96	75	80	96
10.00	74	82	87	74	72	65
20.00	66	66	75	67	68	59
40.00	62	32	51	44	35	49
80.00	57	31	38	45	17	33
IC_{50} (µg mL^{-1})	100.5	30.5	45.3	36.9	31.9	39.9

The ethyl acetate fraction showed least IC_{50} against both carcinoma cells MCF7 and HCT cells (30.5 and 31.9 μg mL^{-1}, respectively) followed by n-butanol fraction which showed IC_{50} 45.3 μg mL^{-1} against MCF7 while the alcoholic extract showed IC_{50} 36.9 μg mL^{-1} against HCT.

CONCLUSION

Fagonia indica Burm F. plant collected from UAE desert was found to contain a moderately high percentage (3%) of flavonoid. The alcoholic extract of the whole plant possesses analgesic action which is probably mediated through both central and peripheral mechanisms and does not seem to involve opioid receptors. Moreover, the antitumor and the antimicrobial activities of the alcoholic extract and its fractions were also documented. These results provide some pharmacological rationale for the traditional use of the plant as analgesic and antitumor in folk medicine.

ACKNOWLEDGMENT

The authors thank Prof. Dr. Samia Shoman, Biochemistry Department, National Cancer Institute, Cairo, Egypt for carrying the antitumor activity of *Fagonia* extract.

A special acknowledgement to the fourth year students of the research groups of Pharmacognosy and Pharmacology Departments, Academic year (2008-2009), for their interesting attention and assistance of the research done in DPC.

REFERENCES

Abdel Khalik, S.M., T. Miyase, H.A. El-Ashaal and F.R. Melek, 2000. Triterpenoid saponins from *Fagonia cretica*. Phytochemistry, 54: 853-859.

Adesina, S.K. and J.A. Reisch, 1985. Triterpenoid glycoside from *Tetrapleura tetraptera* fruit. Phytochemistry, 24: 3003-3006.

Agarawal, P.K., 1989. Carbon-13 NMR of Flavonoids. 1st Edn., Elsevier, Amsterdam, pp: 165.

Armitage, P. and G. Berry, 1987. Comparison of Several Groups. Statistical Methods in Medical Research. 2nd Edn., Blackwell Scientific Publications, Oxford, pp: 186-213.

Beier, B.A., J.A.A. Nylander, M.W. Chase and M. Thulin, 2004. Phytogenetic relationships and biogeography of the desert plant genus *Fagonia*, inferred by parsimony and Bayesian model averaging. Mol. Phytogenet. Evol., 33: 91-108.

Chopra, R.M., K.L. Handa, L.D. Kapur and L.C. Chopra, 1982. Indogenous Drugs of India. 2nd Edn., Academic Publisher, New Delhi, pp: 507.

Danial, J.P. and R.J. Carl, 1969. Organic Structure Determination. Prentice-Hall Inc., Englewood Cliffs.

Dickenson, A.H., 1995. Spinal cord pharmacology of pain. Br. J. Anaesthesia, 75: 193-200.

Eddy, N.B. and D. Leimbach, 1953. Synthetic analgesics. II. Dithienylbutenyl-and dithienylbutylamines. J. Pharmacol. Exp. Ther., 107: 385-393.

El-Wakil, E.A., 2007. Phytochemical and molluscicidal investigations of *Fagonia arabica*. Z. Naturforsch. C, 62: 661-667.

Fields, H.L., 1987. Analgesic D rugs. In: Pain, Day, W. (Ed.). 1st Edn., MacGraw Hill, USA., pp: 272.

Galati, E.M., M.T. Monforte, S. Kirjavainen, A.M. Forestieri, A. Trovato and M.M. Tripodo, 1994. Biological effects of hesperidin, a citrus flavonoid. (Note I): Antiinflammatory and analgesic activity. Farmaco, 40: 709-712.

Gehlot, D. and A. Bohra, 2000. Antibacterial effect of some leaf extracts on *Salmonella typhi*. Indian J. Med. Sci., 54: 102-105.

Gibbona, S. and M.A. Oriowo, 2001. Antihypertensive effect of an aqueous extract of *Zygophyllum coccineum* L. in rats. Phytother. Res., 15: 452-455.

Goad, L.J. and A. Toshihiro, 1997. Analysis of sterols. 1st Edn., Blackie Academic and Professional, London, New York.

Ibrahim, L.F., S.A. Kawashty, A.M. El-Hagrassy, M.I. Nassar and T.J. Mabry, 2007. A new kaempferol trigglycosid from *Fagonia taeckholmiana*, cytotoxic activity of its extracts. Carbohydr. Res., 343: 155-158.

Jayaram, A., P. Singh and H.M. Carp, 1995. An enkephalinase inhibitor, SCH 32615, augments analgesia induced by surgery in mice. Anesthesiology, 82: 1283-1287.

Khanna, N. and S.B. Sharma, 2001. Anti-inflammatory and analgesic effect of herbal preparation: Septilin. Ind. J. Med. Sci., 55: 195-202.

Kim, I.T., Y.M. Park, J.H. Won, H.J. Jung, H.J. Park, J.W. Choi and K.T. Lee, 2005. Methanol extract of *Xanthium strumarium* L. possesses anti-inflammatory and anti-nociceptive activities. Biol. Pharm. Bull., 28: 94-100.

Koster, R., M. Anderson and E.J. De Beer, 1959. Acetic acid for analgesic screening. Fed. Proc., 18: 412-418.

Lamyaa, F.I., A.K. Salwa, M.E.H. Ali, I.N. Mahmoud and J.M. Tom, 2008. A new Kaempferol triglycoside from *Fagonia taechholmiana*, cytotoxic activity of its extract. Carbohydrate Res., 343: 155-158.

Lorian, V., 1991. Antibiotics in Laboratory Medicine. 4th Edn., Williams&Wilkins, Baltimore, London.

Mabry, T.J., K.R. Markham and M.B. Thomas, 1970. The Systematic Identification of Flavonoids. Springer Verlag, Berlin, Heidelberg, New York.

Malhotra, R.S., N. Khanna, S.K. Patni and K.K. Sharma, 1997. Antinociceptive activity of potassium channel openers and morphine: A common mechanism of action. Ind. J. Pharmacol., 29: 57-57.

Mandeel, Q. and A. Taha, 2005. Assessment of *in vitro* antifungal activities of various extracts of indigenous bahraini medicinal plants. Pharm. Biol., 43: 340-348.

Natorska, J. and B. Plytycz, 2005. Strain-specific differences in modulatory effects of morphine on peritoneal inflammation in mice. Folia Biol., 53: 189-195.

Raj, P.P., 1996. Pain Medicine: A Comprehensive Review. Mosby, Missouri, pp: 12-23.

Ramesh, M., Y.N. Rao, A.V. Rao, M.C. Prabhakar, C.S. Rao, N. Muralidhar and B.M. Reddy, 1998. Antinociceptive and anti-inflammatory activity of a flavonoid isolated from *Caralluma attenuata*. J. Ethnopharmacol., 62: 63-66.

Sarra, G.M., F.G. Sarra, F.C. Schlichtenbrede, P. Trittibach and S. Estermann *et al.*, 2005. Effect of steroidal and non-steroidal drugs on the microglia activation pattern and the course of degeneration in the retinal degeneration slow mouse. Ophthalmic Res., 37: 72-82.

Sawynok, J., A.R. Reid and G.J. Doak, 1995. Caffeine antinociception in the rat hot-plate and formalin tests and locomotor stimulation: Involvement of noradrenergic mechanisms. Pain, 61: 203-213.

Shaker, K.H., M. Bernhardt, M.H. Elgamal and K. Seifert, 1999. Triterpenoid saponins from *Fagonia indica*. Phytochemistry, 51: 1049-1053.

Skehan, P., R. Storeng, D. Scudiero, A. Monks and J. McMahon *et al.*, 1990. New colorimetric cytotoxicity assay for anticancerdrug screening. J. Nat. Cancer Inst., 82: 1107-1112.

Soomro, A.L. and N.A. Jafarey, 2003. Effect of *Fagonia indica* on experimentally produced tumours in rats. J. Pak. Med. Assoc., 53: 440-441.

Talarek, S. and S. Fidecka, 2002. Role of nitric oxide in benzodiazepines-induced antinociception in mice. Polish J. Pharmacol., 54: 27-34.

Vogel, H.G. and W.H. Vogel, 1997. Analgesic, Anti-Inflammatory and Anti-Pyretic Activity: Drug Discovery and Evaluation. Springer-Verlag, Berlin Heidelberg, Germany.

Verma, V., P. Mediratta and K.K. Sharma, 1997. Interaction of mu and kappa agonists with calcium channel blockers in experimental pain models. Ind. J. Pharmacol., 29: 45-45.

Wibool, R., S. Chutha, R. Wantana and W. Malinee, 2008. Antinociceptive activity of the methalonic extract of *Kaempferia galangal* Linn. in experimental animals. J. Ethnopharmacol., 118: 225-230.

Yeung, M.F. and C.T. Che, 2009. A review of presence of Oleanolic acid in natural products. Sample Review for Natura Proda Medica, pp: 62.