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ANTI-INFLAMMATORY POTENTIAL OF JUSTICIA GENANDRUSSA BURM F. AND CHARACTERIZATION OF SOME PHYTOCONSTITUENT BY GC-MS.

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ABSTRACT

Justicia genandrussa Burm f. is a small tree in *Acanthaceae* family, traditionally used in treatment of several diseases viz., inflammation, diabetes, hypertension, wounds, pain, asthma, rheumatism etc. The present study was carried out to investigate the anti-inflammatory activity of leaves of *Justicia genandrussa Burn f*. The powdered leaves were successively extractedwith petroleum ether and chloroform. The result of the study showed 69.43% inhibition. Acute oral activity of crude extracts shows nil results. The GC-MS study Showed the presence of phytoconstituent like, Caryophyllene (2.13%), 5-(7a-isopropenyl-4, 5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-enal (1.10%), 13Docosenamide, (Z) (21.86%), Squalene (1.64) etc.

KEYWORDS: Anti-inflammatory**,** rheumatism etc.

INTRODUCTION

It is a well-known fact that traditional systems of medicines always played important role in meeting the global health care needs (Ravishankar, 2007). India has the unique distinction of having six recognized systems of medicine and they are Ayurveda, Siddha, Unani, Yoga,

Naturopathy and Homoeopathy. Botanicals are the vital part of these traditional medicines. Although the traditional Indian system of medicine has a long history of use, they required adequate scientific validation (WHO, 1998). Siddha system of medicine is one of the oldest systems of medicine practiced in Akole, India especially in Maharashtra. This *Justicia genandrussa Burn f*. traditionally used for the treatment of Rheumatic arthritis, in old joint pain. The present research work is designed to identify the bioactive compounds which are present in the polyherbal formulation.

MATERIAL AND METHODS

Collection and Authentication of Plant material

The botanical species of *Justicia genandrussa Burn f*. was collected from rural areas of Akole tahsil in Ahmednagar district in Maharashtra, India and it was authenticated by Botanical Survey of India (BSI), Pune.

Preparation of the sample

In house formulation of sample was prepared by leaves were collected and dried under shade for 7 days. After drying, each plant material was finely powdered and sieved (40mesh). After sieving it was stored in airtight container and used for further studies.

Extraction of leaves

The powdered leaves sample of *Justicia genandrussa Burn f*. was extracted with pet-ether (40:60) by using soxhlet apparatus followed by chloroform until the extraction was completed. After the completion of the extraction, the extract was filtered and the solvent was removed by evaporating on Rota evaporator (Heidolph Laborota 400 efficient). Dark green colour residue was obtained. The percentage yield of the extract was 18.50 %. It was used for anti-inflammatory activity and further GC-MS analysis.

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

GC-MS analysis was carried out using GC of make Thermo trace 1300 GC and Ms of make Thermo TSQ 8000. TG 5MS capillary column (30m X 0.25mm, 0.25µm) employing for separation of components and operating in electron impact Ionization mode at 70eV. Helium was used as carrier gas at a constant flow of 1.0 ml/min and an injection volume of 1.0µL was employed. The temperature of Injector and ion source were maintained at 230˚C. The oven temperature was programmed from 60^0C for 2 min, 280^0Cfor 10 min. Mass spectra were obtained at 70eV. A scan interval is 0.5 seconds and fragments from 40 to 450 Da. The

amount of each component was calculated on relative percentage basis comparing its average peak area to the total areas. The Total Ion Chromatogram (TIC) was created by summing up intensities of all mass spectral peaks. The detector used is MS TSQ 8000.

Anti-inflammatory Activity

1. *In vitro* anti-inflammatory activity by Inhibition of protein denaturation method.

Test Solution (0.5 ml): 0.45 ml of BSA (Bovine serum albumin) (5% w/v aqueous solution) and 0.05 ml of extract solution (250 µg/ml).

Test Control solution (0.5 ml): 0.45 ml of BSA (5% w/v aqueous solution) and 0.05 ml of distilled water.

Standard Control Solution (0.5 ml) : 0.45 ml of BSA (5% w/v aqueous solution). And 0.05 ml of standard drug (Diclofenac sodium) solution (250 µg/ml).All the above solution were adjusted to pH 6.3 using 1N HCl , The samples were incubated at 37˚C for 20 minutes and the temperature was increased to keep the sample at 57˚C for 3 minutes. After cooling, 2.5 ml phosphate buffer saline was added to the above solutions. The absorbance was measured using UV spectrophotometer at 416 nm. The percentage inhibition of protein denaturation was calculated as -

$$\% \text{ Inhibition} = [(\text{Ab of Control} - \text{Ab of Test})/\text{Ab of Control}] \times 100$$

The control represents 100% denaturation. The results compared with Diclofinac sodium(250 µg/ml).

2. *In vivo* anti-inflammatory activity by carrageenan-induced rat paw edema.

The extract was evaluated for the anti-inflammatory activity. Acute inflammation was produced by sub-plantar injection of 0.1 ml of 1% Carrageenan in normal saline in the right hind paw of the rats, 1h after the administration of the drug/extract. The paw diameter was measured by using digital verneir calipers at the intervals of 1, 3 and 5 hrs after the Carrageenan injection. Indomethacin (10 mg/kg, orally) was used as standard drug and extract administered (100 mg/kg, 200 mg/kg and 400 mg/kg) as test sample. The anti-inflammatory activity was calculated as percentage inhibition of Carrageenan induced paw edema using the following formula.

$$\text{Percent inhibition} = 1 - [(\text{paw diameter in treated}/\text{paw diameter in control}) \times 100].$$

RESULTS

GC-MS spectra of pet ether extract

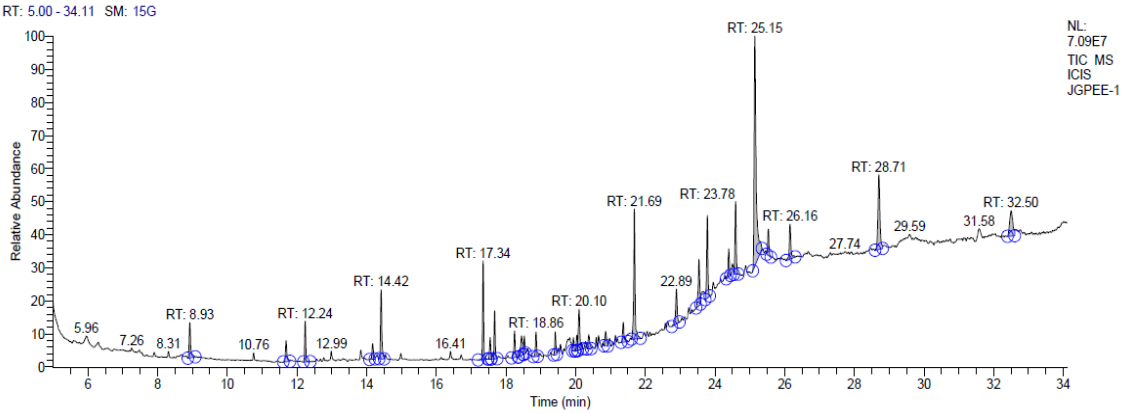

Figure 1: GC-MS spectra of pet-ether extract.

About 25 chemical constituent were identified from pet ether extract of *Justicia genandrussa Burm f.* using GCMS analysis.The phytoconstituents were identified in pet-ether extracts using GC-MS Analysis.These phytoconstituents were recognize to mass spectrum attach to Gas chromatogramcompounds were recognized through mass spectrum attached with Gas chromatogram. The identified phytoconstituent are tabulated in table-1.

Table 1: Phytoconstituent identified in pet- ether extract of *Justicia genandrussa Burm f.*

Sr. No.	Retention Time	Name of phytoconstituents	Molecular Formula	Peak Area
1	8.93	1-Dodecene	$C_{12}H_{24}$	2.51
2	11.69	Cyclotetradecane	$C_{14}H_{28}$	1.41
3	12.24	Caryophyllene*	$C_{15}H_{24}$	2.13
4	14.17	Cetene	$C_{16}H_{32}$	1.15
5	14.42	Globulol	$C_{15}H_{26}O$	4.41
6	17.34	5-(7a-isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol	$C_{20}H_{34}O$	5.18
7	17.54	Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol,4,8,12,15,15-pentamethyl	$C_{20}H_{34}O$	1.11
8	17.68	5-(7a-isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-enal	$C_{20}H_{22}O$	2.75
9	18.25	Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol,4,8,12,15,15-pentamethyl	$C_{20}H_{34}O$	1.74
10	18.45	Hexadecanoic acid,2-methyl-,methyl ester	$C_{18}H_{36}O_2$	2.08
11	18.53	1-naphthalenepropanol	$C_{20}H_{36}O_2$	1.04
12	18.86	Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol,4,8,12,15,15-pentamethyl	$C_{20}H_{34}O$	1.33
13	19.42	5-(7a-isopropenyl-4,5-dimethyl-octahydroninden-4yl)-3-methyl-pent-2-en-1-ol	$C_{20}H_{34}O$	1.32
14	19.94	5-(7a-isopropenyl-4,5-dimethyl-octahydroninden-	$C_{20}H_{32}O$	0.77

		4yl)-3-methyl-pent-2-enal		
15	20.03	n-propyl 9,12-octadecadecadienoate	$C_{12}H_{38}O_2$	0.76
16	20.10	9,12,15-octadecatrienoic acid, ethyl ester,(Z,Z,Z)	$C_{20}H_{34}O$	3.28
17	20.38	5-(7a-isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-enal*	$C_{20}H_{32}O$	1.10
18	20.86	Cedran -diol,(8S,14)	$C_{15}H_{26}O_2$	1.17
19	21.37	Cedran, 8-propoxy-	$C_{18}H_{32}O$	1.45
20	21.69	2-Heptanone	$C_{13}H_{20}O_3$	8.79
21	22.89	7-(acetyloxy)decahydro-2,9,10-trihydroxy-3,6,8,8,10-pentamethyl	$C_{28}H_{40}O_{12}$	2.55
22	23.54	4(2,2,6 Trimethylbicyclo[4.1.0] (hept1yl) butan 2 one	$C_{14}H_{24}O$	3.31
23	23.78	2-Pentenoic acid	$C_{20}H_{32}O_2$	5.12
24	24.40	4(2,2,6Trimethylbicyclo[4.1.0](hept1yl)butan-2 -one	$C_{14}H_{24}O$	1.59
25	24.59	2 Pentanoic acid	$C_{20}H_{32}O_2$	4.60
26	25.15	13Docosenamide,(Z)*	$C_{22}H_{43}NO$	21.86
27	25.53	Squalene*	$C_{30}H_{50}$	1.64
28	26.16	Tetratetracontane	$C_{44}H_{90}$	2.80
29	28.71	Triacontane	$C_{30}H_{62}$	7.28
30	32.50	Tetracosane 32.50	$C_{24}H_{50}$	3.76

The nature and structure of the compounds were identified at different time intervals using mass spectrometer. The heights of the different peaks indicate the relative concentration of the different components present in the sample. The finger prints of the compound which can be identified from The National Institute of Standard and Technology (NIST) library database.

Acute oral toxicity study

Table 2: indicatesthat the pet–ether extract is non- toxic for further use.

Sr. No.	Dose (mg/kg)	Leathality		
		P_1	P_2	P_3
1	5 mg/kg	0/3	0/3	0/3
2	5 mg/kg	0/3	0/3	0/3
3	50 mg/kg	0/3	0/3	0/3
4	50 mg/kg	0/3	0/3	0/3
5	300 mg/kg	0/3	0/3	0/3
6	300 mg/kg	0/3	0/3	0/3
7	2000 mg/kg	0/3	0/3	0/3
8	2000 mg/kg	0/3	0/3	0/3

Anti-inflammatory activity

Table 3: *In-vivo* anti-inflammatory activity by using Caragenan induced paw edema

S. No.	Sample	Concentration	% Inhibition
1.	Diclofenac sodium (Standard)	250 µg/ml	87.25±0.238
2.	P_1 Pet-Ether	250 µg/ml	69.43±0.384
3.	C1 Chloroform	250 µg/ml	16.46±0.332
4.	P_2 Pet-Ether	250 µg/ml	61.47±0.192
5.	C2 Chloroform	250 µg/ml	28.14±0.377
6.	P_3 Pet-Ether	250 µg/ml	76.84±0.316
7.	C3 Chloroform	250 µg/ml	22.91±0.384

% Inhibition of various extracts

% Inhibition = (Abs of Control – Abs of Test)/Abs of control

Table-1. Indicate the activity with comparison with Diclofenac sodium (Standard).The pet-ether extract shown strong activity in three samples. It is considered for further study of anti-inflammatory compounds.

Paw diameter (mm) of sample P_1.

Table 4: indicates the percentage inhibition of pet- ether extract of *Justicia genandrussa Burm f.*

Group	Paw diameter (mm)			% Inhibition
	1hr	3hr	5hr	
Control	2.93±0.134	3.99±0.132	3.41±0.354	--
Standard	0.76±0.042	1.10±0.091	0.57±0.060	83.23
Pet-ether extract(100mg/kg)	2.72±0.022	3.69±0.044	2.60±0.019	23.52
Pet-ether extract(200mg/kg)	2.45±0.013	3.09±0.016	2.11±0.019	37.94
Pet-ether extract(400mg/kg)	1.58±0.134	2.44±0.036	1.28±0.109	62.64

DISCUSSION

Identification of compounds

The compounds were identified by interpretation of the spectrum of the unknown compounds with the spectrum of the known compounds mentioned in The National Institute of Standard and Technology (NIST) library database.

Anti-inflammatory activity

The present results indicate that pet-ether and chloroform extracts of *Justicia genandrussa Burm f*. have anti-inflammatory activity on animal by paw edema model. The both extracts

showed activity against acute and chronic inflammation. The pet ether and chloroform extract showed anti-inflammatory activity in the chronic inflammatory test.

CONCLUSION

1. The extraction protocols developed coupled with GC–MS used in this study allowed us to analyze target compounds at concentrations of nanograms per gram with a high sensitivity and selectivity. Our results showed that pet-ether and Chloroform extracts were effective in the extraction of Anti-inflammatory compounds. Considering the hazards of solvent volume and toxicity associated which shows.

2. It can be concluded that the pet-ether and chloroform extract of leaves of *Justicia genandrussa Burm f*. possess anti-inflammatory activity thus validating the ethnopharmacological claims. This knowledge could be tapped to formulate new agents to treat inflammatory and allergic ailments.

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