World Journal of Chemistry 4 (1): 73-82, 2009
ISSN 1817-3128


# Evaluation of Antiulcer Activity of *Chenopodium moquinianum* and Kaempferol Glycosides in Rats

[1]*Usama W. Hawas,* [1]*Lamyaa F. Ibrahim,* [2]*Abdel Razik H. Farrag,* [3]*Jihan S. Hussein and* [3]*Ihsan Elshiekh*

[1]Department of Phytochemistry and Plant Systematic, National Research Centre, Dokki, Cairo, Egypt
[2]Department of Pathology, National Research Centre, Dokki, Cairo, Egypt
[3]Department of Medical Biochemistry, National Research Centre, Dokki, Cairo, Egypt

**Abstract:** In our screening program for anti-ulcerogenic effect of plants, the flowering aerial parts of *Chenopodium moquinianum* Aellen (*Chenoodiaceae*), an Egyptian plant grown in the western Mediterranean costal region, were analyzed. Isolation of phenolic constituents was preformed using chromatographic techniques. Eleven phenolic compounds, caffeic acid (1), rosmarinic acid (2), kaempferol (3), kaempferol 3-*O*-β-glucopyranoside (4), kaempferol 3-*O*-α-rahmnopyranoside (5), kaempferol 3,7-*O*-α-di-rhamnopyranoside (6), kaempferol 3-*O*-(4-β-xylopyranosyl)-α-rhamnopyranoside-7-*O*-α-rhamno-pyranoside (7), kaempferol 3-*O*-(4-β-apiofuranosyl)-α-rhamnopyranoside-7-*O*-α-rhamnopyranoside (8), quercetin 3-*O*-β-glucopyranoside (9), rutin (10) and isorhamnetin 3-*O*-rutinoside (11) were isolated for the first time from *C. moquinianum*. The structures were established through chemical and spectral analysis. *In vivo* antiulcerogenic property of the *C. moquinianum* aqueous methanolic extract and the pure isolated Kaempferol glycosides (6) and (7) was investigated using ethanol-induced gastric ulcer in rats. Malondialdehyde (MDA) in rat gastric mucosa, reduced glutathione (GSH) and Superoxide dismutase (SOD) were determined and used as biochemical markers of the oxidative status. Histopathological examination of rat stomachs demonstrated that *C. moquinianum* extract and kaempferol glycosides (6) and (7) induced an increase in gastric mucus production and a reduction of the depth and severity of ethanol-induced mucosal lesions. Thus, the gastro-protective effect of *C. moquinianum* extract and kaempferol glycosides (6) and (7) was probably due to the increased mucus production and interference with oxidative stress development as evidenced by decreased gastric mucosal MDA and increased antioxidant parameters (SOD&GSH).

**Key words:** *Chenopodium moquinianum* · Anti-ulcerogenic effect and kaempferol glycosides

## INTRODUCTION

Gastroduodenal ulcer is a very common global problem today [1]. Stress, smoking, nutritional deficiencies and ingestion of nonsteroidal-anti-inflammatory drugs (NSAIDs) and alcohol are all factors which increase the incidence of gastric ulcer [2]. In traditional medicine, several plants and herbs have been used to treat gastrointestinal disorders, including gastric and peptic ulcers [3-5].

*Chenoodiaceae* is a large family comprising about 100 genera and 1500 species [6-8] shows an interesting geographical distribution determined by the fact that they are almost halophytic. Vegetables of this family generally contain 1-*O*-feruloyl-*β*-D-glucose [9]. Plants of the genus *Chenopodium* have been reported to have therapeutic and edible properties [10-12]. In the folk medicine, *Chenopodium* species are widely used as a sedative, antihysteric, antispasmodic, antirheumatic and emmenagogue. Infusion of leaves is used as digestive, carminative, stimulant, stomachic, antiasthmatic and diuretic [13]. Also the essential oils in *Chenopodium* species were found to be responsible for the anthelmintic properties and considered being valuable compounds used as antifungal [14]. Previous phytochemical studies on genus *Chenopodium* have demonstrated different groups of secondary metabolites of which the most important are lipids [15], carbohydrates [16], flavonoids [17], terpens and steroids [18].

**Corresponding Author:** Dr. Usama Hawas, Phytochemistry and Plant Systematic Department, National Research Centre, Dokki-12311, Cairo, Egypt

Thus, it was deemed of interest to study the phytochemical investigation of phenolic compounds (1-11) from the aqueous methanolic extract of the flowering aerial parts of *C. moquinianum* and to evaluate the gastroprotective activity and the antioxidant effect of the crude extract and the major pure isolated compounds, kaempferol glycosides (6) and (7) in rats.

## MATERIALS AND METHODS

**General:** UV/VI: Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC, Egypt). $^1$H-NMR spectra: Varian Unity Inova 500 and 300 (500 and 300 MHz); $^{13}$C-NMR spectra: Varian Unity 500 (125 MHz) and Varian Unity 300 (75.4 MHz) (South Africa University and Germany). MS (Finnigan MAT SSQ 7000, 70 ev). Polyamide (MN-polyamide SC6, Machery Nagel, for column chromatography); Paper Chromatography (PC), Whatman No. 3 MM (46 × 57 cm); Sephadex LH-20 (Pharmacia Fine Chemicals) and Solvent mixtures, BAW (*n*-butanol:acetic acid:water 4:1:5 upper phase) were used.

**Plant Material:** Flowering aerial parts of *Chenopodium moquinianum* were collected on spring mornings from the Egyptian Mediterranean coast, 90 km west of Alexandria in March 2007. The plant was identified by Prof. Salwa Kawashty, Department of Phytochemistry and Plant Systematic, National Research Centre. Voucher specimen was kept in the herbarium of the National Research Center (CAIRC).

**Extraction and Isolation:** The ground-dried aerial parts of *C. moquinianum* plant was extracted with 70% methanol by percolation until exhaustion, the extract was filtered and the solvent was evaporated under reduced pressure at a low temperature until dryness. The extract was defatted with petroleum ether and applied to a polyamide column using water/methanol mixtures of decreasing polarities as eluent. Each fraction was subjected separately to preparative paper chromatography (3 mm) using BAW, 15% acetic acid/water and water as eluents. The separated bands were scraped off and eluted with 70% methanol to get the pure compounds (1-11), which were further purified by repeated Sephadex LH-20 column using methanol/water and methanol.

**Chemical Characterization of the Pure Isolated Compounds**

**Caffeic Acid (1):** white powder, PC $R_f$ 80 (BAW) and 59 (15% HOAc). UV λmax (MeOH): 218, 244, 298, 324. $^1$H-NMR (DMSO-$d_6$, 300 MHz): δ 9.82 (2H, br, 2OH), 7.41 (1H, d, $J$ = 16 Hz, H-7), 7.05 (1H, d, $J$ = 2 Hz, H-2), 6.94 (1H, d, $J$ = 8 and 2 Hz, H-6), 6.76 (1H, d, $J$ = 8 Hz, H-5), 6.18 (1H, d, $J$ = 16 Hz, H-8).

**Rosmarinic Acid (2):** white powder, $R_f$, 85 (BAW) 64 (HOAc). $[\alpha]^{20}_D$ = -2.3 ($c$ = 0.35, MeOH). UV (MeOH): $\lambda_{max}$ (nm): 225, 277, 328. $^1$H-NMR (DMSO-$d_6$, 500 MHz): δ 7.56 (1H, d, $J$ = 16 Hz, H-7'), 7.18 (1H, d, $J$ = 1.5 Hz, H-2'), 6.97 (1H, d, $J$ = 8 and 1.5 Hz; H-6'), 6.82 (1H, d, $J$ = 8 Hz; H-5'), 6.73 (1H, d, $J$ = 1.8 Hz, H-2), 6.67 (1H, d, $J$ = 8 Hz, H-5), 6.58 (1H, d, $J$ = 8 and 1.8 Hz, H-6), 6.28 (1H, d, $J$ = 16 Hz, H-8'), 5.16 (1H, dd, $J$ = 7 and 5 Hz; H-8), 2.96 (2H, m; 7-$CH_2$). EI-MS: 360, 198, 194, 177, 164. (-)ESI-MS: *m/z* (%) = 359 ([M-H]$^-$, 100), (-)-ESI-MS/MS: *m/z* (%) = 359 ([M-H]$^-$, 100).

**Kaempferol (3):** yellow powder, PC $R_f$ 82 (BAW) and 8 (15% HOAc); EI-MS: *m/z* 286; UV λmax (MeOH): 265, 366; (NaOMe): 267, 317, 406; ($AlCl_3$): 262, 269, 310, 367; ($AlCl_3$/HCl): 263, 268, 302, 344; (NaOAc): 274, 382; (NaOAc/$H_3BO_3$): 267, 368. $^1$H-NMR (DMSO-$d_6$, 500 MHz): δ 12.5 (1H, s, 5-OH), 8.05 (2H, d, $J$ = 8 Hz, H-2′, 6′), 6.91 (2H, d, $J$ = 8 Hz, H-3′, 5′), 6.45 (1H, d, $J$ = 2.2 Hz, H-8), 6.15 (1H, d, $^3J$ = 2.2 Hz, H-6).

**Kaempferol 3-O-β-glucopyranoside (Astragalin) (4):** yellow amorphous powder, PC $R_f$ 0.57(BAW), 0.11($H_2O$) and 0.42(15% HOAc); $^1$H-NMR: δ = 12.5 (s, 1H, 5-OH), 8.0 (d, $J$ = 8 Hz, 2H, H-2′,6′), 6.82 (d, $J$ = 8 Hz, 2H, H-3′,5′), 6.40 (d, $J$ = 2.2 Hz, 1H, H-8), 6.15 (d, $J$ = 2.2 Hz, 1H, H-6), 5.5 (d, $J$ = 7.5 Hz, 1H, H-1′′).

**Kaempferol 3-O-α-rahmnopyranoside (5):** yellow powder, PC $R_f$ 62 (BAW) and 36 (15% HOAc). UV λmax (MeOH): 266, 346; (NaOMe): 274, 327, 401; ($AlCl_3$): 274, 304, 349, 396; ($AlCl_3$/HCl): 274, 345, 394; (NaOAc): 274, 305, 392; (NaOAc/$H_3BO_3$): 267, 352. $^1$H-NMR (DMSO-$d_6$, 500 MHz): δ 7.82 (2H, d, $J$ = 8.5 Hz, H-2',6'), 6.91 (2H, d, $J$ = 8.5 Hz; H-3',5'), 6.44 (1H, d, $J$ = 2 Hz; H-8), 6.24 (1H, d, $J$ = 2 Hz, H-6), 5.5 (1H, s, H-1"), 1.12 (3H, d, $J$ = 5.8 Hz; $CH_3$-rhamnose).

**Kaempferol 3,7-*O*-α-di-Rhamnopyranoside (6):** yellowish needle crystals, m.p. 201-204°C, PC $R_f$ 56 (BAW) and 50 (15% HOAc). UV λmax (MeOH): 265, 343; (NaOMe): 246, 387; ($AlCl_3$): 274, 299, 360, 433; ($AlCl_3$/HCl): 271, 359, 431; (NaOAc): 265, 357; (NaOAc/$H_3BO_3$): 265, 344. $^1$H-NMR (DMSO-$d_6$, 500 MHz): δ 7.75 (2H, d, $J$ = 8.5 Hz, H-2',6'), 6.89 (2H, d, $J$ = 8.5 Hz; H-3',5'), 6.76 (1H, d, $J$ = 2 Hz; H-8), 6.44 (1H, d, $J$ = 2 Hz, H-6), 5.53 (1H, s, H-1"), 5.27 (1H, s, H-1'"), 1.12 (3H, d, $J$ = 5.8 Hz; $CH_3$-rhamnose),

0.79 (3H, d, $J$ = 6 Hz; $CH_3$-rhamnose). $^{13}$C-NMR: δ 178.8 (C-4), 162.5 (C-7), 161.8 (C-5), 161 (C-4'), 158.6 (C-2), 156.9 (C-9), 135.4 (C-3), 131.6 (C-2',6'), 121.2 (C-1'), 116.3 (C-3',5'), 106.6 (C-10), 102.7 (C-1''), 100.3 (C-6), 99.2 (C-1'''), 95.4 (C-8), 72.4 (C-4''), 71.9 (C- 2'',4'''), 71.5 (C-3'',2'''), 71.1 (C-5''), 70.9 (C-3'''), 70.6(C-5'''), 18.8 (C-6'''), 18.3 (C-6'').

**Kaempferol 3-*O*-(4-β-xylopyranosyl)-α-rhamnopyranoside-7-*O*-α-rhamnopyrano-side (7):** pale yellow crystal, m.p. 230-233°C, PC $R_f$ 28 (BAW) and 60 (15% HOAc). UV λmax (MeOH): 265, 344; (NaOMe): 265, 388; ($AlCl_3$): 274, 299, 347, 394; ($AlCl_3$/HCl): 274, 340, 394; (NaOAc): 265, 352; (NaOAc/$H_3BO_3$): 265, 346. $^1$H-NMR (DMSO-$d_6$, 500 MHz): δ 12.7 (1H, s, 5-OH), 7.78 (2H, d, $J$ = 8.2 Hz, H-2',6'), 6.95 (2H, d, $J$ = 8.2 Hz; H-3',5'), 6.75 (1H, d, $J$ = 2 Hz; H-8), 6.42 (1H, d, $J$ = 2 Hz, H-6), 5.55 (1H, s, H-1''), 5.39 (1H, s, H-1'''), 4.19 (1H, d, $J$ = 8 Hz, H-1''''), 1.14 (3H, d, $J$ = 6 Hz; $CH_3$-rhamnose), 0.85 (3H, d, $J$ = 6 Hz; $CH_3$-rhamnose). $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) ): δ 178 (C-4), 161.7 (C-7), 160.9 (C-5), 160.5 (C-4'), 157.9 (C-2), 156.1 (C-9), 134.5 (C-3), 130.6 (C-2',6'), 120 (C-1'), 115.5 (C-3',5'), 106.4 (C-1''''), 105.7 (C-10), 100.9 (C-1''), 99.4 (C-6), 98.5 (C-1'''), 94.6 (C-8), 80.5 (C-4''), 79.1 (C-3''''), 76.3(C-2''''), 73.7(C- 2'',4'''), 71.7 (C-3'',2'''), 70.6 (C-5''), 70.4 (C-3'''), 69.3 (C-5'''), 70.1 (C-4''''), 69.3 (C-5''''), 17.9 (C-6'''), 17.4 (C-6''); Negative FAB-Ms m/z: 709 $[M-H]^{-1}$ (100%), 563 [M-H-rhamnose]$^{-1}$(55%).

**Kaempferol 3-*O*-(4-β-apiofuranosyl)-α-rhamnopyranoside-7-*O*-α-rhamnopyrano-side (8):** yellow powder, m.p. 222-224°C, PC $R_f$ 25 (BAW) and 65 (15% HOAc). UV λmax (MeOH): 265, 342; (NaOMe): 267, 386; ($AlCl_3$): 275, 300, 349, 396; ($AlCl_3$/HCl): 275, 300, 342, 394; (NaOAc): 265, 352; (NaOAc/$H_3BO_3$): 265, 343. $^1$H-NMR (DMSO-$d_6$, 500 MHz): δ 12.5 (1H, s, 5-OH), 7.72 (2H, d, $J$ = 8 Hz, H-2',6'), 6.85 (2H, d, $J$ = 8 Hz; H-3',5'), 6.65 (1H, d, $J$ = 2 Hz; H-8), 6.35 (1H, d, $J$ = 2 Hz, H-6), 5.61 (1H, d, $J$ = 4 Hz, H-1''), 5.54 (1H, d, $J$ = 1.2 Hz, H-1'''), 5.31 (1H, d, $J$ = 1.2 Hz, H-1''''), 1.12 (3H, d, $J$ = 6 Hz; $CH_3$-rhamnose), 0.85 (3H, d, $J$ = 6 Hz; $CH_3$-rhamnose). $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) ): δ 177.9 (C-4), 162.1 (C-7), 161.8 (C-5,4'), 157.8 (C-2), 156.4 (C-9), 134.6 (C-3), 130.7 (C-2',6'), 119.2 (C-1'), 116.1 (C-3',5'), 106.6 (C-1''''), 106.1 (C-10), 101(C-1''), 99.8 (C-1'''), 98.6 (C-6), 94.4 (C-8), 80.9 (C-3''''), 76.4 (C-4''), 73.9 (C-2''''), 71.8 (C-4''''), 71.6 (C-4'''), 70.7 (C-2''), 70.4 (C-3''), 70.2 (C-2'''), 69.6 (C-3'''), 69.5 (C-5'''), 67.6 (C-5''), 66 (C-5'''), 18.1(C-6'''), 17.7 (C-6'').

**Quercetin 3-*O*-β-glucoside (Isoquercitrin) (9):** yellow amorphous powder, PC $R_f$ 0.47 (BAW), 0.12 ($H_2O$) and 0.45 (15% HOAc); (-) ESI-MS: *m/z* 463 $[M-H]^-$. $^1$H-NMR: δ = 12.6 (b, 1H, 5-OH), 7.55 (d, $J$ = 1.2 Hz, 1H, H-2´), 7.55 (d, $J$ = 8 Hz, 1H, H-6´), 6.85 (d, $J$ = 8 Hz, 1H, H-5´), 6.38 (d, $J$ = 2 Hz, 1H, H-8), 6.18 (d, $J$ = 2 Hz, 1H, H-6), 5.43 (d, $J$ = 7.5 Hz, 1H, H-1´´). $^{13}$C-NMR: δ = 178 (C-4), 165 (C-7), 162 (C-9), 157.4 (C-5), 157 (C-2), 149 (C-4´), 145 (C-3´), 134 (C-3), 122.5 (C-1´), 121.5 (C-6´), 117 (C-5´), 116 (C-2´), 105 (C-10), 103 (C-1´´), 99.8 (C-6), 94 (C-8), 78 (C-5´´), 77.5 (C-3´´), 75 (C-2´´), 71 (C-4´´), 62 (C-6´´).

**Rutin; Quercetin 3-*O*-rutinoside; Quercetin 3-*O*-α-rhamnosyl (1´´´?6´´)-β-glucoside (10):** yellow amorphous powder, PC $R_f$ 0.5(BAW) and 0.64(15% HOAc). (-) ESI-MS: *m/z* 609 $[M-H]^-$; (+) ESI-MS: *m/z* 633 $[M+Na]^+$; (+)ESI-MS/MS: m/z 633, 487 $[M-rhamnosyl+Na]^+$, 330 [M-quercetin+Na] $^+$. UV λmax (MeOH): 257, 266, 358; (NaOMe): 272, 328, 408; ($AlCl_3$): 271, 299sh, 402; ($AlCl_3$/HCl): 268, 298, 364, 400; (NaOAc): 273, 324, 389; (NaOAc/$H_3BO_3$): 261, 379. $^1$H-NMR: δ = 12.5 (s, 1H,5-OH), 7.5 (d, $J$ = 8 Hz, 2H, H-2´,6´), 6.8 (d, $J$ = 8 Hz, 1H, H-5´), 6.35 (d, $J$ = 2.2 Hz, 1H, H-8), 6.15 (d, $J$ = 2.2 Hz, 1H, H-6), 5.34 (d, $J$ = 7.5 Hz, 1H, H-1´´), 4.38 (d, $J$ = 1.2 Hz, 1H, H-1´´´), 1.1 (d, $J$ = 6 Hz, 1H, $CH_3$-rhamnosyl).

**Isorhamnetin 3-*O*-rutinoside; isorhamnetin 3-*O*-α-rhamnosyl (1´´´?6´´)-β-glucoside) (11):** yellow amorphous powder, PC $R_f$ 0.45 (BAW) and 0.61 (15% HOAc). UV λmax (MeOH): 255, 264, 357; (NaOMe): 271, 327, 413; ($AlCl_3$): 269, 277sh, 300sh, 367sh, 401; ($AlCl_3$/HCl): 268, 275sh, 299sh, 360sh, 398; (NaOAc): 273, 320, 392; (NaOAc/$H_3BO_3$): 258, 360. $^1$H-NMR: δ = 7.95 (d, $J$ = 2 Hz, 1H, H-2´), 7.65 (d, $J$ = 8 Hz, 1H, H-6´), 6.82 (d, $J$ = 8 Hz, 1H, H-5´), 6.41 (d, $J$ = 2.2 Hz, 1H, H-8), 6.19 (d, $J$ = 2.2 Hz, 1H, H-6), 5.25 (d, $J$ = 7.5 Hz, 1H, H-1´´), 4.52 (d, $J$ = 1.2 Hz, 1H, H-1´´´), 3.94 (s, 3H, 3'-$OCH_3$), 1.14 (d, $J$ = 6 Hz, 1H, $CH_3$-rhamnosyl). $^{13}$C-NMR: δ = 179.5 (C-4), 165.7 (C-7), 163.2 (C-2), 159.1 (C-5), 158.7 (C-9), 151 (C-4´), 148.5 (C-3´), 135.8 (C-3), 124.2 (C-6´), 123.3 (C-1´), 116.2 (C-5´), 114.9 (C-2´), 106.3 (C-10), 104.7 (C-1´´´), 102.9 (C-1´´´), 100.1 (C-6), 95.1 (C-8), 78.4 (C-5´´), 77.9 (C-3´´), 76.1 (C-2´´), 74.1 (C-4´´´), 72.3 (C-4´´), 72.3 (C-2'''), 71.8 (C-3´´´), 70 (C-6´´´), 68.8 (C-5´´´), 57 (3'-$OCH_3$), 18.2 ($CH_3$-rhamnosyl).

**Experimental Animals:** Adult Female Sprague–Dawley rats weighing 120-150 g were used for this study. The animals were obtained from the Lab Animal House, National Research Centre, Cairo, Egypt. The animals were

acclimatized for one week in an animal room where the temperature was maintained at 22 ± 3°C and there was a 12-h light: 12-h dark cycle. All animals were deprived of food for 18 h before induction of ulcer and were allocated to different experimental groups. The animals had free access to food and water unless stated otherwise. All animals received humane care in compliance with the ethics in the use of animals issued by the National Research Centre.

**Experimental Design:** The experiment was performed according to the method of Morimoto *et al.* [19]. The animals were divided into six groups, each consisting of six rats. Group 1 represented the control group, which received distilled water orally. Groups 3, 4, 5 and 6 received *C. moquinianum* aqueous methanolic extract and isolated compounds (5) and (6) (800 mg/kg) and Ranitidine (40 mg/kg), respectively. One hour after treatment, all rats of gastric groups 2, 3, 4, 5 and 6 received 1mL of 99.5% ethanol to induce ulcer. One hour later, the animals were sacrificed with an over-dose of diethyl ether. The abdomen of each rat was dissected and the stomach was slightly taken out. Then the oesophagus was tied as a knot nearest the cardia by a surgical suture. From the duodenum side, 10 ml of 10% formalin solution was injected into the stomach. The distended stomach was immediately tied on the pyloric sphincter using another surgical suture to avoid leakage of the formalin solution.

**Measurement of Gastric Mucosal Damage:** The distended stomach was removed from the abdomen, inflated with 10 ml of 10% formalin solution and immersed in the same solution to fix the outer layer of the stomach. Each stomach was then dissected along the greater curvature, rinsed with tap water to remove gastric contents and blood clots and examined under a dissecting microscope (20 × 6.3) to assess the formation of ulcers. The sum of length (mm) of all lesions for each stomach was used as the ulcer index (UI) and the inhibition percentage was calculated by the following formula:

$$\text{Inhibition (\%)} = [\text{UI control} - \text{UI treated}] / \text{UI control} \times 100.$$

**Histological and Histochemical Methods:** The stomach of the rats submitted to gastric ulcers was fixed in 10% formalin for 24 h at 4°C. The samples were routinely processed for embedding in paraffin and cut into 7µm-thick sections that were stained with periodic acid-Schiff (PAS) [20] and hematoxylin eosin [21]. The samples were analyzed with a Leica microscope in association with Leica Qwin 500 Software (Leica-England) in Pathology Department, National Research Centre.

**Biochemical Analysis:** The role of oxidative stress in gastric lesions of all groups was evaluated. For this end, 0.5 g tissue (Stomach tissue) was treated with 4.5 ml of phosphate buffer. The mixtures were homogenized on ice for 15 min. Homogenates were filtered and centrifuged at 4°C, then; these supernatants were used for determination of antioxidant and oxidative stress parameters.

**Malondialdehyde (MDA):** The extent of gastric MDA (as a product of lipid peroxidation) was estimated using the method described by Ohkawa *et al.* [22].

**Superoxide Dismutase (SOD) Activity:** Stomach SOD estimation was based on the generation of superoxide radicals producing γ xanthine and xanthine oxidase, which reacts with nitro blue tetrazolium (NTB) to form formazan dye [23]. SOD activity was then measured at 560 nm by the degree of inhibition and was expressed as mmol/min/mg tissue.

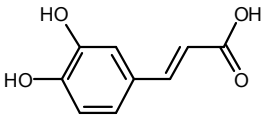
**Reduced Glutathione (GSH):** The amount of stomach GSH was measured according to the method described by Tashima *et al.* [24]. Absorbance was measured at 412 nm. The results of GSH level in stomach were expressed as nmol/g tissue.

**Statistical Analysis:** Results were expressed as means ± standard errors (SE). The statistical difference between the treated group and that of the control was calculated by using ANOVA and Student's t-test. The degree of significance was set at $p < 0.05$.

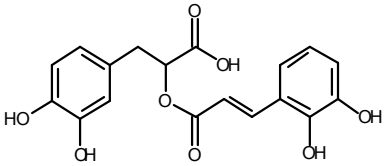
## RESULTS AND DISCUSSION

Preliminary screening of the aqueous methanolic (25%) extract of the flowering aerial parts of *Chenopodium moquinianum* by paper chromatography indicated the presence of mixture of phenolic compounds. The concentrated extract was fractionated over polyamide column using water/methanol mixtures of decreasing polarities.

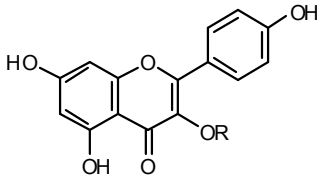
Eleven known phenolic compounds (Fig. 1) were isolated and purified by standard methods. Compounds (1) and (2) were found to exhibit chromatographic properties; fluorescent blue spots on



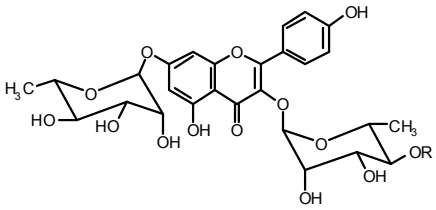
1



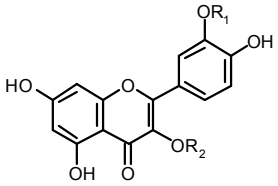
2



3: R = H
4: R = glucose
5: R = Rhamnose

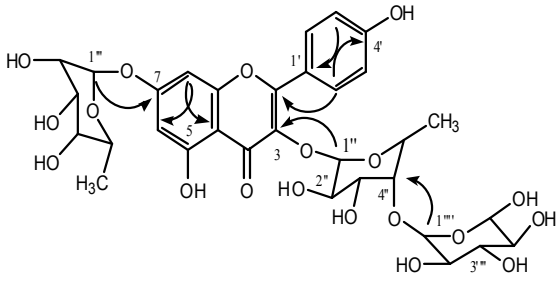


6: $R_1$ = H
7: $R_1$ = Xylose
8: $R_1$ = Apiose

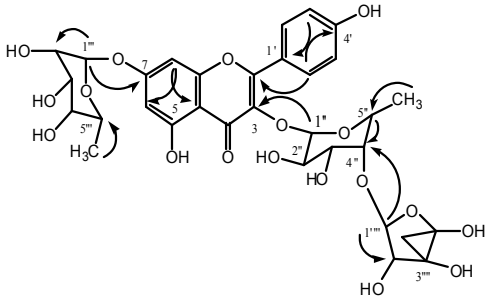


9: $R_1$ = H, $R_2$ = glucose
10: $R_1$ = H, $R_2$ = rutinoside
11: $R_1$ = $CH_3$, $R_2$ = rutinoside

Fig. 1: Phenolic compounds isolated from *Chenopodium moquinianum*



7



8

Fig. 2: Selected HMBC correlations for compounds 7 and 8

paper chromatograms (PC) under UV light, changing to bright canary yellow colour on fuming with ammonia, results of $FeCl_3$ colour reaction (intense green) and UV spectral analysis in MeOH typical for caffeic acid and its esters [25]. Flavonoid compounds (3-11) were detected by spraying its spots on PC with alcoholic aluminum chloride where compound (3) appeared as a yellow spot under UV light and compounds (4-11) appeared as dark purple spots, changing to yellow when exposed to ammonia vapour [26]. Chemical investigations; complete acid hydrolysis for *O*-glycosides and hydrogen peroxide oxidation for triglycosides (7) and (8) were carried out and followed by paper co-chromatography with authentic samples to identify the hydrolytic flavonoid glycoside products whether aglycones and sugar moieties. The structures of the isolated phenolic compounds were determined from UV, MS and NMR spectral data. One-dimension ($^1$H- and $^{13}$C-) NMR spectroscopy were used to elucidate the structure of compounds (1-6) and (9-11) while the structure of compounds (7) and (8) were elucidated by 1D- and 2D-NMR (HHCOSY, HSQC and HMBC) to determine the linkage between the sugar moieties.

The isolated phenolic compounds were identified as caffeic acid (1) [27], rosmarinic acid (2) [28], kaempferol aglycone (3) [26], kaempferol 3-*O*-β-glucopyranoside (4) [29], kaempferol 3-*O*-α-rhamnopyranoside (5) [29], kaempferol 3,7-*O*-α-di-rhamnopyranoside (6) [30], isoquercitrin (9) [26], rutin (10) [30] and isorhamnetin 3-*O*-rutinoside (11) [26,30].

Chemical and spectral analysis of compound (7) showed consistence with the presence of compound (6) and xylose as additional sugar moiety. HSQC experiment was first employed to determine the direct H/C correlation and assigned protonated carbons. The anomeric protons of the rhamnose moieties at $\delta_H$ 5.55 (H-1") and 5.39 (H-1"') were correlated with $\delta_C$ 100.9 (C-1") and 98.5 (C-1"'), respectively and the anomeric proton of xylose at $\delta_H$ 4.19 (H-1"") was correlated with $\delta_C$ 106.4 (C-1""). In the HMBC spectrum, the rhamnose protons H-1" and H-1"' showed correlations with $\delta_C$ 134.5 (C-3) and 161.7 (C-7), respectively, while xylose proton H-1"" was correlated with $\delta_C$80.5 (C-4") indicated that xylose is a terminal sugar through ether linkage (1-4) with rhamnose moiety on C-3. Thus, compound 7 was identified as kaempferol 3-*O*-(4-β-xylopyranosyl)-α-rhamnopyranoside-7-*O*-α-rhamno-pyranoside [31,32].

Compound 8 exhibited similar spectral and chemical analysis of compound 7 with the exception that it contains apiose instead of xylose as a sugar moiety. In 2D-NMR of 8, the anomeric carbon (C-1"") of apiose moiety was assigned at $\delta_C$ 106.6 through the HSQC correlation with the anomeric proton (H-1"") at $\delta_H$ 5.31, this proton was correlated in the HMBC spectrum with C-4" of rhamnose moiety on C-3 of kaempferol aglycone. Thus, compound 8 was identified as Kaempferol 3-*O*-(4-β-apiofuranosyl)-α-rhamnopyranoside-7-*O*-α-rhamnopyranoside [31,32].

Table 1: Effects of *C. moquinianum* extract and kaempferol glycoside (6) and (7) and Ranitidine on MDA, GSH and SOD activities in tissue of stomach

| Groups | MDA nmol/g tissue | GSH nmol/mg tissue | SOD mmol/mgtissue |
|---|---|---|---|
| Control | 21±0.5 | 3.25±0.05 | 125.5±2.8 |
| Ethanol | 59±0.9 * | 2.35±0.02 * | 91.5±1.2 * |
| *C. moquinianum* extract | 28±0.7 ** | 2.85±0.06 ** | 117.5±2.6 ** |
| Kaempferol glycoside (5) | 32±0.3 ** | 3.00±0.01 ** | 118.5±2.0 ** |
| Kaempferol glycoside (6) | 31±0.4 ** | 2.75±0.09 ** | 119.5±4.6 ** |
| Ranitidine | 23±0.6** | 3.35±0.04 ** | 124.0±3.5 ** |

Data represented as means±standard errors and Ethanol group were compared with the control group and other groups.
* Significant at $p < 0.05$ according to control.
** Significant at $p < 0.05$ according to ethanol group.

Table 2: Effects of *C. moquinianum* extract and Kaempferol glycosides (6) and (7) and Ranitidine on gastric ulcers in rats

| Groups | Ulcer index (mm) | Protection (%) |
|---|---|---|
| Control | - | - |
| Ethanol | 28.1±1.7 | - |
| *C. moquinianum* extract | 9.3±0.37* | 66.90 |
| Kaempferol glycoside (5) | 8.4±0.77* | 70.10 |
| Kaempferol glycoside (6) | 7.9±0.41* | 71.88 |
| Ranitidine | 4.3±0.37* | 84.69 |

Data represented as means±standard errors and Ethanol group were compared with the other groups.
* Significant at $p < 0.05$ in comparison with ethanol group

*Chenopodium* species were considered slightly toxic and have no ulcerogenic and irritation effects in the stomach of rats at 800 mg/kg b.w [17]. Therefore, the possible antiulcerogenic effect of the *C. moquinianum* aqueous methanolic extract and the pure isolated kaempferol glycosides (6) and (7) was investigated using ethanol induced ulcer model in rats and Ranitidine as a reference drug.

The results of this study showed that the intragastric administration of absolute ethanol produced an intense gastric mucosal damage, which was indicated by the biochemical, morphmetrical, histopathological and histochemical examinations.

The biochemical investigation of the tissue of the stomach of rats administered with ethanol showed a significant elevation in MDA levels (Table 1), while GSH and SOD levels were significantly decreased.

No mucosal lesions were detected in rats which were not treated with ethanol. The intragastric administration of ethanol caused gastric ulcer areas in rats Ulcer index (28.1 ± 1.7mm) (Table 2).

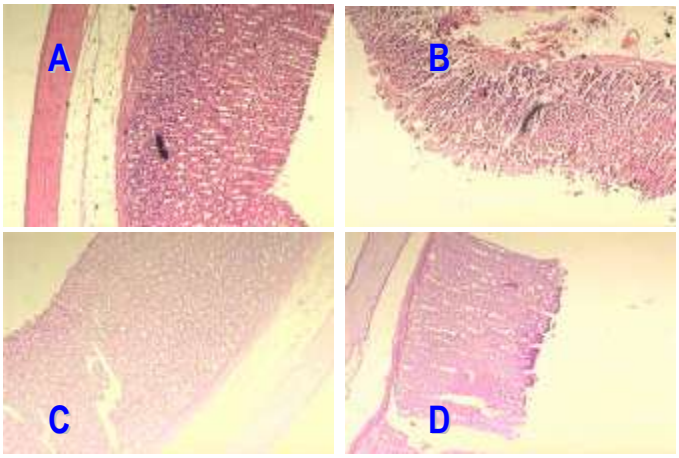



Fig. 3: A) Stomach of control rat, B) Stomach of rat treated with ethanol (H&E X150), C) Stomach of control rat and D) Stomach of rat treated with ethanol (PAS X150)

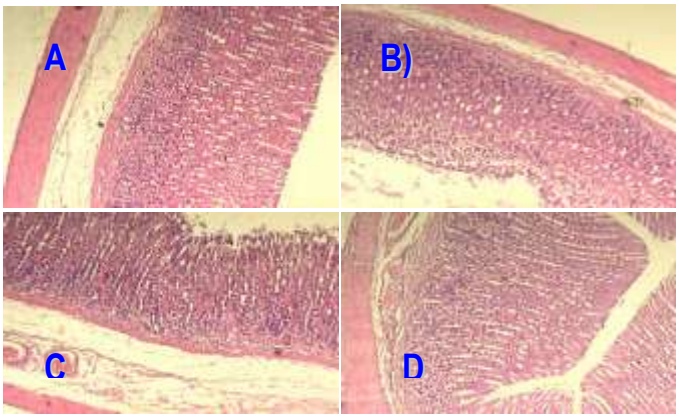



Fig. 4: A) Stomach of rat treated with ethanol and Rantidine, B) Stomach of rat treated with ethanol and crude extract, C) Stomach of rat treated with ethanol and kaempferol glycoside (6) and D) Stomach of rat treated with ethanol and kaempferol glycoside (7) (H&E X150)

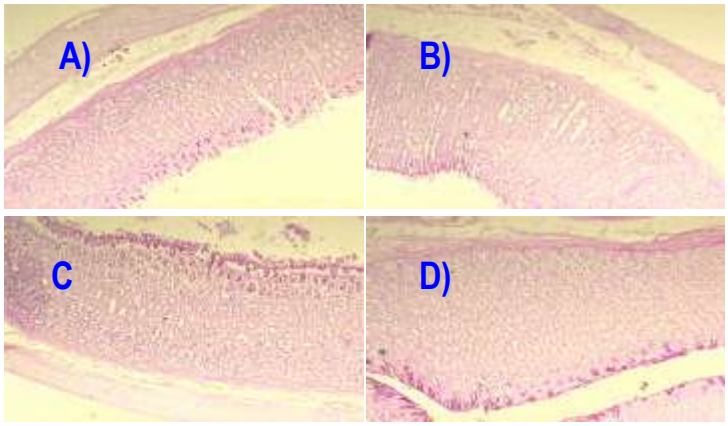



Fig. 5: A) Stomach of rat treated with ethanol and Rantidine, B) Stomach of rat treated with ethanol and crude extract, C) Stomach of rat treated with ethanol and kaempferol glycoside (5) and D) Stomach of rat treated with ethanol and kaempferol glycoside (6) (PAS X150)

Histopathological examination of the stomach sections revealed that absolute ethanol led to extensive mucosal lesions. These lesions were various; some extended into the mucosa involving the surface mucous cells at different depths and some extended to the cells lining the gastric pits or into the gland area. Mucosa was highly necrotized, large segment of necrotic mucosa was partially detached and was floating in lumen and there were submucosal hemorrhage and necrotic focuses (Fig. 3B), when compared with non-treated rats (Fig. 3A). The histochemical examination indicated that mucus production in the zones of the lesions was reduced (Fig. 3C) while the zones without lesions were preserved and mucus was even increased as compared with the control one (Fig. 3D). Similar results were found by Szabo *et al*. [33] and Kwiicien *et al*. [34].

Ethanol produces gastric lesions by direct action on the mucosa [33], impairment of gastric defensive factors and enhancement of lipid peroxidation products [35]. Ethanol is also able to induce direct oxidative damage against gastric mucosal tissues. It increases hydroxyl radical production and lipid peroxidation in the gastric mucosa [36].

The formation of gastric mucosal lesions by necrotizing agents, such as ethanol, has been reported to involve the depression of these gastric defensive mechanisms [37]. Oral treatment with ethanol causes focal edema, necrosis and submucosal hemorrhage, as well as circulatory disturbances [38]. The formation of gastric mucosal lesions following ethanol administration involves several mechanisms which reduce the flow of gastric blood, thereby contributing to the development of hemorrhage and necrosis and the solubilization of mucus constituents in the stomach. These actions resulted in an increased flow of $Na^+$ and $K^+$, increased pepsin secretion and a loss of $H^+$ ions and histamine into the lumen [33].

On the other hand, animals treated with *C. moquinianum* extract, kaempferol glycosides (6) and (7) and Ranitidine as a reference drug showed significant reduction in the incidence and severity of ulceration in ethanol-induced ulcer and significant reduction in lipid peroxidation levels as compared with the ethanol group. Antioxidant parameters (SOD & GSH) and lipid peroxidation (MDA) levels were found to be closer to the normal level in treated groups of animals compared to the control group.

Pre-treatment with *C. moquinianum* extract, pure isolated kaempferol glycosides (6) and (7) and the reference drug (Ranitidine) significantly reduced the ulcer area by 66.90%, 70.10%, 71.88% and 84.69%, respectively, compared with that given ethanol only. Histopathological examination of stomach sections revealed that pretreatment with *C. moquinianum* extract, pure isolated kaempferol glycosides (6) and (7) and Ranitidine reduced the degenerative changes that were induced by ethanol (Fig. 4A, B, C and D).

The histochemical examination showed an increase in mucus production which was most demonstrated in rats that were not treated with ethanol. In these rats the mucus appeared as a thick continuous layer covering the mucosal surface (Fig. 5A, B, C and D).

The possible protections of the *C. moquinianum* extract containing flavonoids and kaempferol glycosides (6) and (7) as flavonoid compounds that contain catecholic groups in their structures and their ability to scavenge radicals, thus preventing some deleterious processes [36]. Therefore, it is possible that phenolic compounds (1-11) as antioxidants present in *C. moquinianum* extract, might play a relevant role in mediating gastroprotective action of this extract [39,40]. Another possibility of the gastroprotective reason of the aqueous methanolic extract and the isolated pure kaempferol glycosides is through increasing the production of mucus as indicated by the histochemical examination. A number of factors appear to influence ulcer healing; a process for which mucus and bicarbonate secretion may be important since the mucus/bicarbonate layer protects newly formed cells from acid and peptic injury [41].

Moreover, oxygen radicals and lipid peroxidation are thought to be involved in the ethanol-induced gastric damage. As a consequence of their extreme chemical reactivity, reactive oxygen species (ROS) can cause severe changes at the cellular level which can culminate in cell death. At the molecular level, they attack essential cell constituents, such as proteins, lipids and nucleic acids, which can cause the loss of their biological function and formation of toxic compounds [42]. GSH is present in the stomach at high concentrations and plays an important role in maintaining the integrity of the gastric mucosa [43]. Excessive peroxidation causes increased GSH consumption [44]. The anti-oxidant activity of the extract and the pure compounds would have prevented excessive peroxidation.

Scrutinising the biochemical results showed that there were no significant different effects between the aqueous methanolic extract and kaempferol glycosides (6) and (7), while the histopathological and histochemical results indicated that Kaempferol 3-*O*-β-xylopyranosyl-(1→4)-α-rhamnopyranoside-7-*O*-α-rhamno-pyranoside (7) gave better results than the aqueous methanolic extract and Kaempferol 3,7-*O*-α-di-rhamnopyranoside (6).

## CONCLUSIONS

The present study clearly demonstrates that *C. moquinianum* extract and pure isolated kaempferol glycosides, Kaempferol 3,7-*O*-α-di-rhamnopyranoside (6) and Kaempferol 3-*O*-β-xylopyranosyl-(1→4)-α-rhamnopyranoside-7-*O*-α-rhamno-pyranoside (7) have antioxidant effect, gastro-protective and healing properties against ethanol induced oxidative stress and ulcer. The effects observed on chronic gastric lesions suggest influences on free-radical-scavenging properties and an increase of mucus production.

## REFERENCES


1. Jainu, M. and C.S.S. Devi, 2006. Antiulcerogenic and ulcer healing effects of *Solanum nigrum* (L.) on experimental ulcer models: Possible mechanism for the inhibition of acid formation. J. Ethnopharmacol., 104: 156-163.
2. Belaiche, J., A. Burette, M. De-Vos, E. Louis, M. Huybrechts and M. Deltenre, 2002. Study Group of NSAID-GI Complications. Observational survey of NSAID-related upper gastro-intestinal adverse events in Belgium. Acta Gastroenterologic Belgiam 65: 65-73.
3. Singh, S. and D.K. Majumdar, 1999. Evaluation of the gastric antiulcer activity of fixed oil of Ocimum sanctum (Holy Basil). J. Ethnopharmacol., 65: 13-19.
4. Rates, S.M.K., 2001. Plants as source of drugs. Toxicon., 39(5): 603-613.
5. Yesilada, E. and I. Gurbuz, 2003. A compilation of the studies on the antiulcerogenic effects of medicinal plants. In: Singh, Surender, Singh, V.K., Govil, J.N. (Eds.), Phytochemistry and Pharmacology. Recent Progress in Medicinal Plants, vol. II. SCI Tech Publishing LLC, Houston, TX, pp: 111-174.
6. Bailey, L.H., 1949. Manual of Cultivated plants, (Vol. 1), Macmillan. New York, pp: 352.
7. Zohary, M., 1966. *Flora Plasatina*, (Vol. 1). The Israel Academy of Sciences and Humanities, pp: 13.
8. Darnley, R.G., 1974. Chemotaxonamy of Flowering Plants, (Vol. II), McGill-Queens University Press, Montreal and London, pp: 1236.
9. Reschke, A. and K. Herrmann, 1982. Vorkommen von 1-*O*-Hydroxicinnamyl-β-D-glucosen im Gemüse. Zeitschrift für Lebensmittel-Untersuchung und-Forschung, 174: 5-8.
10. Bailey, L.H., 1942. The Standard Cyclopedia of Horticulture, (Vol. 1), Macmillan. New York, pp: 29.
11. Chopra, R.N., S.L. Mayar and I.C. Chopra, 1956. Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research, New Delhi, 27: 130.
12. Chopra, R.N., 1958. *Indigenous Drugs of India.* The Ant Press. Calcutta 2nd, 545: 607.
13. Boulos, L., 1983. Medicinal plants of North Africa. Inc., Algonac, Michigan, USA, pp: 286.
14. Bliss, A.R., 1925. A pharmacodynamic study of the anthelmintic properties of western oils of Chenopodium. J. of AVMA, 19: 625-630.
15. De Pascual, T.J., I.S. Bellido, C. Torres, B.A. Sastre and M. Grand, 1981. Phellandrene endoperoxides from the essential oil of *Chenopodium multifidum*. Phytochemistry, 20(1): 163-165.
16. Baydoun, E.A.H. and C.T. Bretr, 1985. Comparison of cell wall compositons of a desert xerophyte and a related mesophyte. Phytochemistry, 24(7): 1595-1597.
17. Ibrahim, L.F., S.A. Kawashty, A.R. Baiuomy, M.M. Shabana, W.I. El-Eraky and S.I. El-Negoumy, 2007. A Comparative Study of the Flavonoids and Some Biological Activities of two *Chenopodium* species. Chem. Nat. Comp., 43: 24-28.
18. Mata, R., A. Navarrete, L. Alvarez, R. Pereda-Miranda, G. Delgado and A. Romo De vivarf, 1987. Flavonoids and terpenoids of *Chenopodium graveolens*. Phytochemistry, 26: 191-193.
19. Morimoto, Y., K. Shimohara, S. Oshima and T. Sukamoto, 1991. Effects of the new anti-ulcer agent KB-5492 on experimental gastric mucosal lesions and gastric mucosal defensive factors, as compared to those of teprenone and cimetidine. Jpn. J. Pharmacol., 57: 495-505.
20. Vacca, L.L., 1985. Laboratory Manual of Histochemistry. Raven Press, New York, pp: 578.
21. Behmer, O.A., E.M.C. Tolosa and A.G. Freitas Neto, 1976. *Manual de técnicas para histologia normal epatológica*. EDART-Editora da Universidade de S&O Paulo, pp: 241.
22. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
23. Sun, Y., L.W. Oberley and Y. Li, 1988. A simple method for clinical assay of superoxide dismutase. Clin. Chem., 34: 497-500.
24. Tashima, K., A. Fujita and K. Takeuchi, 2000. Aggravation of ischemia/reperfusion-induced gastric lesions in streptozotocin-diabetic rats. Life Sciences, 67(14): 1707-1718.
25. Harbon, J.B., 1973. Phytochemical Methods. Chapman & Hall, London.

26. Mabry, T.J., K.R. Markham and M.B. Thomas, 1970. The Systematic Identification of Flavonoids, Springer-Verlag, New York, USA.
27. El-Mousallamy, A.M.D., U.W. Hawas and S.A.M. Hussein, 2000. Teucrol, a decarboxyrosmarinic acid and it's 4'-O-triglycoside, teucroside from *Teucrium pilosum*. Phytochemistry, 55: 927-931.
28. Statke, T., K. Kamiaya, Y. Saiki, T. Hama, Y. Fujimoto, S. Kitanaka, Y. Kimura, J. Uzawa, H. Endang and M. Umar, 1999. Studies on the constituents of fruits of *Helicteres isora* L. Chem. Pharm Bull., 47(10): 1444-1447.
29. Markham, K.R., 1982. Techniques of Flavonoid Identification, Academic Press, London.
30. Harborne, J.B., 1994. The Flavonoids-Advances in Research Science 1986, Chapman and Hall, London, UK.
31. Bock, K., C. Pedersen and H. Pedersen, 1983. Carbon-13 nuclear magnetic resonance spectroscopy data for oligosaccharides. Adv. Carbohydr. Chem. Biochem., 42: 193-225.
32. Gohar, A.A., G.T. Maatooq and M. Niwa, 2000. Two flavonoid glycosides from *Chenopodium murale*. Phytochemistry, 53(2): 299-303.
33. Szabo, S., J.S. Trier and C.H. Allan, 1987. Ethanol induced damage to mucosal capillaries of rat stomach. Ultrastructural features and effects of prostaglandin E2 and cysteamine. Gastroenterology, 92: 13-22.
34. Kwiicien, S., T. Brzowski and S.J. Konturek, 2002. Effects of reactive oxygen species action on gastric mucosa in various models of mucosal injury. J. Physiol. Pharmacol., 53: 39-50.
35. Soll, A.H., R. Goyal and C.R. Kahn, 1990. Pathogenesis of peptic-ulcer and implications for therapy. N. Engl. J. Med., 322: 909-916.
36. Repetto, M.G. and S.F. Llesuy, 2002. Antioxidant properties of natural compounds used in popularmedicine for gastric ulcers. Braz. J. Med. Biol. Res., 35: 523-534.
37. Kinoshita, M., N. Tsunehisa and H. Tamaki, 1995. Effect of a combination of ecabet sodium and cimetidine on experimentally induced gastric-lesions and gastric-mucosal resistance to ulcerogenic agents in rats. Biol. Pharm. Bull., 18: 223-226.
38. Oates, P.J. and J.P. Hakkinen, 1988. Studies on the mechanism of ethanol-induced gastric damage in rats. Gastroenterology, 94(1): 10-21.
39. Vilegas, W., M. Sanommiya, L. Rastrelli and C. Pizza, 1999. Isolation and structure elucidation of two new flavonoid glycosides from the infusion of m*aytenus aquifolium leaves*. Evaluation of the antiulcer activity of the infusion. J. Agric. Food Chem., 47(2): 403-406.
40. Coelho, R.G., F.G. Gonzalez, M. Sannomiya, L.C. Di Stasi and W. Vilegas, 2009. Gastric anti-ulcer activity of leaf fractions obtained of polar extract from *Wilbrandia ebracteata* in mice. Nat. Prod. Res., 23(1): 51-59.
41. Tarnawski, A., I.L. Szabo, S.S. Husain and B. Soreghan, 2001. Regeneration of gastric mucosa during ulcer healing is triggered by growth factors and signal transduction pathways. J. Physiol Paris, 95: 337-344.
42. Kaharaman, A., N. Erkasap, T. Koken, M. Serteser, F. Aktepe and S. Erkasap, 2003. The antioxidative and antihistaminic properties of quercetin in ethanol-induced gastric lesions. Toxicol., 183: 133-142.
43. Altinkaynak, K., H. Suleyman and F. Akcay, 2003. Effect of nimesulide, rofecoxib and celecoxib on gastric tissue glutathione level in rats with indomethacin-induced gastric ulcerations. Pol. J. Pharmacol., 55: 645-648.
44. Banerjee, S., C. Hawksby, S. Miller, S. Dahill, A.D. Beattie and K.E. McColl, 1994. Effect of *Helicobacter pylori* and its eradication on gastric juice ascorbic acid. Gut., 35: 317-322.