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Phytochemical investigation and evaluation of anti-inflammatory and anti-arthritic activities of essential oil of *Strobilanthus ixiocephala* Benth.

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Column chromatographic fractionation of essential oil obtained by hydrodistillation from the flowering tops of *S. ixiocephala* resulted in the isolation of β-caryophyllene, fenchyl acetate, T-cadinol and a new sesquiterpene alcohol for which a name ixiocephol has been proposed. The β-caryophyllene and fenchyl acetate were identified by Co-TLC with authentic samples whereas T-cadinol and ixiocephol were structurally elucidated by UV, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data. The GC-MS analysis of the essential oil has also revealed the presence of various monoterpenoids and sesquiterpenoids. The essential oil of *S. ixiocephala* demonstrated a dose dependant anti-inflammatory activity in carrageenan-induced rat paw oedema. It has also revealed good activity in cotton pellet granuloma and adjuvant induced arthritis model in rats.

Keywords: Anti-arthritic activity, Anti-inflammatory activity, Essential oil of *Strobilanthus*, *Strobilanthus ixiocephala*

Strobilanthus ixiocephala Benth. family: Acanthaceae also named as *Thelepaepale ixiocephala* (Benth) Bremek commonly known as Waiti (Mar.) is a small straggling shrub found in Konkan, the Deccan and Kanara in India. It is a very common, abundant and gregareous Khandala plant found in large patches about half way down St. Xavier's Ravine, at an altitude of 500 m. in Khandala and at Brahmagiri hills of Nashik. It is found to flower once in seven years[1-3]. Twigs with floral heads yield an essential oil having a refreshing camphoraceous odour. The scent of the oily substance exuded by the glandular hairs is most penetrating and persistent. *Strobilanthus heyneanus*, another species of genus *Strobilanthus* is also an aromatic shrub found in South India particularly in Kerala is reported to be used as a remedial measure against neurological disorders, oedema, itiching, skin diseases and ulcers[4]. Very interestingly the essential oil prepared from the whole plant of *S. heyneanus* is reported to be effective in inflammatory conditions[5,6]. Another plant *Strobilanthus callosus* is reported to be useful in treating inflammatory disorders[7]. Being chemotaxanomically related to *S. heyneanus* and *S. callosus*, the essential oil of floral heads of *S. ixiocephala* has been undertaken in present study for its phytochemical investigation and evaluation for anti-inflammatory and anti-arthritic activity studies.

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Materials and Methods

Plant material—Fresh flowering tops of *S. ixiocephala* were collected from the Brahmagiri hills of Trimbakeshwar, Nashik. Authentication of *S. ixiocephala* was carried out with the help of Botanical Survey of India, Pune and the voucher specimen of the plant has been deposited in their office. *S. ixiocephala* Benth authenticated with Voucher Specimen field no. 153115 and Accession no. 103082.

Reagents and apparatus—All reagents were of puriss or analytical quality unless otherwise specified. Puriss grade solvents were distilled prior to use. Silica gel for column chromatography had an average size of 60-120 mesh (E. Merck, Darmstadt, FRG). The FT-IR spectra (KBr disc) were recorded on JASCO 5300 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on instrument AMX-500 (500 MHz) by Bruker using deuteriated solvent like $CDCl_3$. Mass spectrum were obtained from Mass spectrometer instrument Varian VXR-300 (300 MHz). The essential oil was subjected to GC/MS analysis on the GC-MS of HP CHEM Model HP G 18008 (EI, 2965 ev) gas chromatographic detector (GSD) equipped with HP-5 cross linked 5% PhMe silicone column (30 m×0.25 m; 0.25 μm film thickness) using helium as a carrier gas. The flow rate adjusted was 0.7 ml/min. with the temperature program from 80° to 150°C at 5°C/min. and then subsequently up to 300°C at 30°C/min.

Quantitative data were obtained from electronic integration of TIC data. Hydrodistillation of essential oil was carried out by using Clavenger's apparatus[8].

Extraction and isolation of phytoconstituents— The freshly collected flowering tops of *S. ixiocephala* were chopped and subjected to hydrodistillation by using Clavenger's type essential oil hydrodistillation apparatus. The column chromatographic fractionation of the oil using petroleum ether and benzene as eluent for the isolation of essential oil constituents Isolated constituents were identified by using chromatographic and spectroscopic methods. The oil was also subjected to GC-MS analysis which revealed the presence of various mono- and sesquiterpenoids.

Testing for anti-inflammatory and antiarthritic activity— Experiments were performed on albino rats of either sex (Wistar Strain) weighing about (120-160g body weight). Test drug was freshly prepared as a fine homogenized suspension in tween-80 (2% w/v) in distilled water. Diclofenac Sodium (10 mg/kg) was used as standard drug.

Carrageenan-induced oedema in rats[9]— Odema was induced by injecting 0.1 ml of carrageenan (1% w/v) in distilled water into the subplantar region of the left hind paw after 1 hr (oral) drug administration. One group was used as a control and given only vehicle (2% w/v) Tween-80. The volume of the paw was measured with a volume differential meter model 7140 UGO Basile after 3 hr and 24 hr of carrageenan injection. Results were determined as the percent inhibition of oedema compared with the control group.

Cotton pellet granuloma in rats[10]— Autoclaved cotton pellets 50±1 mg were implanted by subcutaneous incision on the back under ether anaesthesia. Drugs were administered daily orally for 7 days. Animals were sacrificed on day 7 and the granuloma was dried in an oven at 60°C and weighed to determine the percent inhibition of granuloma.

Adjuvant-induced arthritis in rats[11,12]— (Therapeutic test) Arthritis was induced in rats in groups of six animals by injecting 0.05 ml of a 0.5% (w/v) suspension of killed *Mycobacterium tuberculosis* (Difco) in paraffin oil into the left hind limb. Paw volume was measured till 12th day by using UGO Basile plethismometer 7140. Drug treatment was started on day 13 and terminated on day 21. At 22nd day blood was withdrawn through heart puncture of all groups by anaesthetizing the animals with diethyl ether and the biochemical parameters like haemoglobin content, total WBC count, differential WBC count, ESR and total protein content (albumin and globulin) were analysed . The difference in paw volume on day 3 and day 21 were considered as oedema volume and the percent inhibition of oedema was determined.

Data analysis— Data are expressed as mean ± SD. Statistical analyses were carried out by using Student's t test.

Results

The hydrodistillation of the fresh callises of *S. ixiocephala* yielded 0.5% (v/w) of essential oil which was greenish yellow in colour with pleasant aromatic odour. TLC study of essential oil on silica gel-G in pure benzene using vanillin/concentrated sulphuric acid (1%) as spraying reagent revealed four major spots having R_f value 0.96, 0.87, 0.63 and 0.45 and 12 minor components. The essential oil afforded four major constituents from column chromatographic fractionation using petroleum ether and benzene as eluent.

The GC-MS analysis of essential oil (Table 1) revealed the presence of a new sesquiterpene alcohol as a major constituent (23.4%) and T-cadinol (17.6%). Sesquiterpene hydrocarbons like γ-cadinene (4.8%), β-cadinene (1.6%) and β-caryophyllene (6.6%) were also present. The monoterpenoids mainly fenchone (3.0%), α-fenchol (7.6%), isoborneol (8.3%) and α-fenchyl acetate (2.2%) were also detected from the GC-MS data of essential oil.

Pharmacological effects of essential oil

(i) The essential oil of *S. ixiocephala* demonstrated a dose related anti-inflammatory activity in acute model of inflammation. In oral doses of 0.5, 1.0, 2.0 and 4 ml/kg it showed a dose dependant activity to the extent of 37.45%,

Table 1 — Chemical composition of *Strobilanthus ixiocephala* oil by GC-Mass

Constituent	RI	Area %	Identification
Fenchone	1087	3.0	GC/MS
α-Fenchol	1112	7.6	GC/MS
Isoborneol	1156	8.3	GC/MS
α-Fenchyl acetate	1220	2.2	GC/MS, Co-TLC
β-Caryophyllene	1418	6.6	GC/MS, Co-TLC
γ-Cadinene	1513	4.8	GC/MS
β-Cadinene	1531	1.6	GC/MS
Ixiocephol*	—	23.4	GC/MS, IR, ^{1}H-, ^{13}C-NMR, Mass
T-Cadinol	1640	17.6	GC/MS, IR, ^{1}H, ^{13}C- NMR

50.58%, 64.48%, and 75.68% ($P<0.001$) inhibition (Table 2) where as standard drug diclofenac sodium in dose of 10 mg/kg produced 76.83% inhibition after 24 hr. ($P<0.001$).

(ii) The essential oil in cotton pellet granuloma (a chronic model of inflammation) when administered orally in the dose of 1 ml/kg showed 35.73% inhibition of granuloma where as 56.28% inhibition of granuloma was shown by standard diclofenac sodium 10 mg/kg dose ($P<0.001$). (Table 3).

(iii) The essential oil in Freund's adjuvant induced arthritis in rats at the oral dose of 1 ml/kg inhibited the rat paw oedema by 35.56% after 21 days where as diclofenac sodium (10 mg/kg) produced 54.22% inhibition of rat paw oedema after 21 days ($P<0.001$) (Table 4). In biochemical study (Table 5) essential oil and standard drug showed increase in haemoglobin content as compared to adjuvant positive control group ($P<0.001$). The increased WBC count was significantly suppressed by essential oil and

Table 2 — Effect of essential oil of *S. ixiocephala* in carrageenan induced rat paw oedema

Group No.	Group specification	Mean paw volume			Mean difference		Percent inhibition	
		Initial	3 hr	24 hr	3 hr	24 hr	3 hr	24 hr
I	Control tween-80 (2%)	0.717 ± 0.0163	1.019 ± 0.0028	0.976 ± 0.0045	0.303 ± .0185	0.259 ± .01135	—	—
II	Standard (Diclofenac sodium 10 mg/kg)	0.727 ± 0.0121	0.823 ± 0.0031	0.786 ± 0.0058	0.097 ± .0114	0.060 ± 0.0111	67.99*	76.83*
III	Essential oil (0.5 ml/kg)	0.732 ± 0.0183	1.007 ± 0.0159	0.893 ± 0.0157	0.276 ± 0.0050	0.162 ± 0.0047	8.91	37.45*
IV	Essential oil (1.0 ml/kg)	0.700 ± 0.0141	0.960 ± 0.0191	0.828 ± 0.0122	0.260 ± 0.0071	0.128 ± 0.0091	14.19	50.58*
V	Essential oil (2.0 ml/kg)	0.717 ± 0.0186	1.914 ± 0.0204	0.807 ± 0.0100	0.194 ± 0.0053	0.092 ± 0.0104	35.97*	64.48*
VI	Essential oil (4.0 ml/kg)	0.718 ± 0.0204	0.897 ± 0.0173	0.781 ± 0.0039	0.178 ± 0.0059	0.063 ± 0.0174	41.45*	75.68*

No. of rat = 6 per group, tabular value represents mean ± SD.
*$P<0.001$ (Comparison of I with II, III, IV, V and VI)

Table 3 — Effect of essential oil of *S. ixiocephala* on cotton pellet granuloma

Group No.	Group specification	Initial wt (mg)	Wt after implantation (mg)	Difference in wt (mg)	Percent inhibition
I	Control Tween-80 (2%)	51.33 ± 0.8165	241.17 ± 1.6021	189.83 ± 1.4720	—
II	Diclofenac sodium (10 mg/kg)	50.5 ± 1.0488	133.5 ± 3.1464	83.00 ± 3.7947	56.28*
III	Essential oil (1 ml/kg)	50.5 ± 1.0489	172.5 ± 1.8708	122.00 ± 2.0976	35.73*

No. of rat = 6 per group, tabular value represents mean ± SD.
* $P<0.001$ (Comparison of I with II and III)

Table 4 — Effect of essential oil on adjuvant induced arthritis

Group No.	Group specification	Mean paw volume			Mean difference		Percent inhibition
		Initial	After 3 days	After 21 days	After 3 days	After 21 days	After 21 days
I	Control Tween-80 (2%)	1.123 ± 0.0234	1.428 ± 0.0041	1.348 ± 0.0256	0.305 ± 0.0212	0.225 ± 0.0294	—
II	Diclofenac sodium (10 mg/kg)	1.160 ± 0.0014	1.452 ± 0.0279	1.263 ± 0.0047	0.292 ± 0.0027	0.103 ± 0.0042	54.22*
III	Essential oil (1 ml/kg)	1.303 ± 0.0225	1.610 ± 0.02	1.448 ± 0.0242	0.307 ± 0.0216	0.145 ± 0.0035	35.56*

No. of rat = 6 per group, tabular value represents mean ± SD.
*$P<0.001$ (Comparison of I with II and III).

Table 5 — Effect of essential oil of *S. ixiocephala* on biochemical parameters studied in adjuvant induced arthritis

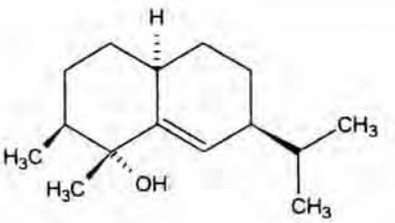
SR. no.	Parameters	Control Tween-80 (2%)	Standard diclofenac sodium (10 mg/kg)	Test drug essential oil (1 ml/kg)
1	Haemoglobin	10.55g/dl ± 0.0609	12.21 g/dl* ± 0.1145	11.83 g/dl* ± 0.1084
2	Total WBC count	10900/cu.mm ± 89.4427	8233.33/cu.mm* ± 163.299	8783.33/cu.mm* ± 132.92
3	Differential WBC count			
a)	Neutrophils	16.67% ± 1.2111	43.83%* ± 1.602	42.33%* ± 1.506
b)	Lymphocytes	81.16% ± 1.1690	52.67%* ± 1.9666	55.33%* ± 1.6329
c)	Eosinophils	1.667% ± 0.5164	2.16% ± 0.7528	1.833% ± 1.1690
d)	Basophils	0.0% ± 0.0	0.0% ± 0.0	0.0% ± 0.0
e)	Monocytes	0.5% ± 0.5477	1.33% ± 0.8615	0.5% ± 0.5477
4	ESR	11.33 mm ± 1.03after 1 hr	2.0 mm* ± 0.0 after 1 hr	2.0 mm* ± 0.0 after 1 hr
5	Total protein content	8.005 gm % ± 0.0351	5.65 gm %* ± 0.0351	7.358 gm % ± 0.1319
a)	Serum albumin	3.405 gm % ± 0.0345	2.15 gm %* ± 0.02	2.567 gm %* ± 0.1506
b)	Serum globumin	4.60 gm % ± 0.0141	3.495 gm %* ± 0.0234	4.792 gm % ± 0.1390

No. of rat = 6 per group, tabular value represents mean ± SD.
*$P < 0.001$ (Comparison of all parameters of Control group with Std and Test group).

standard diclofenac sodium ($P < 0.001$). The increased lymphocyte count in adjuvant control group was significantly restored back to normal by test and standard drug ($P < 0.001$). The significant increase of ESR in adjuvant control was also restored back to normal by the essential oil and standard drug. The increased protein content of adjuvant control group was remarkably decreased by standard drug ($P < 0.001$) but essential oil did not show much effect on total protein content but suppressed only the serum albumin content as compared to adjuvant control group.

Discussion

Column chromatographic fractionation of essential oil on silica gel afforded four major components in pure form with trace impurities. The isolated pure constituents were identified as β-caryophyllene, fenchyl acetate and T-cadinol by Co-TLC with authentic samples. T- cadinol was elucidated on the basis of its UV, IR, ^1H, ^{13}C NMR and mass spectral data which was found to be in accordance with the literature data of T-cadinol[13,14]. The fourth compound afforded from chromatographic fractionation has been



Ixiocephol

found to be a new sesquiterpene alcohol, a white crystalline compound, mp 62°-64°C, λ_{max} 259.4 nm, IR (KBr) cm^{-1}:3368 (-OH), 2961, 1595, 1458, 1352, 904 and 760. ^1H NMR ($CDCl_3$) : 2.12 (4H, m, C_1-CH_2), 1.73 (2H,m,C_2-CH_2), 1.41 (2H,m, C_3-CH_2), 5.47 (1H,d C_6-CH), 2.12 (1H, m, C_7-CH), 1.58 (2H,m, C_8-CH_2), 1.89 (2H,m C_9-CH_2), 2.21 (1H, m, C_{10}-CH), 1.37 (1H,m, C_{11}-CH), 0.95 (3H,d, C_{12}-CH_3), 0.96 (3H,d, C_{13}-CH_3), 1.19 (3H,s, C_{14}-CH_3) and 0.86 (3H,d, C_{15}-CH_3). ^{13}C-NMR ($CDCl_3$) : δ 37.01 (C_1), 21.33 (C_2), 51.13 (C_3), 75.39 (C_4), 148.09 (C_5), 123.86 (C_6), 43.65 (C_7), 24.97 (C_8), 42.47 (C_9), 37.43 (C_{10}), 32.98 (C_{11}), 21.07 (C_{12}), 21.13 (C_{13}), 23.80 (C_{14}), and 15.09 (C_{15}). MS m/e (%) 222 (0.5), 204 (17.0), 161 (100), 147 (6.0), 133 (9.0), 105 (19.5), 91 (15.0), 79 (9.0), 55 (5.5) and 41 (9.0). The name

Ixiocephol has been proposed for this new sesquiterpene alcohol obtained from the essential oil of *S. ixiocephala*[15].

The essential oil of *S. ixiocephala* have shown significant anti-inflammatory activity in acute as well as chronic model and also remarkable antiarthritic activity in adjuvant induced arthritis model. In therapeutic test model of adjuvant arthritis primary lesions are generally seen on day 3 and from day 12 onwards secondary lesions are observed. The right hind paw volume was measured on day 0 and on day 21. Therefore in present study, the difference between the above two values has been considered to be the oedema volume. The standard drug and essential oil did not suppress the primary lesions on day 3 but shown a significant inhibition of secondary swelling in the ajuvant injected and non-injected paw on day 21. T-lymphocytes have been reported to play a central role in the pathogenesis of rheumatoid arthritis. These cells comprise the majority of the lymphoid cells found in the rheumatoid synovium[16]. The inhibition of secondary inflammation in adjuvant arthritic rats by the essential oil was further strengthened by the biochemical parameters studied. Increased lymphocyte count in arthritic rats was significantly suppressed in the standard and essential oil treated group indicating its immunosuppressant nature. Arthritis condition generally results in accumulation of leucocytes and release of lysosomal enzymes, the main mediators in arthritis[17]. In present study the migration of leucocytes into the inflammed area is significantly suppressed by the essential oil as seen from the significant decrease in total WBC count. Most of the non-steroidal anti-inflammatory agents exert their beneficial effect by inhibiting either release of lysosomal enzymes or by stabilizing lysosomal membrane which is responsible for inflammatory process[6]. Possibly essential oil would be acting by the similar mechanism as it reduced the total protein content especially serum albumin content. The ESR count which drastically increased in arthritic control group has been remarkably counteracted by the standard and essential oil, restoring it back to normal, thus justifying its significant role in arthritic conditions. In conclusion, remarkable anti-inflammatory and antiarthritic activity of essential oil of *S. ixiocephala* could be correlated to other chemotaxonomically related species like *S. callosus* and *S. heyneanus* for the treatment of various inflammatory conditions.

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