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Anti-ulcerogenic Activity of Extract and Some Isolated Flavonoids from *Desmostachia bipinnata* (L.) Stapf

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Abstract: Five main flavonoid glycosides were isolated, for the first time, from the ethanol extract of *Desmostachia bipinnata* (L.)Stapf (Gramineae). They were identified as kaempferol(**1**), quercetin(**2**), quercetin-3-glucoside(**3**), trycin(**4**) and trycin-7-glucoside(**5**). The structure elucidation was based on UV, Electrospray ionization mass spectrometry (ESIMS), ^{1}H and ^{13}C NMR, proton- proton correlation spectroscopy (^{1}H-^{1}H Cosy), distortionless enhancement by polarization transfer (DEPT), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlations spectrum (HMBC). The total extract (200 and 300 mg/kg) and two of the isolated compounds (trycin and trycin-7-glucoside.100 mg/kg each) showed a very promising antiulcerogenic activity with curative ratios; 68.31, 70.54, 77.39 and 78.93%, respectively.

Keywords: Antiulcerogenic; *Desmostachia bipinnata* (L.)Stapf; quercetin-3-glucoside; quercetin and trycin-7-glucoside.

1. Introduction:

Family Gramineae comprise 660 genera and 10000 species. This family is of great economic and medicinal importance as it includes all cereals, bamboos and sugar cane. The medicinal activities of Gramineae species were very clear in their uses as astringent and in treatment of wound [1], piles and venerable disease *(Cynodon dactylon*), as anti-emetic *(Phragmites australis*) and finally as diuretic, and eye problems. *Saccharum officinarum* (sugar cane).

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Some active chemical groups were isolated from the member of this family such as Alkaloids (such as hordenine, gramine and 5-methoxy –N-methyl tryptamine), flavonoides which have anti-oxidant activities and lower the concentration of blood triglycerides and cholesterol.

Desmostachia bipinnata (L.) Stapf (Gramineae) occurs widely in Egypt. The dried tender leaves of the plant are very active as antimicrobial [2,3]. Previous chemical work on the plant resulted in the isolation of some known coumarins (scopoletine and umbelliferone), sugars, amino acids and carbohydrates [4]. The pharmacological activity of this plant has not been studied before although it has been used in folk medicine in treatment of wounds and abdominal pain [5] .

2. Material and methods

2.1. Plant material

The aerial parts of *Desmostachia bipinnata* (L.) Stapf were collected from the North Sinai during summer 2003. Identification of the plant was verified by Late Prof. N. El-Hadidi, Professor of Taxonomy, Botany Dept., Faculty of Science, Cairo University and by comparison with plant description in Flora of Egypt [6,7]. A voucher specimen of was kept in the herbarium of Desert Research Center. Plant sample was air-dried in shade, reduced to fine powder, packed in tightly closed containers and stored for phytochemical and pharmacological studies

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2.2. Adsorbents and Solvent systems

Aluminium sheets 20x20 Cm Silica gel G 60 F254 Merck KGaA 64271 Darmstadt, Germany, was used for thin layer chromatography (TLC). Silica gel 60 particle size (70-230 mesh) was used for column chromatography. Whattman paper No. 3 for paper chromatography (PC). Solvent systems: (a) chloroform –methanol (9:1), (b) ethyl acetate-methanol-water (30:5:4); (c) ethyl acetate-methanol-acetic acid-water (65:15:10:10); (d) butanol- acetic acid – water (4:1:5) was used for developing the chromatoplates. Visualization of chromatograms was achieved under UV before and after exposure to ammonia vapor or by spraying with aluminum chloride [8]; all solvents used were of analytical grade.

2.3. Apparatus

Mp.s. were determined on a Kofler hot-stage apparatus and are uncorrected; mass spectra (Electrospray negative ion) sample dissolved in acetonitriale on an Micromass Quattro spectrometer .^{1}H and ^{13}C NM spectra, using external electronic referencing through the deuterium resonance frequency of the solvent, were determined at 600.17 or 150.91 MHz respectively with a JEOL ECA 600 spectrometer fitted with an auto 5mm X/H probe. Carbon atom types were established in the ^{13}CNMR spectrum by employing a combination of broad- and proton-decoupled and distortionless enhancement by polarization transfer(DEPT) experiments with 64 K data points over a spectrum width of 17,605.6Hz.[$^{1}J_{C\text{-}H}$] and $^{2}J_{C\text{-}H}$ and $^{3}J_{C\text{-}H}$]. ^{1}H-^{13}C correlations were established by using HMQC and HMBC pulse sequences respectively.^{1}H-^{1}H correlations were by double quantum filtered COSY.

2.4. Extraction and isolation

Defatted powder (2 kg) of the aerial parts of the plant was extracted in a Soxhlet apparatus with 95% ethanol. The ethanol extract was concentrated under reduced pressure (250 g), and diluted with water (300 ml), filtered over a piece of cotton then successively extracted with ether, chloroform, ethyl

acetate, and n-butanol. Each extract was dried over anhydrous sodium sulphate, and concentrated to yield 10, 20, 28.5 and 50 g dry extracts, respectively.

TLC examination of all extracts using systems a& b revealed the presence of the same spots in ether and chloroform extracts. Accordingly both extracts were combined together (20g) and applied on column chromatography packed with silica gel G (410 g) and eluted gradually with chloroform-methanol,100 fraction of 150 ml each were collected and reduced to four sub-fraction each fraction was concentrated under reduced pressure to yield 4,3,5.2 and 6 g respectively. All fractions were subjected to re isolation on silica gill columns from which compounds 1&2 were isolated.

The ethyl acetate and n-butanol extracts were found to have the same spots when chromatographic tested on TLC (system b). Both extracts were collected together (40 g) and subjected to preparative TLC (system b) followed with repeated preparative paper chromatography (PPC) using system (d). Bands corresponding to each flavonoid were separately extracted with methanol, concentrated, they yielded 8 and 10 g, respectively both fractions were reapplied separately on Silica gel columns and eluted with system (b) where compounds 2-5 were isolated.

2.5.Kaempferol ***1****:* (20 mg) yellow crystals, R_f =0.91 (system b), m.p. 277° C, UV, λ max in MeOH: nm 367, 268; ($AlCl_3$): 265, 350, 420; ($AlCl_3$/ HCl): 265, 350, 420; (NaOA): 275, 300 (sh), 380; (NaOAc/H_3BO_3): 267, 319 (sh), 380; (NaOMe): 285, 322, 430 .^{1}H-NMR (DMSO-d_6): δ 8.0 (2H, d, J = 8 Hz, H2` and H6`), δ 6.9 (2H, d, J = 8 Hz, H3` and H5`), δ 6.4 (1H, d, J = 2.5 Hz, H8) and δ 6.2 (1H, d, J = 2.5 Hz, H6). EI-MS m/z (% re. int): 285 (M^+) (100), 258 (15), 229 (16), 184 (8), 121 (22) and 93 (10).

2.6. Quercetin ***2****:* (95 mg) yellow crystals, m.p 322-324 °C, R_f =0.98 in system a. UV: λmax (MeOH): (nm) 255, 301 and 371, (NaOMe) 245, 330, ($AlCl_3$) 272, 301, 454., ($AlCl_3$/ HCl) 270,357, 426, (NaOAc) 275, 324,387, (NaOAc/H_3BO_3) 262,385. ^{1}HNMR(DMSO –d_6) δ 7.64 (1H , d ,J=8.5 H-2'), 7.49 (1H , q, J=8.5 , H-6'), 6.85 (1H , d, J=8.5 ,H-5')., 6.37 (1H ,d ,J=2.5, H-6) and 6.14 (1H , d, J=2.5, H-8). ^{13}C NMR (Methanol-D_6 600 MHz): δ ppm 176.3 (C-4) , 164.5 (C-7), 161.2 (C-5) , 156.6 and 148.2 (C-2 and C-9 respectively), 147.2 (C-4') , 145.5 (C-3'), 136.2 (C-3) , 122.4 (C-6') , 120.4 (C-1') , 116.1(C-2'), 115.5 (C-5') , 103.4 (C- 10), 98.8 and 93.7 (C-6 and C-8 respectively).

2.7. Quercetin -3-glucoside ***3****:* (68 mg) yellow crystals, m.p °C, R_f =0.98 in system a. UV: λmax (MeOH): (nm) 255, 301 and 371, (NaOMe) 245, 330, ($AlCl_3$) 272, 301, 454., ($AlCl_3$/ HCl) 270,357, 426, (NaOAc) 275, 324,387, (NaOAc/H_3BO_3) 262,385 . ^{1}HNMR(DMSO –d_6) δ 7.64 (1H , d ,J=8.5 H-2'), 7.49 (1H , q, J=8.5 , H-6'), 6.85 (1H , d, J=8.5 ,H-5')., 6.37 (1H ,d ,J=2.5, H-6) and 6.14 (1H , d, J=2.5, H-8), 4.48(d, J=3, 1H, H-1''), 3.3-4.13m.sugar proton). ^{13}C NMR (Methanol-D_6 600 MHz): δ ppm 176.3 (C-4) , 164.5 (C-7), 161.2 (C-5) , 156.6 and 148.2 (C-2 and C-9 respectively), 147.2 (C-4') , 145.5 (C-3'), 136.2 (C-3) , 122.4 (C-6') , 120.4 (C-1') , 116.1(C-2'), 115.5 (C-5') , 103.4 (C- 10), 98.8 and 93.7 (C-6 and C-8 respectively), 103.38(C-1″), 62.74(C-6″), 78.4 (C-5″), 78.11(C-″), 75.05(C-2″), 71.34(C-4″).

2.8.Tricin ***4****:* (345 mg) yellow crystals, m.p °C, R_f =0.78 in system a. UV: λmax (MeOH): (nm) 255, 301 and 371, (NaOMe) 245, 330, ($AlCl_3$) 272, 301, 454,($AlCl_3$/ HCl) 270,357, 426, (NaOAc) 275, 324,387, (NaOAc/H_3BO_3) 262,385 . ^{1}HNMR (DMSO –d_6):7.3(s, 2H, H-2'), 6.7(d, 1H, H-8), 6.6(s, 1H, H-3), 6.5(d, 1H, H-6), 3.94(s, 6H, 3', 5' OCH3). ^{13}C NMR (Methanol-D_6 600 MHz): δ ppm,164(C-2), 103(C-3), 181.63(C-4), 157.4(C-5), 98.67(C-6), 163.45(C-7), 94.3(C-8), 161.2(C-9), 121.1(C-10), 140.2(C-1'), 104.4(C-2'), 148.3(C-3'), 164.3(C-4'), 138.2(C-5'),104.5(C-6').

2.9. Tricin 7-glucoside **5**: (536 mg) yellow crystals, m.p °C, R_f =0.54 in system c. UV: λmax (MeOH): (nm) 255, 301 and 371, (NaOMe) 245, 330, ($AlCl_3$) 272, 301, 454., ($AlCl_3$/ HCl) 270,357, 426, (NaOAc) 275, 324,387,(NaOAc/H_3BO_3) 262,385 . ^{1}HNMR (DMSO –d_6):7.3(s, 2H, H-2'), 7.04(d, 1H, H-8), 6.89(s, 1H, H-3), 6.42(d, 1H, H-6), 5.02(d, J=3, 1H, H-1''), 4.64(s, 6H, 3', 5' OCH3) 3.24-3.84.1(m.sugar proton). ^{13}C NMR (Methanol-D_6 600 MHz): δ ppm,164.69(C-2), 103(C-3), 182.56(C-4), 157.4(C-5), 100.00(C-6), 163.45(C-7), 95.83(C-8), 161.61(C-9), 105.88(C-10), 140.2(C-1'), 105.05(C-2'), 148.3(C-3'), 163.61(C-4'), 148.79C-5'), 105.05(C-6'), 100.65(C-1″), 56.88(OCH_3), 56.88(OCH_3), 61.74(C-6″), 77.89(C-5″), 77.02(C-″), 73.68(C-2″), 70.15(C-4″).

3. Pharmacological study

3.1. Preparation of the plant extract

Dried aerial parts of *Desmostachia bipinnata* (L.)Stapf were extracted in a soxhlet apparatus with ethanol 95%. The ethanol extract was completely dried under vacuum, weighed and the residue was used in testing. The dried plant extract was freshly suspended in distilled water just before administration.

3.2. Determination of median lethal dose (LD_{50})

LD_{50} of the ethanol extract was determined as described before [9]. For this purpose, 5 groups of 5 mature mail Swiss albino mice (23-25 g body weight) each were used. The tested extract was administered orally in doses of 200-400 mg/kg b.wt in addition to a group used as a control (given the solvent). Mice were kept under observation for 24 h during which the number of dead animals in each group was recorded.

3.3. Anti-ulcerogenic effect

Forty male Wister albino rats of 190-200 g body weight were obtained from the Laboratory Animal Colonies, Helwan, Egypt. Animals were kept under good hygienic conditions and fed on standard diet and watered *ad libitum*. They were divided into 8 equal groups and starved for 48 h before use to ensure an empty stomach (Galvin & Mikhail 1976). To avoid dehydration during the period of fasting, rats were supplied with sucrose (BDH) 8% (w/v) solution in NaCl (BDH) 0.2% (w/v), which was removed 1 h. before experimentation. The 1st group was kept as a normal control, while the 2nd one was kept as a positive control. Three treatment groups received the ethanol extract of *Desmostachia bipinnata* (L.) Stapf in doses of 150, 250 and 300 mg/kg and another two treatment groups were administered compounds 4 and 5 respectively in a dose of 100 mg/kg. In addition, a group of rats was given ranitidine as a reference drug in a dose of 100 mg kg/kg. The ethanol extract, compounds 4, 5 and ranitidine were given orally via a stomach tube. Two doses were given in the first day at 08:00 h and 16:00 h; a third dose was given on the second day 1.5 h before induction of gastric ulceration. All rats except the normal control were given ethanol (Merck) 50% (v/v) (in distilled water) in a dose of 10 ml/kg orally to induce gastric ulceration. Normal control rats received equivolumes of distilled water only at the same time intervals. One hour after ethanol administration, all rats were killed by an overdose of chloroform and the stomachs were rapidly removed, opened along their greater curvature and gently rinsed under running tap water. Lesions in the glandular part

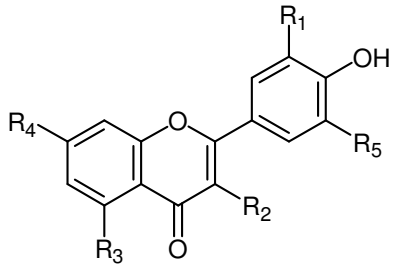
of the stomach were measured under an illuminated magnifying microscope (10 x). Lsions were counted and their lengths were measured. Petechial lesions were counted, and then each five petechial lesions were taken as 1 mm of ulcer [10]. To calculate the ulcer index (mm), the sum of the total length of long ulcers and petechial lesions in each group of rats was divided by its number. The curative ratio was determined according to the formula:

Curative ratio (%) = 100(C-S)/C wherein C is the ulcer index of the control, and S is the ulcer index of the test compound.

The results obtained were statistically analyzed using t- test [11].

4. Results and discussion

Compounds 1-5 were identified as kaempferol(1), quercetin(2), quercetin-3-glucoside(3), trycin(4) and trycin-7-glucoside(5) by comparing their, EI-MS, ^{1}H NMR, ^{13}C NMR, UV spectrum in methanol and different shift reagents with published data [12,13].



Compounds	R_1	R_2	R_3	R_4	R_5
1	H	OH	OH	OH	H
2	OH	OH	OH	OH	H
3	OH	O-gluc	OH	OH	H
4	OCH_3	H	OH	OH	OCH_3
5	OCH_3	H	OH	O-gluc	OCH_3

Figure 1. Structures of the compounds isolated from *Desmostachia bipinnata* (L.) Stapf

Acid hydrolysis of compound 3 and 5 revealed the sugar glucose that was identified by TLC (system c), and an aglycons which was found to be identical with compound 2 and 4 when compared with its TLC, UV shift reagents. It is substituted at position 3 and 7 in both compounds were indicated by their UV spectra upon addition of diagnostic shift reagent and from the correlation in HMBC and HMQC. From the obtained data and by their comparison with other published data [13,14] Compound 5 was identified as trycin-7-glucoside.

No deaths were recorded in mice treated with the alcohol extracts in doses from 0.1 to 5 g/kg body weight. This means that, the studied extracts are considered to be safe for human use, since substances possessing safety test higher than 0.5 g/kg are considered non toxic [15].

Gastric damage induced by ethanol was characterized by both long ulcers and petechial lesions. The number of ulcers and the ulcer index in control rats (received ethanol) were highly significant ($P< 0.001$) when compared to normal untreated animals (received distilled water). Repeated oral administration of the ethanol extract of *Desmostachia bipinnata* (L.)Stapf in doses of 250 and 300 mg/kg, reduced the severity of gastric damage, as the ulcer index was significantly decreased to 2.95 and 2.60 mm, respectively compared to 8.61 mm in the positive control group. The curative ratio was 61.34 and 67.55% following administration of both doses (Table 1). Compounds 4 and 5 in a dose of

100 mg/kg showed the best results. They significantly decreased the ulcer index into 2.00 and 1.60 mm, respectively compared to 8.61 mm in the positive control group and elevate the curative ratio into 79.51 and 80.96%, respectively. The curative ratio following ranitidine administration was 2.43% only. Failure of ranitidine to decrease gastric damage induced by ethanol could be attributed to its mechanism of action, as it blocks the histaminergic receptors, so prevents the stomach from producing excess acid. This mechanism cannot protect the gastric mucosa against the irritant and damaging effects of ethanol.

It could be concluded that the ethanol extract of *Desmostachia bipinnata* (L.)Stapf and the isolated compounds are highly safe for human use. Due to their antiulcerogenic activity, they could be used orally either for prophylaxis or for treatment of gastric ulcer.

Table 1. Antiulcerogenic effect of the ethanol extract of *Desmostachia bipinnata* (L.)Stapf and its isolated compounds in rats (n=5).

Treatment group	Dose (mg/kg b.wt)	Number of ulcers (M±S.E)	Ulcer index (mm)	Curative ratio (%)
Normal control	00	0	0	-
Positive control	00	6.5±0.48 [a]	8.60±0.46 [a]	-
Alcohol extract	150	6.4±0.24	7.61±0.32	15.42
	250	4.8±0.35 **	2.95±0.25 ***	61.31
	300	3.6±0.20 ***	2.60±0.21 ***	67.57
Compound 4	100	2.1±0.17 ***	2.00±0.12 ***	79.49
Compound 5	100	1.8±0.10 ***	1.60±0.10 ***	80.93
Ranitidine	100	6.2±0.30	8.40±0.44	2.43

[a] Compared to normal control (P< 0.001). ** P< 0.01 compared to positive control.
*** P< 0.001 compared to positive control.

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