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Chemical Constituents and Biological Activities of the Aerial Parts of *Stipagrostis plumosa* (L) Munro ex T.Anderson

Mohamed Salaheldin. A. Abdelkader[1*], Mohamed A. A Orabi[2], Sherif M Shama[2], Mahmoud. H. Assaf[3]
[1]*Department of Pharmacognosy, Faculty of Pharmacy, Sohag University, 82524 Sohag, Egypt*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut Branch, Assiut 71524, Egypt*
[3]*Department of Pharmacognosy, Faculty of Pharmacy, Assiut 71526, Egypt*

Abstract

Objectives: This study investigates active constituents and potential biological activities of *Stipagrostis plumosa* aerial parts, which may re-explore this plant as an important medicinal plant rather than being only forage for horses and camels.

Materials and methods: Compounds were isolated by different chromatographic methods. The structural determination was accomplished by the 1D- and 2D-NMR spectroscopic experiments as well as HRESIMS analysis. Cytotoxic activity against HepG-2 and MCF-7 cell lines was assessed using MTT assay, and expressed as IC_{50} (µg/ml) values. The antioxidant activity was performed using DPPH radical scavenging assay and expressed as IC_{50} (mg/ml) values. Carrageenan induced hind paw edema was performed to evaluate anti-inflammatory activity. The analgesic activity was evaluated using acetic acid induced writhing method in mice**.**

Results: Ten known compounds (**1-10**) are isolated and characterized for the first time from the plant. The total phenolic content is ranged from 10.44 to 42.33 mg/g gallic acid equivalents of the dry fraction weight. Compound **3** exerted selective growth inhibitory action against HepG-2 cell line with IC_{50} = 40 µg/mL. Meanwhile, the tested fractions and compounds exhibited moderate to low cytotoxic activities with IC_{50} ranging from 120–670 µg/mL. Ethyl acetate and *n*-butanol fractions showed maximum antioxidant activity with IC_{50} = 0.136 ± 0.005 mg/mL and 0.551± 0.012 mg/mL, respectively. Also, the tested fractions showed significant analgesic and anti-inflammatory activities.

Conclusion: The present study shows that *Stipagrostis plumosa* could be regarded as promising plant source of bioactive phenolic compounds with good antioxidants, moderate cytotoxicity, strong analgesic and anti-inflammatory activities.

Keywords: *Stipagrostis plumosa*; Phenolics; Flavolignans; Antioxidants; cytotoxicity, antimicrobial, anti-inflammatory and analgesic.

INTRODUCTION

Stipagrostis plumosa (L.) T. Andersson, family Gramineae or Poaceae is a perennial grass, 15–50 cm tall and grows wildly in stable or modestly mobile sand [1]. It is distributed in the Africa north of the Sahara, eastwards through Arabia, Turkey and Palestine to Pakistan and northwest India, Mediterranean region and Egyptian desert [1]. Gramineae is the fifth largest family of flowering plants in the world with approximately 660 genera and 10000 species [2]. This family is of great economic and medicinal importance as it includes all cereals, bamboos and sugar cane. Steroids, terpenoids, flavonoids, phenolic acids, fatty acids, hydroxamic acids and alkaloids are the main classes of the bioactive compounds reported from poaceae. The medicinal activities of plant species of Gramineae are very clear including antioxidant, [3], antimicrobial [4], antifungal [5], anticancer and cytotoxic activities [6]. Some of the grass species have been proved to show strong antioxidant properties and have been effective in the treatment of inflammations [7]. Some species exhibited antiparasitic activities, antimalarial [8] and anthelmintic [9], hepatoprotective, [10], antihyperglycemic [11]. Anti-inflammatory, antipyretic and analgesic activities [12] are also among the different reported biological properties of family Poaceae. The genus *Stipagrostis* is of maximum diversity in North American desert. It consists of about 50 species, some of the species belong to this genus are important from forage point of view. Species of the genus *Stipagrostis* are important components of various habitats in desert areas. *S. plumosa* provides excellent forage for camels and horses [13]. The whole plant is prescribed by local peoples to treat gastro-intestinal disorders as antispasmodic. Reviewing the current literatures, nothing could be traced considering phytochemical or biological studies on *S. plumosa* (L.) plant. These encourage us to investigate its active constituents and potential biological activities, which may re-explore this plant as an important medicinal plant rather than being used only by local people and forage for horses and camels.

MATERIALS AND METHODS

Apparatus and Chemicals

^{1}H and ^{13}C NMR spectra were recorded at 25 °C with a Varian Inova 400 MHz NMR spectrometer. High-resolution mass spectra were acquired with a Thermo

scientific LTQ/XL Orbitrap, specifications; analyzer: FTMS, mass range: normal full ms 100-2000, resolution: 30,000. For LC-ESIMS, gradient separation was achieved using a Sun Fire C-18 analytical HPLC column (5 mm, 4.6×150 mm, Waters) with a mobile phase of 0-100% MeOH over 30 min at a flow rate of 1 mL/min. RP-HPLC were carried out on Agilent 1260 Infinity semi-preparative HPLC system with an Agilent Eclipse XDB-C18 column (5 m, 10×250 mm, Agilent technologies, USA) monitored using an Agilent photodiode array detector. Detection was carried out at 220, 254, 280, 350, and 400 nm. TLC was performed on pre-coated TLC plates with silica gel 60 F254 (layer thickness 0.2 mm, Merck, Darmstadt, Germany). The chromatograms were developed using the following solvent systems: HEX:EtOAc (90:10), HEX:EtOAc (95:05), DCM/MeOH (90:10,)and DCM/MeOH (85:15,). The compounds were detected by spraying with 10% v/v H_2SO_4 in EtOH reagent and heating at 110 °C for 1–2 min. Column chromatography (CC) was performed using a silica gel (Kieselgel 60 Å, 40-63 µM mesh size, Fluorochem, UK).

Plant material

The aerial parts of *S. plumosa* L. Munro ex T. Anderson were collected in May 2013 from east desert (35 Km) from Assuit, Egypt. The plant material was identified and authenticated by Prof. Dr. Salah M. El-Naggar Professor of Botany and Plant Taxonomy, Faculty of Science, Assuit University, Assuit, Egypt. A voucher sample (No. STP2013) was kept in the Herbarium of Faculty of Pharmacy, AL-Azhar University, Assuit, Egypt. The aerial parts were air-dried in the shade, and then ground.

Animals Used

Male albino rats (wt. = 100 - 120g) and mice (wt. = 20 – 25g) were used. The animals were housed under standardized environmental conditions in the Pre-Clinical Animal House, Pharmacology Department, Faculty of Medicine, Assiut University. They were fed with standard diet with free access to tap water and kept under a 12/12 hours light/dark cycle.

Extraction and isolation

A 5 kg of the air-dried powdered aerial parts was extracted by maceration in methanol/H_2O (70%, v/v) till complete exhaustion, followed by filtration. The filtrates were combined and evaporated to dryness under reduced pressure to obtain crude dark greenish brown syrupy residue extract. The solvent free residue (340 g, 6.8%) was mixed with 500 mL of distilled H_2O, and subjected to successive solvent fractionation to give *n*-hexane fraction (25 g), dichloromethane fraction (6 g), ethyl acetate fraction (23 g), *n*-butanol fraction (45 g) and aqueous fraction (200 g). The crude ethyl acetate fraction (15 g) was subjected to column chromatography on silica gel using DCM-MeOH gradients. Fractions of 100 ml were collected and the similar fractions were combined and concentrated under reduced pressure to give nine groups labeled SPE-1 to SPE-9. Fraction SPE-5 was chromatographed on silica gel columns using DCM-MeOH, followed by sephadex LH-20 CC using DCM-MeOH (1:1, v/v) as solvent system, and afforded compound **3** (20 mg) and compound **4** (10 mg). Fraction SPE-6 was subjected to repeated silica gel CC using *n*-hexane-EtOAc gradient and DCM-MeOH gradient to afforded two sub-fractions labelled as SPE-6-A and SPE-6-B. The SPE-6-A was chromatographed on silica gel CC using *n*-hexane-EtOAc followed by sephadex LH-20 CC, and afforded compound **5** (9 mg). Fraction SPE-6-B was repeated chromatographed on silica gel columns using DCM-MeOH gradients, and finally purified by RP- HPLC to afford compounds **1** (10 mg) and **2** (12 mg). Fraction SPE-7 was chromatographed on silica gel columns using DCM-MeOH gradient elution that afforded compounds **8** (12 mg). The crude *n*-hexane fraction (20 g) was subjected to column chromatography on silica gel using *n*-hexane-EtOAc mixtures in a manner of increasing polarities. Fractions of 100 ml were collected and monitored on TLC (silica gel) using *n*-hexane-EtOAc mixtures of different polarities as solvent systems, and H_2SO_4 in EtOH (10%, v/v) as a spray reagent. Similar fractions on TLC were combined to yield 8 groups, SPH-1 to SPH-8. Group SPH-4 was subjected to repeated silica gel CC using gradients of *n*-hexane-EtOAc and *n*-hexane-DCM (20:80) isocratic elution which afforded compound **6** (30 mg). Group SPH-5 was subjected to repeated silica gel CC using *n*-hexane-EtOAc gradient which afforded two sub-fractions labeled SPH-5-A and SPH-5-B. The SPH-5-A was chromatographed on silica gel CC using *n*-hexane-EtOAc (98:2) isocratic elution which afforded compounds **9** and **10** (100 mg). The SPH-5-B was chromatographed on silica gel CC using DCM (100%) isocratic elution which afforded compound **7** (20 mg).

Total soluble phenolic compound content

The content of soluble phenols was measured using a modified Folin and Ciocalteu reagent and the method described previously [14]. The method employs the reduction of a phosphowolframate–phosphomolybdate complex to blue products by phenolic compounds. Briefly, 0.5 ml of 1mg/ml solution of the extract, blank or standard was placed in a 25 ml volumetric flask, where the Folin–Ciocalteu reagent (0.5 ml) was added and the mixture was allowed to react for 3 min under continuous stirring, then a solution of sodium carbonate (75 g/l, 10 ml) was added and mixed well. The volume was then made up to 25 ml with distilled water and left standing at room temperature for 1 hour. The absorbance was then measured at 750 nm using a Shimadzu UV-160A spectrophotometer (Kyoto, Japan). The results were expressed as gallic acid equivalents (GAE), using a calibration curve over the range of 50–200 ppm. The concentration of the total

phenolics contents was calculated by using an equation obtained from gallic acid calibration curve.

Cytotoxicity assay

The in vitro cancer growth inhibitory activity of some of the isolated compounds and fractions was determined using MTT colorimetric assay against hepatocellular carcinoma (HepG-2) and human breast adenocarcinoma (MCF-7) cell lines. The evaluation is based on the reduction of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), by the mitochondrial dehydrogenase of viable cells, to give a blue formazan product which can be measured spectrophotometrically. Cells were seeded in 96-well plates at a concentration of 5×10^4 cells/well and incubated for 24 h at 37 °C in a 5% CO2 humidified atmosphere. Plate was incubated at 37 °C and examined frequently for up to 3 days. Cells were checked for any physical signs of toxicity (e.g. partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation. After treatment with various concentrations and dilutions of the test compounds and fractions, the cells were incubated for an additional 48 hours at 37 °C. After that, the medium was removed and cells in each well were incubated with 100 µL of MTT solution (5 mg/mL) for 4 hours at 37 °C. MTT solution was then discarded and 200 µL dimethyl sulfoxide (DMSO) was added to dissolve insoluble formazan crystal and the plates were incubated at 37 °C for 30 min. Optical density (OD) was measured at 560nm and subtract background at 620nm using ELISA reader (Mindray Microplate Reader). Data were obtained from triplicate wells. Cell viability was expressed with respect to the absorbance of the control wells (untreated cells), which were considered as 100% of absorbance. The percentage of cytotoxicity is calculated as [(A - B) / A] × 100, where A and B are the OD560 of untreated and of treated cells, respectively. The 50% cytotoxic concentration (CC50) was defined as the compound's concentration (µg/mL) required for the reduction of cell viability by 50%, which were calculated by regression analysis [15].

Antioxidant activity

The method of Gupta *et al.* [16] was adapted for testing the radical scavenging of the plant extracts using the stable free radical 2,2-diphenyl-1-picrahydrazy (DPPH) spectrophotometry. Stock solution of DPPH was prepared as 0.1 mM DPPH in methanol. In the assay, 0.2 mL different extracts and standard of various serial concentrations (1- 0.0625 mg/mL) were added to 1.8 mL of 0.1 mM DPPH solution and the mixture was shaken vigorously by vortex and allowed to stand for 30 minutes at room temperature in dark. After incubation, absorbance of the solutions was measured against a blank (0.2 ml methanol and 1.8 ml $DPPH^{\cdot}$ only without sample, expressed as 100 % free radicals) at 517 nm spectrophotometery. Antioxidant activities of each extracts were determined based on the reduction of $DPPH^{\cdot}$ absorbance by calculating percentage of antioxidant scavenging activity as the change in absorbance with respect to the blank. The experiments were carried out in triplicate using ascorbic acid as a reference standards and DPPH radical scavenging activity was calculated by using the formula [17].

$$\%\ scavenging = \frac{absorbance\ of\ control - absorbance\ of\ test}{absorbance\ of\ control} \times 100$$

Inhibition Concentration ($IC_{50\%}$), the amount of sample necessary to decrease the absorbance of DPPH by 50% was done by Brand-Williams *et al*.,1995, for the interpretation of the results from $DPPH^{\cdot}$ method. The discoloration of sample was plotted against the sample concentration in order to calculate the $IC_{50\%}$ value [18]

Anti-inflammatory activity:

Carrageenan-induced rat hind paw edema model described by Winter *et al* [19] was performed. This method of testing anti-inflammatory activity depends on measuring the inhibition of edema produced acutely by injection of phlogistic agent into the tissues of the plantar surface of the hind paw of the rat. Seven groups (5 rats/ group) were used. The inflammation was induced in rat paws by injection of 0.1 ml of 1% carageenan suspension in normal saline solution into the sub-plantar tissue of the right hand paw. At the beginning of the experiment, the paws thickness was measured in mm using varinier caliber. The first group was kept as a negative control, injected intraperitoneally by 3% tween 80 in normal saline, while the second group injected by indomethacin (8 mg/kg) as a positive control. The other groups were separately intraperitioneally injected with different extracts of aerial parts of *S. plumosa* (L) at a dose of 400 mg/kg of the body weight. After 30 minutes from administration, the inflammation was induced by injection of the carrageenan suspension in the right paw while the left one was injected by an equal volume of saline solution. The difference between the thicknesses of the two paws was taken as a measure of edema. The anti-inflammatory efficacy of the tested fractions was estimated by comparing the magnitude of paw swelling in the pretreated animals with those induced in control animals receiving saline. The measurements were carried out at 1, 2, 3, 4 and 5 hours after injection of the inflammatory agent. The percentage of edema [20] and percentage of inhibition [21] were calculated as follows:

$$\%\ \text{Variation (edema)} = \frac{(\text{Right paw thickness - Left paw thickness}) \times 100}{\textit{Right}\ \text{paw thickness}}$$

and

$$\%\ \text{Inhibition} = \frac{(V_o - V_t) x 100}{V_o}$$

Where:

V_o: the average paw thickness of control group.

V_t: the average paw thickness of the treated group.

Analgesic activity

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice according to the method of Koster *et al* [22]. Swiss male albino mice (20-25 g) were used. Animal were acclimatized to the laboratory condition for at least one hour before testing. In this method, acetic acid is administered intraperitoneally to the experimental animals to induce pain sensation. Diclofenac sodium (5 mg/kg) was used as a positive control. Different extracts at a dose of 400 mg/kg was administrated orally. Thirty minutes later, 0.6% acetic acid was injected intraperitoneally (0.2%ml/mouse), but Diclofenac sodium was administered 15 minutes prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse of all groups were observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperiotoneal administration of acetic acid solution. The number of writhes in each treated group was compared to that of a control group.

Statistical analysis

All biological assays were performed in triplicate. Results were expressed as mean ± standard error (SEM). Independent t-test and one-way analysis of variance (ANOVA) were used to compare two or more groups of data, p values less than 0.05 were considered significant. SPSS software was used for statistical analysis.

RESULTS AND DISCUSSION

The phytochemical investigations of the aerial parts of methanol extract lead to isolation and identification of ten compounds for the first time from the plant. The identified compounds are: Flavolignans; salcolin A (**1**) and salcolin B (**2**) [23], a flavonoid; tricin (**3**) [24-26], simple phenolics; vanillic acid (**4**) [27, 28] and protocatechualdehyde (**5**) [29, 30], fatty acids; palmitic acid (**6**) [31] and oleic acid (**7**) [32], and phytosterols; β-sitosterol-3-*O*-β-D-glucopyranoside (**8**) [33] and a mixture of β-sitosterol and stigmasterol (**9** and **10**) [34] . All physical and spectral data of these compounds are in agreement with the published data. The different chemical shift and coupling constant parameters of compound **1-5** are summarized in table 1.

Compounds **1** and **2** were isolated as yellow amorphous powder. Both compounds showed the same HRESIMS at m/z: 527.1547 $[M+H]^+$, correspond with the molecular formula $C_{27}H_{26}O_{11}$. The 1D and 2D NMR spectra were similar to the extent that the same sets of resonances were nearly observed in each compound as shown table 1. The only difference was observed is the signals for adjacent chiral centers at C-7" and C-8" Compounds **1** and **2** are diastereoisomers of tricin-4′-*O*-(β-guaiacylglyceryl) ether, rare flavonolignans that have been previously reported in few studies [23, 35]. The stereoisomerism in these compounds arises from the presence of adjacent chiral centers at C-7" and C-8". The assignment of *erythro* and *threo* forms of the two diastereoisomers has been discussed in detail [23]. Generally, adjacent protons of the *erythro* type have been reported to have smaller coupling constants (4.5–5.6 Hz) than those of the *threo* type (5.0–8.2 Hz) in different *d*-solvents [23, 36]. Salcolin A, isolated from *Calamus quiquesetinervius* showed potent platelet aggregation inhibition, compared with aspirin [37].

Compound **3**, tricin, is a common flavone dominant in cereal crop plants; in the wheat grain. Tricin is mainly found as an aglycone in the outer layers: husk, pericarp and aleurone [38]. Because of its wide spectrum of health promoting effects, a metabolic engineering strategy has been proposed to produce tricin in sufficient amounts for further experimentation, and increase its accumulation in wheat grain endosperm as a nutraceutical [39] . Tricin occurrence, biosynthesis, regulation, biological importance, pharmacological effects, and potential role as a chemopreventive and anticancer agent has been previously reviewed [39]. In this study, tricin isolated in sufficient quantities making *S. plumosa* aerial parts a potential source for tricin.

Cytotoxicity assays

Based on Antioxidants screening and total phenolic contents of different plant fractions. Ethyl acetate and *n*-butanol fractions were chosen for cytotoxicity assays. In this study compounds **1-3**, Ethyl acetate and *n*-butanol fractions were evaluated for cytotoxic activity against MCF-7, and HepG2 cell lines by MTT assay. The IC_{50} values are listed in table 2. Compound **3**, tricin, displayed selectively strong growth inhibitory action against HepG-2 cell line with IC_{50} = 40 µg/mL and moderate activity against MCF-7 cell lines with IC_{50} = 206 µg/mL. Although compounds **1** and **2** are diastereoisomers, they exhibited different cytotoxic activity. Compound **1**, *threo* isomer, was

Table 1: ^{1}H and ^{13}C NMR spectroscopic data of compounds **1–5** in DMSO-d_6 (400 and 100MHz).

	1		**2**		**3**		**4**		**5**	
No.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	-	-	-	-	-	-	-	123.6		126.4
1-COOH/CHO	-	-	-	-	-	-	-	169.0	9.73 (1H, s)	193.0
2	-	163.0	-	163.0	-	164.1	7.56 (1H, d, 1.8)	115.8	7.34 (1H, d, 8)	116.2
3	7.03 (1H, s)	104.8	7.02 (1H, s)	104.8	7.02 (1H, s)	103.8	-	148.6		147.1
3-O CH3	-	-	-	-	-	-	3.90 (3H, S)-O CH3	56.4		-
4	-	181.8	-	181.8	-	181.8	-	152.4		153.7
5	-	161.4	-	161.4	-	161. 4	6.84 (1H, d, 8.2)	113.8	6.87 (1H, d, 2.4)	130.8
6	6.20 (1H, br s)	99.1	6.91 (1H, br s)	99.2	6.19 (1H, d, 2.1)	98.8	7.54 (1H, dd, 8.2, 1.8)	125.2	7.38 (1H, dd, 8, 2.4)	115.3
7	-	164.7	-	164.8	-	163.6				
8	6.55 (1H, br s)	94.4	6.53 (1H, br s)	94.5	6.55 (1H, d, 2.1)	94.2				
9	-	157.4	-	157.5	-	157.3				
10	-	103.7	-	103.5	-	103.7				
1`	-	125.3	-	125.3	-	125.3				
2`,6`	7.31 (2H, s)	104.3	7.30 (2H, s)	104.3	7.30 (2H, s)	104.3				
3`,5`	-	152.9	-	153.0	-	148.2				
4`	-	139.9	-	139.5	-	142.2				
3`,5`-O CH3	3.87 (6H, s)	56.4	3.88 (6H, s)	56.4	3.88 (6H, s)	56.4				
1``	-	133.0	-	133.2	-					
2``	6.97 (1H, d, 1.8)	111.0	6.93 (1H, d, 1.8)	111.0						
3``	-	146.9	-	147.0						
4``	-	145.4	-	145.5						
5``	6.71 (1H, d, 8.2)	114.7	6.70 (1H, d, 8.2)	114.7						
6``	6.79 (1H, dd, 8.2, 1.8)	119.2	6.75 (1H, dd, 8.2, 1.8)	119.4						
7``	4.83 (1H, d, 5.2)	71.6	4.80 (1H, d, 5.2)	72.2						
8``	4.26 (1H, m)	87.0	4.35 (1H, m)	86.5						
9``	3.64 (1H dd, 11.7, 4.9)	60.4	3.73 (1H, m)	60.2						
	3.52 (1H, dd, 11.8, 3.4)		3.50 (1H, dd, 11.8, 4.8)							
3``-O CH3	3.74 (3H, s)	55.5	3.74 (3H, s)	55.5						

Table 2: Cytotoxic activity of isolated compounds (**1-3**), ethyl acetate and *n*-butanol fractions against HepG-2 and MCF-7 cell lines.

Compound/extracts	IC_{50} (μg/ml)	
	HepG-2	MCF-7
1	120	215
2	330	570
3	40	206
Ethyl acetate	288	290
***n*-butanol**	460	670
Doxorubicin	0.75	1.28

Table 3: Effect of different extracts of *S. plumosa* (L) on the right hind paw thickness in carrageenan induced hind paw oedema model in rats.

Group	Dose(mg/kg)	Time (hours) after phlogistic agent administration				
		1	2	3	4	5
		Thickness of the right paw (mm) after injection				
Control		**5.49**±0.055	**5.61**±0.030	**5.85**±0.038	**5.87**±0.029	**6.11**±0.050
Indomethacin	8	**4.83**±0.060**	**4.74**±0.055**	**4.67**±0.053**	**4.61**±0.038**	**4.57**±0.023**
Total extract	400	**5.04**±0.029**	**4.86**±0.065**	**4.11**±0.034**	**4.34**±0.066**	**4.72**±0.057**
***n*-Hexane**	400	**5.07**±0.026*	**4.92**±0.033**	**4.88**±0.023**	**5.12**±0.049**	**5.47**±0.072**
Ethyl acetate	400	**5.08**±0.087*	**5.02**±0.093**	**4.96**±0.092**	**5.26**±0.066**	**5.60**±0.041**
***n*-Butanol**	400	**5.04**±0.043**	**4.50**±0.084**	**4.06**±0.026**	**3.95**±0.034**	**4.29**±0.037**
Aqueous	400	**4.70**±0.05**	**4.58**±0.047**	**4.23**±0.029**	**4.04**±0.028**	**4.44**±0.092**

Data are expressed as mean ±S.E, n=5

Differences with respect to the control group were evaluated using two-way ANOVA test.

(*P<0.05, ** P<0.01) S.E. = Standard error n= Number of animals

Table 4: Results of the inhibitory effects of the different fractions of aerial parts of *S. plumosa* (L) on Carrageenan induced edema in rats.

Group	Dose (mg/kg)	Time (hours) after phlogistic agent administration				
		1hr	2hr	3hr	4hr	5hr
		% inhibition of inflammation				
Control	-	_	_	_	_	_
Indomethacin	8	12.02	15.51	20.27	21.52	25.15
Total extract fr.	400	8.14	13.43	29.74	26.06	21.06
***n*-Hexane fr.**	400	7.65	12.24	16.58	12.83	10.53
Ethyl acetate fr.	400	7.47	10.58	15.27	10.45	8.35
Butanol fr.	400	8.26	19.85	30.54	32.77	29.84
Aqueous fr.	400	14.39	18.36	27.75	31.18	27.33

Table 5: Results of analgesic activity of the different fractions of *S. plumosa* on Swiss albino mices using acetic acid induced writhing method.

Treatment	Dose (mg/Kg)	Number of writhes observed	Inhibition percentage
Negative Control.	------	77.33±1.764***	--------
Diclofinac sodium	5	6.67±0.422***	91.38
Total extract	400	21.17±0.477***	72.62
n-Hexane fr.	400	16.50±0.428***	78.66
Ethyl acetate fr.	400	13.50±0.763***	82.54
n-Butanol fr.	400	8.50±0.428***	89.01
Aqueous fr.	400	14.50±0.428***	81.25

Data are expressed as mean ±S.E, n=5

Differences with respect to the control group were evaluated using the students t-test (*P<0.05, **P<0.01, ***P<0.001). S.E. = Standard error n= Number of animals

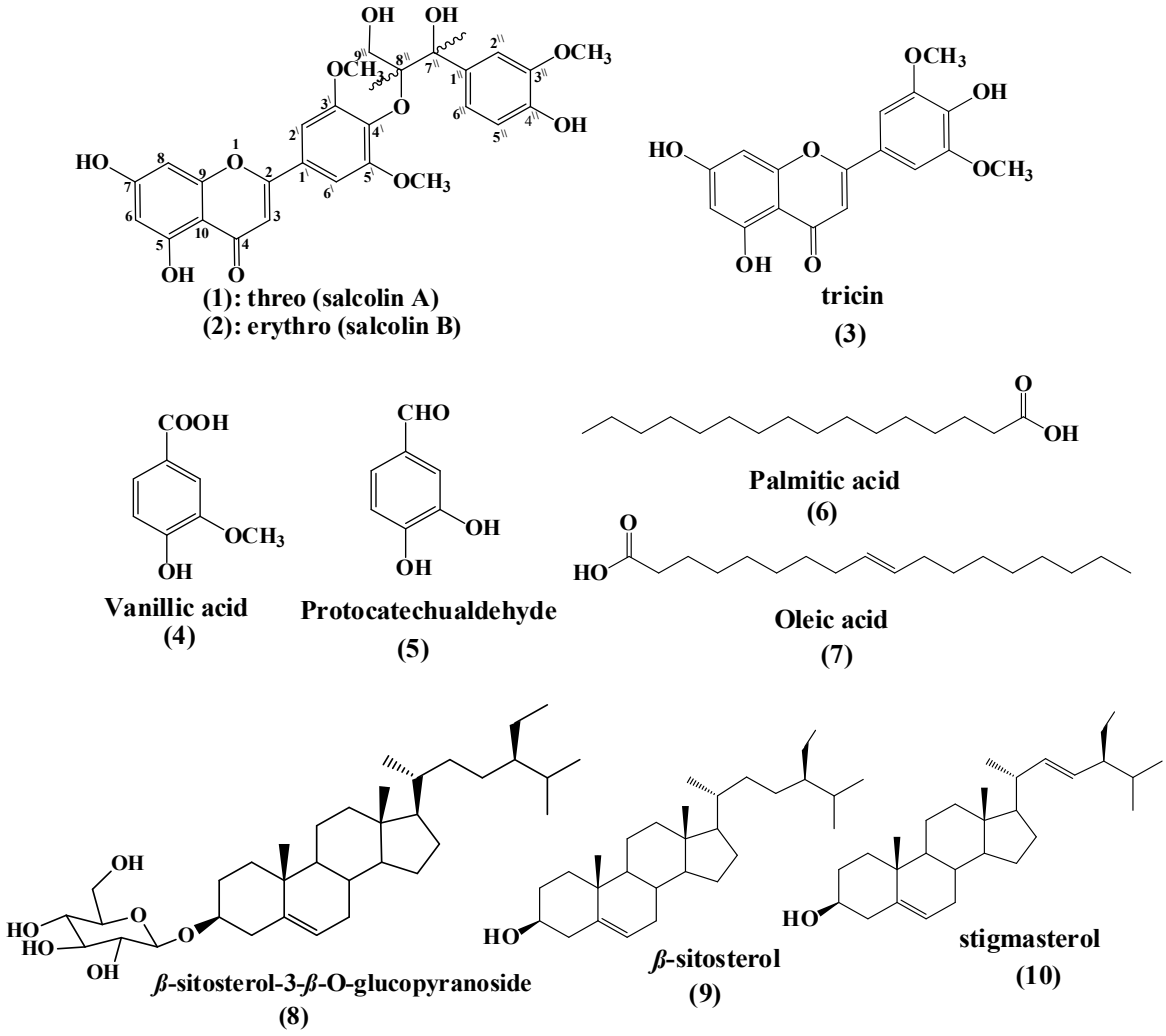

Figure 1: Structures of isolated compounds (**1-10**).

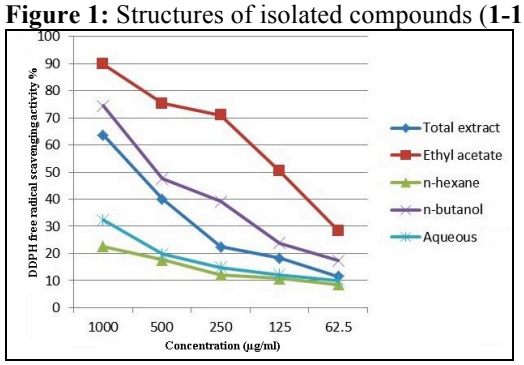

Figure 2: Antioxidant activity of the total methanolic extract and different fractions of *S. plumosa* aerial parts.

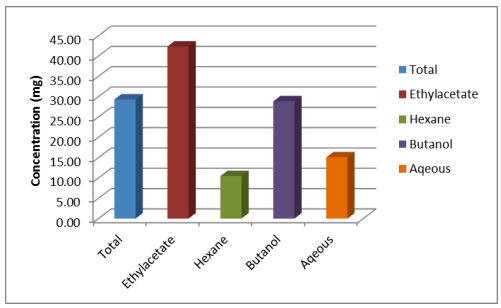

Figure 3: Total phenolic contents of methanolic extract and different fractions *of S. plumosa* aerial parts expressed in terms of gallic acid equivalent (mg/g GAE of extract).

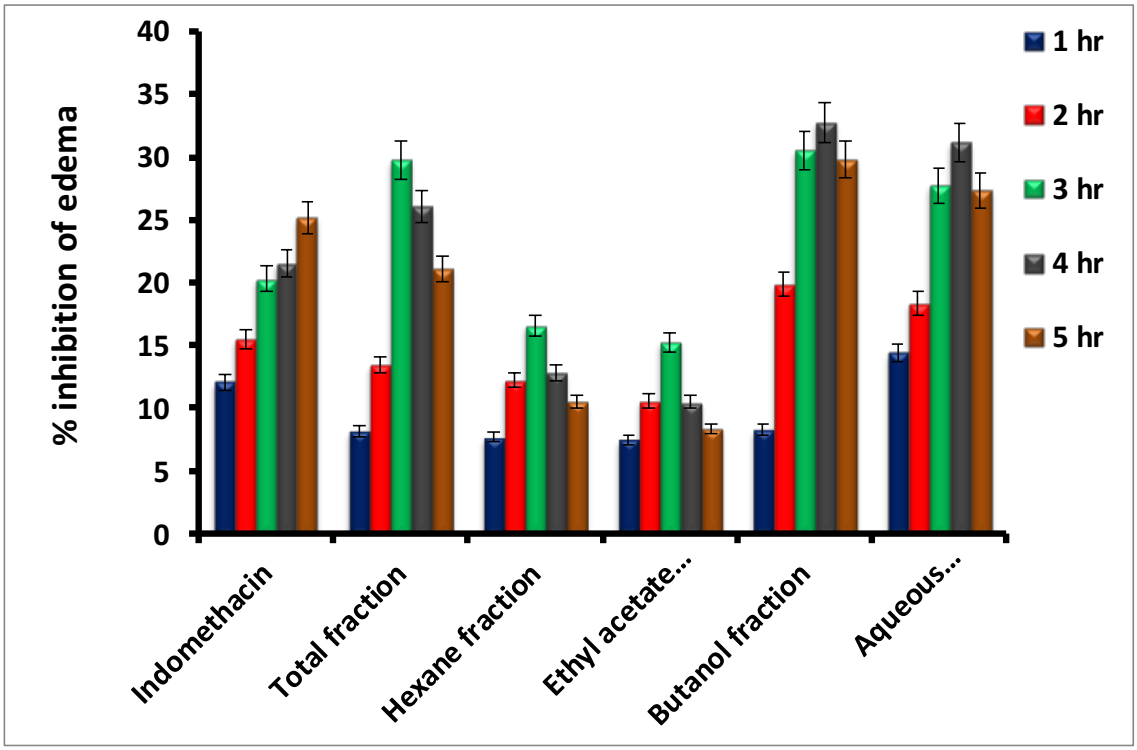

Figure 4: Percentage of edema inhibition of the total methanolic extract and different fractions of *S. plumosa* aerial parts.

more active than compound **2** *erythro* isomer, against HepG-2 cell line with IC_{50} = 120 µg/mL and MCF-7 cell line with IC_{50} = 215 µg/mL. Meanwhile, compound **2**, showed less cytotoxic activity with IC_{50} = 330 µg/mL and 570 µg/mL against HepG-2 and MCF-7 cell lines, respectively. Of the two tested fractions, ethyl acetate showed more cytotoxic activity than *n*-butanol fraction, with IC_{50} = 288 µg/mL and 290 µg/mL against HepG-2 and MCF-7 cell lines respectively. *n*-butanol fraction showed less cytotoxic activity with IC_{50} = 460 µg/mL and 670 µg/mL against HepG-2 and MCF-7 cell lines respectively. Tricin reported to have selective potent anticancer against colon and liver cancers [40]; this explain its potent activity against HepG-2 ell line and its moderate activity against MCF-7 cell lines in our study. Several studies attempted to explain the mechanism(s) of action of tricin; the US National Cancer Institute has recently considered tricin as one of the most promising new chemopreventive agents. Its target organ was identified as the colon, and its mechanism seems to be through the inhibition of both cyclooxygenase-2 (COX) and phosphatidylinositol 3-kinase (PI3K) activities [41]. It was explained that the two methoxyl groups present on the B-ring of tricin appears to be responsible for its lipophilicity, which seems to play a key role in its biological activity, cellular uptake and *in vivo* stability, especially in the intestine and colon [39 and references therein].

Total Phenolic Contents and Antioxidant activity

The content of phenolics was calculated from the regression equation of the calibration curve (R2 =0.9833, y = 0.0044x + 0.0384), expressed in GAE as milligrams per gram of the extract or fraction (GAE mg /g extract or fraction). Among all the fractionated extracts, the ethyl acetate fraction had the highest TPC (42.33 ± 0.57 mg GAE/g) followed by total extract (29.33± 0.57 mg GAE/g), *n*-butanol fraction (28.84 mg GAE/g) and aqueous fraction (15.06 mg GAE/g). Meanwhile hexane fraction contains the lowest contents of phenolic ((10.44 mg GAE/g). mg/g). The antioxidant activity of the methanol extract and its fractions (Hexane, ethyl acetate, *n*-butanol and aqueous) was investigated using DPPH method. As shown in figure 2, indicated good scavenging activity of the total extract, and tested fractions towards DPPH in comparison with reference standard ascorbic acid. Ethyl acetate and *n*-butanol fractions showed maximum antioxidant activity with and respectively. The ethyl acetate fraction showed maximum antioxidant activity with IC_{50} = 0.136 ± 0.005 mg/mL, followed by *n*-butanol with IC_{50} = 0.551± 0.012 mg/mL, total methanolic with IC_{50} = 0.731 ± 0.010 mg/mL, aqueous with IC_{50} = 1.771 ±

0.029 mg/mL and finally *n*-hexane fractions which showed lowest activity with IC_{50} = 2.81 ± 0.076 mg/mL. None of the tested fractions was active as ascorbic acid which showed IC_{50} = 0.045 ± 0.001 mg/mL. The higher antioxidant activity of ethyl acetate and *n*-butanol fractions are attributed to the presence of flavonoids, flavolignans and simple phenolic compounds which are isolated form this fraction. There was a linear correlation between the values of the total phenolic contents and the antioxidant activities of plant extracts.

The applications of antioxidants are industrially widespread in order to prevent polymers oxidative degradation, auto-oxidation of fats, synthetic and natural pigments discoloration, etc. In the past few years, there is an increased interest for antioxidants from natural sources rather than from synthetic sources because of the health risks and toxicity of synthetic antioxidant [42]. Plants are being utilized frequently as sources of natural antioxidants, and some of the compounds present in plants have significant antioxidative properties and health benefits [43].

Anti-inflammatory activity:

All the tested extracts and fractions at dose of 400 mg/kg possess varying significant anti-inflammatory activity in carrageenan-induced edema in rats. The results of the measurements of the paw thickness and percentage anti-inflammatory activity are listed in (Tables 3 & 4) respectively. The effect begins within the 2nd hour and becoming highly significant in the 3rd and the 4th hours and continues till the 5th hour in some fractions. The *n*-butanol fraction as shown in Figure 4 exerted higher percentage of edema inhibition in comparison with the other fractions through the whole 5 hours. The total methanlic extract and aqueous fractions showed a potent anti-inflammatory activity comparable to that of indomethacin. They reduced the carrageenan-induced edema with maximum effect being obtained after 3–4 hours after carrageenan injection, after which it begin to decline. The *n*-hexane and ethyl acetate, fractions showed lowest anti-inflammatory effect. Carrageenan model of inflammation is said to be biphasic with the first phase attributed to the release of histamine, serotonin and kinins in the first hour, while the second phase is attributed to release of prostaglandins and lysosome enzyme [44]. The tested extracts inhibited both the first and the second phases of inflammation. The anti-inflammatory activity of *S. plumosa* extracts may be attributed to presence of sterols, triterpenes, flavonoids and tannins, which are detected in phytochemical screening of the plant (data not presented).

The analgesic activity:

The tested extracts of the plant showed significant analgesic action compared to the reference drug diclofenac sodium against acetic acid induced pain in mice at a dose of 400 (mg/kg). Analgesic effect of the extracts was demonstrated in the experimental models using acetic acid Induced Writhing Test (Chemical Stimulation). The decrease in writhing are generally considered an important parameter of analgesic activity in acetic acid induced Writhing Test. All fractions significantly ($P<0.001$) reduced the number of acetic acid induced writhing, compared with control as shown in table 4. The *n*-butanol and ethyl acetate fractions showed high potency. The reduction of writhing and stretching induced by *n*-butanol and ethyl acetate fraction was 89.01% and 82.54%, respectively, which was comparable to that of diclofinac sodium which showed 91.38% reduction. The other fractions showed variable activities; aqueous fraction showed 81.25% inhibition followed by *n*-hexane fraction 78.66% and total methanolic extract 72.62%.

CONCLUSIONS:

Based on the results of this study, it may be conclude that *S. plumosa* plant can be regarded as promising natural plant sources of bioactive compounds especially phenolics. Ten compounds were isolated from the *n*-hexane and ethyl acetate fractions of aerial part methanolic extract of *S. pulmosa* including two flavolignans of rare occurrence. These compounds are recorded for the first time from the plant. The structural determination was accomplished by the 1D- and 2D-NMR spectra as well as HRESIMS analysis. Ethyl acetate fraction showed the highest total phenolic content followed by total extract and *n*-butanol fraction. A linear correlation was obtained between the values of the total phenolic contents and the antioxidant activities of plant extracts. Different tested fractions showed good antioxidants, moderate cytotoxicity, strong analgesic and anti-inflammatory activities. Further studies of this plant species should be directed to phytochemical investigation of *n*-butanol fraction which exhibited alongside ethyl acetate fraction the highest biological activities.

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