PHYTOCHEMICAL STUDY AND EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF SOME MEDICINAL PLANTS GROWING IN EGYPT

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SUMMARY: The anti-inflammatory activity of different extracts of five plants abundantly growing in Egypt, namely Ipomoea palmata Forsk. (Convolvulaceae), Alstonia scholaris R.Br. (Apocynaceae), Salix subserrata Willd., Salix tetrasperma Roxb. and Populus nigra Linn. (Salicaceae) has been studied. Phytochemical study on selected bioactive extracts was carried out as well as their possible mechanism of action. The results revealed a significant anti-inflammatory activity of the extracts under investigation to different degrees. A chromatographic study of the bioactive lipoidal extracts of A. scholaris and I. palmata was carried out and the results revealed the presence of unsaturated fatty acids (linoleic and linolenic). Beta-sitosterol and campesterol were present in A. scholaris and I. palmata, respectively. Chromatographic and spectral investigation of the flavonoids in the bioactive aqueous extract of I. palmata revealed the presence of luteolin, quercetin 7-glycoside and apigenin. The anti-inflammatory activity may be due to the presence of these phytochemical constituents.

Key Words: Alstonia scholaris, Populus nigra, Salix subserrata, Salix tetrasperma, Ipomoea palmate, Anti-inflammatory activity, Phytochemical constituents.

INTRODUCTION

The present study dealt with evaluation of the anti-inflammatory activity of the extracts of some Egyptian plants, and their phytochemical constituents as well as their possible mechanism of action. The side effects of steroidal and non steroidal anti-inflammatory drugs currently used for management of chronic inflammatory diseases may be more difficult to manage than the disease itself. Therefore there is a need for new safe approaches for such diseases. The natural botanical sources in Egypt may supply us with natural anti-inflammatory

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Table 1: Mean thickness of inflammation (cm) of the hind paw at different time intervals of carrageenan injection after administration of different successive extracts of *Alstonia*.

Groups		Hours					
		0.5	1.0	1.5	2.0	3.0	4.0
Control	Mean	0.1	0.122	0.135	0.13	0.133	0.16
	± SE	0.019	0.009	0.01	0.015	0.03	0.02
Pet. ether extract of *Alstonia*	Mean	0.115	0.115	0.185	0.201	0.225	0.225**
	± SE	0.011	0.02	0.02	0.032	0.032	0.024
	% Inhibition	15	5	5	34	7	15
Ether extract of *Alstonia*	Mean	0.11	0.11	0.11	0.14	0.17	0.19
	± SE	0.013	0.01	0.013	0.01	0.011	0.005
	% Inhibition	10	0	8	7	19	37
Control	Mean	0.1	0.11	0.12	0.15	0.21	0.3
	± SE	0.013	0.015	0.011	0.013	0.025	0.015
Chloroform extract of *Alstonia*	Mean	0.1	0.13	0.13	0.13	0.172	0.25**
	± SE	0.008	0.01	0.012	0.017	0.033	0.008
	% Inhibition	0	18	8	13	17	17
50% Aqeous methanol extract of *Alstonia*	Mean	0.05	0.04***	0.03*****	0.02*****	0.02*****	0.13****
	± SE	0.02	0.013	0.015	0.015	0.018	0.037
	% Inhibition	50	64	75	87	91	17
Control	Mean	0.1	0.122	0.135	0.13	0.133	0.16
	± SE	0.019	0.009	0.01	0.015	0.03	0.02
Water extract of *Alstonia*	Mean	0.06	0.09*	0.12	0.17*	0.19	0.19
	± SE	0.01	0.009	0.017	0.008	0.013	0.022
	% Inhibition	40	18	0	13	10	37

Values significantly differ from the control: *: p<0.05, **: p<0.025, ***: p<0.010, ****: p<0.005, *****: p<0.001

agents which may have minimal draw backs. Reviewing the folk medicine as well as the literature some species of the genus *Ipomoea* and *Alstonia* have been shown to possess antirheumatic and anti-inflammatory activities (1-5). Some Salicaceae species such as *Salix subserrata*, *Salix tetrasperma* and *Populus nigra* were also used in folk medicine as antirheumatic sedative and analgesic (6, 7).

The genus *Ipomoea* is related to the (Family Convolvulaceae) which includes 400 species all over the world from which *I. palmata* Forsk. or *I. caircia* L. grow abundantly in Egypt. *Ipomoea cairica* L. Sweet is used in Brazilian folk medicine for the treatment of rheumatism and inflammations through inhibition of the release of mediators induced edema, such as histamine (8). The major bioactive constituents previously isolated from the genus *Ipomoea* were lipoidal matters (9) and phenolic compounds (10). The genus *Ipomoea* has been reported

Table 2: Mean thickness of inflammation (cm) of the hind paw at different time intervals of carrageenan Injection after administration of different successive extracts of *Ipomea*.

Groups		Hours					
		0.5	1.0	1.5	2.0	3.0	4.0
Control	Mean	0.1	0.122	0.135	0.13	0.133	0.16
	± SE	0.019	0.009	0.01	0.015	0.03	0.02
Pet. ether extract of *Ipomea*	Mean	0.12	0.13	0.14	0.15	0.17	0.19
	± SE	0.025	0.017	0.024	0.026	0.02	0.017
	% Inhibition	20	18	17	0	19	37
Ether extract of *Ipomea*	Mean	0.05*	0.06**	0.08	0.09*	0.11	0.13
	± SE	0.015	0.02	0.024	0.02	0.041	0.02
	% Inhibition	50	45	33	40	48	57
Chloroform extract of *Ipomea*	Mean	0.0.04**	0.06*****	0.086**	0.103	0.103	0.13
	± SE	0.008	0.008	0.013	0.013	0.014	0.014
	% Inhibition	60	45	28	31	51	57
Control	Mean	0.1	0.11	0.12	0.15	0.21	0.3
	± SE	0.013	0.015	0.011	0.013	0.025	0.015
50% Aqeous methanol extract of *Ipomea*	Mean	0.09	0.05**	0.02*****	0.04****	0.09**	0.12*****
	± SE	0.010	0.015	0.010	0.024	0.038	0.03
	% Inhibition	10	55	83	73	59	60
Water extract of *Ipomea*	Mean	0.11	0.1	0.12	0.14	0.14	0.15
	± SE	0.015	0.014	0.017	0.02	0.016	0.018
	% Inhibition	10	9	0	7	10	50

Values significantly differ from the control: *: p<0.05, **: p<0.025, ***: p<0.010, ****: p<0.005, *****: p<0.001

to possess many biological activities. Pongprayoon *et al.* (2) showed a significant analgesic effect attributed to the petroleum ether, chloroform and ethanol extracts of *I. palmata* in albino rats. Pongprayoon *et al.* (2) also, reported that the extract of the leaves of *I. pes-caprae* L. was effective in the treatment of dermatitis caused by jelly fish sting and the edema induced by ethyl phenyl propiolate in experimental animals.

The genus *Alstonia* (Family Apocynaceae) is introduced all over the World for out-door planting. The most common species growing in Egypt is *A. scholaris*. Kweifio-Okai (3) and Kweifio-Okai *et al.* (5) reported the beneficial use of the crude aqueous extract of *A. boonei* root bark when included within a herbal preparation in the treatment of rheumatoid arthritis. Several constituents were isolated from *A. scholaris* such as sterols (11) and alkaloids (12-14).

The genus *Populus* possesses an ancient name of disputed origin belonging to Family Salicaceae, it was called also poplar, popcorn, and aspen. Toma et al.(6)

Table 3: Mean thickness of inflammation (cm) of the hind paw at different time intervals of carrageenan injection after administration of different successive extracts of *Populus*.

Groups		Hours					
		0.5	1.0	1.5	2.0	3.0	4.0
Control	Mean	0.1	0.14	0.11	0.1	0.12	0.16
	± SE	0.02	0.02	0.008	0.013	0.011	0.015
Pet. ether extract of *Populus*	Mean	0.07	0.11	0.07*	0.11	0.14	0.17
	± SE	0.013	0.017	0.013	0.017	0.011	0.018
	% Inhibition	30	21	36	10	17	6
Ether extract of *Populus*	Mean	0.05	0.04**	0.08	0.1	0.08	0.15
	± SE	0.016	0.016	0.011	0.018	0.016	0.013
	% Inhibition	50	64	14	9	43	12
Chloroform extract of *Populus*	Mean	0.07	0.1	0.1	0.08	0.075	0.15
	± SE	0.017	0.027	0.0	0.021	0.017	0.013
	% Inhibition	30	29	9	20	38	6
Control	Mean	0.1	0.122	0.135	0.13	0.133	0.16
	± SE	0.019	0.009	0.01	0.015	0.03	0.02
50% Aqeous methanol extract of *Populus*	Mean	0.084	0.087	0.064****	0.125	0.129	0.154
	± SE	0.009	0.016	0.017	0.02	0.024	0.31
	% Inhibition	16	29	53	4	4	4
Water extract of *Populus*	Mean	0.047*	0.077	0.077***	0.107	0.108	0.097**
	± SE	0.01	0.019	0.014	0.014	0.02	0.007
	% Inhibition	53	37	43	18	23	39

Values significantly differ from the control: *: p<0.05, **: p<0.025, ***: p<0.010, ****: p<0.005, *****: p<0.001

prepared a surgical spray bandage containing a mixture of poplar bud together with other ingredients which possess an antiallergic, anti-inflammatory local anesthetics effects and was useful in the treatment of cuts, bites, burns, stings, post operative sores, ulcers and wounds. Shen (15) reported that populin isolated from the leaves of *P. tomentosa* showed antipyretic and analgesic activities. Von Kruedener *et al.* (16) proved that *P. tremula* has anti-inflammatory and antirheumatic properties, which are mainly due to its salicylate components. The effect is comparable to non steroidal anti-inflammatory drug but with little or no side effects.

The leaves of *S. subserrata* Willd. or S. safsaf Forsk. abundantly present near water on the Nile banks in Egypt was used topically in folk medicine as antirheumatic remedy. Phenolic compounds was identified in the leaves of *S. aegyptiaca* as gallic acid, caffeic acid, vanillin, *p*-coumaric acid, myricetin, catechin, epigallocatechin gallate, rutin and quercetin as well as salicin. This indicated the presence of high amounts of phenols and flavonoids in the leaves of *S. aegyptiaca* species thereby may be utilized as a source of health promoting antioxidants (17). In a previous study we have demonstrated the anti-inflammatory activity of the aque-

Table 4: Mean thickness of inflammation (cm) of the hind paw at different time intervals of carrageenan injection after administration of salicin and 50% aqueous methanol of *Salix tetrasperma*.

Groups		Hours					
		0.5	1.0	1.5	2.0	3.0	4.0
Control	Mean	0.1	0.14	0.11	0.1	0.12	0.16
	± SE	0.02	0.02	0.008	0.013	0.011	0.015
Salicin	Mean	0.04**	0.06**	0.09	0.06**	0.09	0.07***
	± SE	0.008	0.017	0.025	0.018	0.035	0.021
	% Inhibition	60	51	33	54	32	56
Control	Mean	0.1	0.14	0.11	0.1	0.12	0.16
	± SE	0.02	0.02	0.008	0.013	0.011	0.015
50% Aqeous methanol extract of *Salix tetrasperma*	Mean	0.08	0.11	0.1	0.07	0.05**	0.1***
	± SE	0.011	0.016	0.008	0.011	0.018	0.008
	% Inhibition	20	21	9	30	58	38

Values significantly differ from the control: **: p<0.025, ***: p<0.010

ous methanol extract of *S. subserrata* in adjuvant arthritis in rats as a model of experimental chronic inflammation (18).

The objective of the present study was to assess the anti-inflammatory effect of the different successive extracts of some species belonging to the previously mentioned genus abundantly grow in Egypt, in acute inflammation using carrageenan model in rats. The aim included phytochemical study of selected bioactive extracts as well as their possible mechanism of action.

MATERIALS AND METHODS

I: Phytochemical analysis

Plant materials

Samples of the aerial parts of *I. palmata* Forsk (Convolvulaceae), *A. scholaris* R.Br. (Apocynaceae) and the leaves of *S. subserrata* Willd., *S. tetrasperma* Roxb. and *P. nigra* Linn. (Salicaceae) were collected from the Zoo and Orman garden. Giza, Egypt and were authenticated by Agriculture Engineer Badia Diwan Consultant of Plant Taxonomy at the Ministry of Agriculture, Cairo, Egypt. A voucher specimen was deposited in the National Research Centre Herbarium with a registration number of 1025-1029 for *I. palmata* Forsk., *A. scholaris* R.Br., the leaves of *S. subserrata* willd., *S. tetrasperma* Roxb. and *P. nigra* Linn., respectively. The collected parts of each of the plants under investigation were separately air dried, reduced to No. 36 powder and kept in tightly closed containers.

Instruments

PU 9712 Infrared Spectrophotometer Pye Unicam, Cambridge, England was used for running the IR spectra of the test substances in KBr discs. UV-Visible spectrophotometer Beckman DU, and Shimaszu were used for recording UV spectra and measuring the absorbance in UV and visible range. Gas chromatograph-Hewlett Packard model 5790 was used.

Investigation of the lipoidal matter in the bioactive extracts

a. Preparation of lipoidal matter

The air dried powder of the aerial parts (200 g) of each plant under investigation namely *A. scholaris* and *I. palmata* were separately and exhaustively extracted with light petroleum ether (60-80°C) in a continuous extraction apparatus. The solvent was evaporated under vacuum.

b. Preparation of unsaponifiable matter and fatty acids

The solvent free extract 3 g of each of the two plants under investigation was saponified by refluxing with alcoholic potassium hydroxide (10%). After dilution with water the unsaponifiable matter fraction was extracted with ether. The ether was evaporated while USM was weighed and kept for further investigation. The aqueous mother liquor was acidified with (10%) hydrochloric acid and the liberated fatty acids were extracted with ether. Ether was evaporated and the residue was weighed and kept for studying the fatty acids (19, 20).

Table 5: The mean volume of inflammation of the hind paw (c.c) at different time intervals of carrageenan injection after administration of 50% aqueous methanol of *Salix subserrsta*.

Groups		Hours					
		0.5	1.0	1.5	2.0	3.0	4.0
Control	Mean	0.14	0.278	0.39	0.466	0.55	0.52
	± SE	0.023	0.034	0.028	0.041	0.035	0.043
50% Aqeous methanol extract of *Salix subserrsta*	Mean	0.018*****	0.141****	0.338	0.418	0.448**	0.368
	± SE	0.013	0.016	0.028	0.043	0.023	0.03
	% Inhibition	87	49	13	10	19	29

Values significantly differ from the control: **: p<0.025, ****: p<0.005, *****: p<0.001

c. Investigation of unsaponifiable matter

TLC analysis Aliquots of the (USM) of each of the two species under investigation were separately dissolved in chloroform, spotted on silica gel G plates alongside with solution of authentic, β-sitosterol, campesterol, β-amyrin, and cholesterol and developed with solvent system (benzene: ethyl acetate) (86: 14) (v/v). The developed chromatoplates were sprayed with vanillin-sulphuric acid reagent, and heated at 100°C for 5 min for detection.

GLC of unsaponifiable matter

GLC conditions Column (10%) OV-101 packed column, stationary phase, chromosorb W-HP detector temperature 290°C; injector temperature, 280°C, carrier gas N_2, flow-rate 30 ml/min, air flow rate, 300 ml/min, H_2 flow rate, 30 ml/min; detector FID, chart speed 0.5 cm/min, oven program: initial temperature 70°C, final temperature 270°C, programmed: 4°C/min for 50 min maintained for 35 min at 270°C, total time 85 min.

d. Methylation of fatty acids

The fatty acid fraction (0.5 g) of each of the two plants species under investigation was subjected to methylation by refluxing with 50 ml absolute methanol and 3 ml concentrated sulphuric acid for 2 hours. Complete methylation was checked by TLC on silica gel "G" using solvent system (light petroleum: chloroform: acetic acid) (60: 40: 1) (v/v/v) and visualization was carried out by exposure to iodine vapour. The methylated fatty acids were extracted with diethyl ether. After evaporation of the ether the fatty acid methyl esters were ready for GLC analysis.

GLC conditions of fatty acid methyl esters

Stationary phase (10%) diethylene glycosuccinate (DEGS) packed column, oven temperature 170°C, detector temperature 300°C, injector temperature 250°C, carrier gas N_2, flow rate 30 ml/min, air flow rate, 350 ml/min, H_2 flow rate 350 ml/min, detector FID, chart speed 2 cm/min. Identification of the fatty acid methyl esters was carried out by direct comparison of retention times of each of the separated compounds with those of certain available authentic samples of the fatty acid methyl esters analyzed under the same conditions. Quantization was based on peak area integration.

Investigation of flavonoids in the bioactive extracts

Extraction and isolation

The defatted aqueous methanol extract of *I. palmata* (75 g) was subjected to thin layer chromatographic study using ready made cellulose "F" plates (Merck) as adsorbent and *n*-butanol: acetic acid: water (4: 1: 5) (v/v/v) and acetic acid : water (1: 1) (v/v) as solvent systems (21). The developed chromatograms were located under UV light before and after spraying with (2%) ethanolic aluminium chloride reagent (22) and exposure to ammonia vapors. Three compounds were detected namely I_1, I_2 and I_3 with HRf of (76, 65), (36, 78) and (87, 53), respectively in the two previously mentioned solvent systems. The aqueous methanol fraction (10 g) containing the three compounds (I_1, I_2 and I_3) was subjected to preparative column chromatography using cellulose microcrystalline (Merck) as adsorbent, elution was carried out with saturated n-butanol, fractions 50 ml each were collected and separately concentrated to a small volume and subjected to paper chromatographic investigation using n-butanol: acetic acid: water (4: 1: 5) (v/v/v) as solvent system. The similar collected fractions containing compounds I_1, I_2 and I_3 were resubjected to preparative paper chromatography Whatmann No. 3 using n-butanol: acetic acid: water (4: 1: 5) (v/v/v) as solvent system and detection was carried out under UV light and after spraying with 2% ethanolic $AlCl_3$. All compounds were passed over Sephadex LH-20 in methanol for purification. The purity of the compounds isolated (I1, I2 and I3)were tested by comparison with different flavonoidal authentic compounds using the two solvent systems previously

Table 6: Acute oral lethal toxicity of dried aqueous methanol extract of Salix subserrsta in mice.

Group No.	Dose (mg/kg)	No. of animals/group	No. of dead animals	Z	D	Z.D
1	1000	8	0	0	1000	0
2	2000	8	0	0	2000	0
3	4000	8	0	0	2000	0
4	6000	8	0	0.5	2000	1000
5	8000	8	1	1.5	2000	3000
6	10000	8	2	5	2000	10000
7	12000	8	8	4	0	0

Z: Half the sum of dead mice from two successive doses.

D: Difference between two successive doses.

Z.D: Product of Z and D.

used and sprayed by 2% ethanolic $AlCl_3$. No impurities were detected and each compound gave a single spot in the two systems. The concentrated eluate from these compounds in methanol was left for crystallization in a refrigerator where crystalline substances were separated. From the previous methods of isolation, three compounds (I_1, I_2 and I_3) were isolated, and identified by co-chromatography, physical and spectral analysis (23) as well as compared with published data (24).

II: Biological part

Anti-inflammatory activity test

a. Animals

All animal procedures were preformed after approval by the Ethics Committee of the National Research Centre and in accordance with recommendations for the proper care and use of laboratory animals (NIH publication NO. 85-23, revised 1985).

White female albino rats of 150 gm average body weight were used. The animals were housed individually in wire bottomed cages at room temperature.

Male and female albino mice of (21-25 g) body weight were used. The animals were kept individually in wire bottomed cages at room temperature of (25 ± 2°C).

b. Plant extracts

Different successive extracts; petroleum ether, ether, chloroform (50%) aqueous methanol and water extracts of the aerial parts of *I. palmata* and *A. scholaris* and the leaves of *P. nigra* were prepared. On the other hand, 50% aqueous methanol extract of the leaves of S. subserrata, and *S. tetrasperma* and salicin have also been prepared.

c. Pure salicin was obtained from Sigma, USA.

d. Chemicals used for induction of inflammation

Carrageenan type IV (Sigma, USA) was used for induction of acute inflammation in rats.

Methods

Preparation of plant doses

Petroleum ether, ether and chloroform extracts were separately emulsified in water using gum acacia. The water and the aqueous methanol extracts were dissolved in water. One dose level (500 mg/kg rat body weight) of each extract and pure salicin were tested.

1. Acute inflammation test (25)

The rats were fasted for 16 hours before starting the experiment and divided into twenty two groups, each comprised six rats. Eighteen groups were used as test groups and four groups served as control. The different prepared extracts of the different plant materials were given separately to the rats of the eighteen tested groups in a dose of (500 mg/kg) rat body weight. The four control groups were not given any extracts. After one hour of the oral administration, all rats were injected into the subplanter surface of the right hind paw with 0.1 ml carrageenan (1% w/v) in saline. Paw volumes were measured using differential volume meter (or paw thickness were measured using vernier caliper) immediately before the injection of carrageenan and after 30 min. and 1, 1.5, 2, 3 and 4 hr of carrageenan injection. The mean increase of the hind paw volumes (or thickness) of rats given the different plant extracts (inflammation) were calculated and compared with that of the control inflammed rats. Statistical analysis was carried out using student's *t*-test.

2. Acute oral toxicity test

Acute lethal toxicity test of the aqueous methanol extract of Salix was carried out according to Goodman *et al.* (26). Mortality counts after 24 hrs among equal sized groups of lethally intoxicated mice (8 animals/group) receiving progressively increasing oral dose levels of the dried aqueous methanol extract was recorded and tabulated. The LD_{50} was calculated according to Paget and Barnes (27).

RESULT AND DISUCUSSION

Carrageenan induced edema of rat foot is used widely as a working model of acute inflammation in the search for new anti-inflammatory agents (28). The edema which develops in rat paw after carrageenan injection is a biphasic event (29). The edema is mediated by histamine and 5-hydroxy tryptamine during the first hour, after which the increased vascular permeability is maintained by the kinin release up to 2.5 hr, from 2.5-6.0 hr, the inflammatory mediator appears to be prostaglandin which is closely associated with migration of leucocytes into the inflamed site (30, 31).

The results of acute inflammation test are present in Tables (1-5).The different successive extracts of the aerial parts of *A. scholaris* showed variable anti-inflammatory activity from which the aqueous methanol extract was the most promising, it showed a significant anti-inflammatory effect starting one hour following carrageenan injection with 64% inhibition ($P < 0.01$) and continued all over the experiment with maximum inhibition of (91%) ($P < 0.001$) after three hours. Concerning the successive extracts of the aerial parts of I. palmata, the ether, chloroform and aqueous methanol extracts showed different significant anti-inflammatory activities which are more pronounced in case of the aqueous methanol extract, the anti-inflammatory effects starting one hour following carrageenan injection with 55% inhibition ($P < 0.025$) and continued all over the experiment with a maximum inhibition of 83% ($P < 0.001$) after 1.5 hr.

The results of the anti-inflammatory activity of the studied *Salicaceae* species are summarized in tables 3-5. It can be noticed that the different successive extracts of *P. nigra* leaf, produced variable anti-inflammatory activity. The water successive extract proved to be the most significant where it produced a percentage of inhibition after 1.5 hr equal to 43 % ($P < 0.01$). On the other hand, a comparison of the anti-inflammatory activity between the aqueous methanol extract of *S. subserrata* and S. tetrasperma, revealed a significant anti-inflammatory activity in case of *S. tetrasperma* after (3 and 4 hr) following carrageenan injection (58% inhibition, $P < 0.025$) and (38% inhibition, $P < 0.010$), respectively, while in case of *S. subserrata* a more significant anti-inflammatory activity was observed after 0.5, 1, 3 and 4 hours following carageenan injection. It showed a percentage of inhibition of 87% ($P < 0.001$), 49% ($P < 0.005$), 19% ($P < 0.025$) and 29% ($P < 0.025$), respectively. Salicin the major phenolic glycoside found in the

Table 7: GLC of USM of Alstonia scholaris and Ipomoea palmate (as area % of total USM).

USM constituents	*Alstonia scholaris*	*Ipomoea palmata*
Dodecane (C12)	5.696	0.018
Tetradecane (C14)	-	0.333
Pentadecane (C15)	0.417	0.968
Hexadecane (C16)	-	0.886
Octadecane (C18)	0.217	0.236
Nonadecane (C19)	-	0.778
Eicosane (C20)	0.480	1.862
Heneicosane (C21)	0.183	0.268
Decosane (C22)	-	8.571
Tricosane (C23)	0.500	1.453
Tetracosane (C24)	-	0.328
Pentacosane (C25)	0.335	1.016
Hexacosane (c26)	1.306	0.978
Octacosane (C28)	0.453	0.905
Nonacosane (C29)	1.020	0.885
β-amyrin	0.507	-
Triacontane (C30)	8.759	1.210
Cholesterol	3.990	-
Campesterol	-	0.582
β-sitosterol	0.433	-

aqueous methanol extract of Salix species has been tested separately for its anti-inflammatory activity in a dose of 500 mg/kg of body weight. The results revealed a significant anti-inflammatory activity after 0.5, 1, 2 and 4 hours following carrageenan injection, with 60% inhibition ($P < 0.025$), 51% inhibition ($P < 0.025$), 54% inhibition ($P < 0.025$) and 56% inhibition ($P < 0.010$), respectively.

The anti-inflammatory activity profile of the aqueous methanol extracts of *Alstonia* and *Ipomoea* plants is suggested to involve a reduction of kinin and prostaglandin E_2 (30, 31) while the anti-inflammatory activity of the aqueous methanol extract of *S. subserrata* might be related to reduction of histamine, 5-hydroxy, tryptamine and prostaglandin E_2. The anti-inflammatory activity of the water extract of *P. nigra* involved the inhibition of edema mediated by histamine and 5-hydroxy tryptamine then kinin release and at the end continued by inhibited prostaglandin release. The anti-inflammatory activity of the aqueous methanolic extract of *S. tetrasperma* is started 3 hours following carrageenan injection, and continued till 4 hours which might means that the anti-inflammatory activity is due to its inhibitory effect on prostaglandin E_2 release. It has been reported previously (32) that non-steroidal anti-inflammatory drugs mainly suppressed the last phase of carrageenan model (prostaglandin phase) which correlates with their ability to suppress mononuclear leucocytes. The non steroidal anti-inflammatory drugs block prostaglandin and thromboxane formation by inhibiting cyclooxygenase and arachidonic acid (33, 34).

The results of acute toxicity test (Table 6) showed that the aqueous methanol extract of *S. subserrata* was safe up to 4 g/kg rat body weight. The LD_{50} of the extract was calculated to be (10.250 g/kg) of mice body weight.

The bioactive extracts of the different plants under investigation were subjected to phytochemical examination for the identification of their active constituents starting with the less polar bioactive extracts of *A. scholaris* and *I. palmata* which were rich in lipoidal matter as determined by chemical and chromatographic tests. The lipoidal matter was prepared from the two plants as previously described. The results revealed that the lipoidal matter of *A. scholaris* (3.3%) was more than that of *I. palmata* (1.9%). The percentage of unsaponifiable matter in *A. scholaris* was more (73.19 %) than in *I. palmata* (60.63%). The percentage of the total fatty acids fraction was higher in *I. palmata* (37.4%) than *A. scholaris* (21.2%). Qualitative investigation carried out by thin layer chromatography revealed the presence of β-sitosterol and cholesterol in *A. scholaris* while campesterol was found only in *I. palmata*. GLC analysis of unsaponifiable matter showed that the percentage of hydrocarbons identified in unsaponifiable matter of *A. scholaris* was 14.366% while it was 20.695% in I. palmata. Triacontane (C_{30}) was the main hydrocarbon identified in unsaponifiable matter of *A. scholaris* (8.759%) but decosane (C_{22}) (8.271%) is the main hydrocarbon identified in the unsaponifiable matter of I. palmata. In the unsaponifiable matter of *A. scholaris* three triterpenoidal matter were identified (β-amyrin, cholesterol and β-sitosterol) in a total percentage of (4.930%) while in I. palmata, campesterol was identified only in a percentage of 0.582%. GLC analysis of the total fatty acids of *I. palmata* and *A. scholaris* showed the presence of lauric, myristic and linolenic acids in both plants under investigation. Palmitic and arachidic acids were present in *A. scholaris*. Oleic, linoleic and behinic acids were present only in *I. palmata*. Arachidic acid was the major fatty acid in *A. scholaris* (34.28%)

Table 8: GLC Analysis of methyl esters of fatty acids of Alstonia scholaris and Ipomoea palmata (as area % of total fatty acids).

Fatty acids	*Alstonia scholaris*	*Ipomoea palmata*
Lauric acid (12:0)	0.31	1.82
Myristic acid(14:0)	0.3	2.21
Palmitic acid(16:0)	2.8	-
Oleic acid(18:1)	-	14.05
Linoleic acid(18:2)	-	2.6
Linolenic acid(18:3)	25.21	18.4
Arachidic acid((20:0)	34.28	-
Behinic acid(22:0)	-	17.16

while linolenic acid was the major fatty acid present in *I. palmata* (18.4%). The percentage of saturated fatty acids in *A. scholaris* was 37.685% where arachidic acid was the major one (34.28%) while the percentage of unsaturated fatty acids was equal to 25.212% and they were presented only by linolenic acid. The percentage of saturated fatty acids of *I. palmata* was 21.2%, the major one was behinic acid (17.16%) while the unsaturated fatty acids percentage was 35.047%. The percentage of saturated fatty acids in *A. scholaris* (37.685%) was more than in *I. palmata* (21.2%) while the unsaturated fatty acids in *I. palmata* (35.047%) was more than in *A. scholaris* (25.212%). From the previous results, it can be concluded that the anti-inflammatory activity of the petroleum ether and ether extracts of the two plants under investigation might mainly due to the presence of different unsaturated fatty acids such as linoleic and linolenic acids series. α-Linolenic acid can be converted in the body to eicosapentaenoic acid which can act as a competitive inhibitor of arachidonic acid conversion to the inflammatory mediators prostaglandin E_2 and leukotriene B_4 (35). Ingestion of ω-3 fatty acids decreases the levels of certain pro-inflammatory chemokines thereby delaying the onset and severity of autoimmune symptoms (36). It was reported previously (37, 38) that essential fatty acids may have anti-inflammatory therapeutic activity. Fatty acids also have direct effect on cell function which is independent of products formed through the action of cyclooygenase and lipooxygenase. On the other hand the anti-inflammatory activity may also ascribed to the unsaponifiable matter represented by phytosterols, β-sitosterol, campesterol and β-amyrin. These compounds have been shown previously to possess antioxidant and anti-inflammatory activity (39-41). Phytosterols block inflammatory enzymes by modifying prostaglandin pathway (42).

From the previous biological results the aqueous methanol extract of *I. palmata* proved a remarkable and significant anti-inflammatory activity. Phytochemical and chromatographic screening of this bioactive extract revealed the presence of flavonoids. Therefore it was of interest to isolate these compounds by different chromatographic tools (TLC, CC and PPC) as previously reported in details. The results revealed the presence of compounds I_1, I_2 and I_3. The three flavonoidal compounds isolated from the aqueous methanol extract of I.palmata were identified as luteolin, quercetin 7-glycoside and apigenin. The identification was based on standard chemical, physical and spectral methods and comparison with authentic samples and reported literature.

Concerning the bioactive extracts of *Salicaceae* species, previous phytochemical examination of the bioactive aqueous methanolic extracts of *Salicaceae* species, proved the presence of phenolic glycosides mainly salicin as well as the flavonoids luteolin, quercetin and rutin (22).

Majority of flavonoids have been reported to possess anti-inflammatory activity (43, 44). So the anti-inflammatory activity of different polar extracts in the present study may be due to presence of flavonoids.

The anti-inflammatory activity of the two aqueous methanol extracts of the aerial parts of *I. palmata* and the leaves of *Salix* species might be attributed to the presence of the favonoids. The mechanism of action may be ascribed to the inhibition of pathway of arachidonic acid metabolism. The role of these flavonoids was previously studied (38) in comparison with Indomethacin and Hydrocortisone as reference anti-inflammatory drugs. The results proved that these compounds exerted a significant anti-inflammatory activity, luteolin appear to be the most active, where its activity is similar in potency to Inomethacin and can be attributed to the inhibition of pathway of arachidonic acid metabolism. While the anti-inflammatory activity of apigenin might be due to inhibition of histamine release (45) and that of quercetin might be through inhibition of lipooxygenase leading to blockage of the production of leukotrienes. Quercetin also blocked histamine release (37).

Salicin, the major phenolic glycoside present in the bioactive extracts in *Salix* species is considered to be the pharmacologically active principle due to its structure similarity to aspirin.

From the previous results it can be concluded that all plants' extracts under investigation possess a significant anti-inflammatory activity with different degrees.

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