

Chemical Constituents, Antimicrobial And Anti-Inflammatory Activities Of *Anaphalis triplinervis* Essential Oil

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Abstract

The essential oil of *Anaphalis triplinervis* has been isolated through hydro-distillation and analysed by gas chromatography-mass spectroscopy (GC-MS). The *in-vitro* antimicrobial activity has been investigated by well-diffusion method against gram-positive and gram-negative bacteria as well as pathogenic fungus and *in-vivo* anti-inflammation activity has been evaluated by using complete Freund's adjuvant (CFA) induced model into the left hind paw oedema. A total of nineteen components have been observed which constitute 93.2% of the total oil, observed major group components are fatty acids (40%), sesquiterpene hydrocarbons (29%), oxygenated sesquiterpenes (19.7%), fatty acids methyl esters (1.1%), diterpene (2.7%), monoterpene (0.8%) and phthalate contamination (0.6%). The main constituents of the oil are n-hexadecanoic acid (33.7%), cis- β -franesene (17.1%), phytone (7.1%), α -humulene (3.8%), elemol (3.6%), caryophyllene oxide (3.4%), δ -cadinene (3.3%) and β -caryophyllene (3%). This essential oil has shown effective antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli* bacteria as well as pathogenic fungus *Aspergillus niger* at 400 ppm concentration and also has shown the reduction in paw volume and haematological parameters in inflammatory rats at 200 mg/kg dose.

Keywords: *Anaphalis triplinervis*, Essential oil, Sesquiterpene, Antimicrobial activity, Anti-inflammatory activity

Introduction

A. triplinervis belongs to Asteraceae family and is commonly known as Bhukhiphul, Raktskandana, Pearly everlasting etc. in India and has more than 80 species (Chopda & Mahajan, 2009; Khakurel et al., 2014). It is an herbaceous perennial type plant mainly distributed in the tropical and subtropical region (Saxena et al., 1984). It is widespread throughout Afghanistan, Bhutan, India, Nepal, and Tibet to south-west China at an altitude of 1800-3300 m in the Himalayan region (Tiwari et al., 2016). *A. triplinervis* is an erect herb with stout and poorly branched stem entirely cottony or woody about 25-50 cm tall. Leaves of *A. triplinervis* are elongate, smooth near the stem gripping, upper leaves are small (1.2-1.5 cm) while lower leaves are 4-6 cm long, 3-5 veined with or without a different stalk. Its flowers are shining white petals with a yellow colour in the centre. The month of flowering is July to October with clusters of more than 15 and as flower heads of 1 cm across (Malla et al., 2015).

Aerial parts of *A. triplinervis* are employed to treat swelling, an illness, fever, menstrual disorder, cough, cold, diuretic, tonsillitis, edema, laceration of toes, dressing wounds, skin problems etc. (Dorji et al., 2017; Singh & Chauhan, 2005; Kommu et al., 2013; Bhat et al., 2013; Bisht & Purohit, 2010). Its flowers are used for the treatment of epilepsy by preparing a mixture which is known as Pam-brey in Arunachal Pradesh, India (Balami, 2006). In Nepal, its flowers and leaves are used as an antiseptic and, its flowers and leaves are used to stop vomiting in Pakistan (Ahmed et al., 2014; Hailu, 2004).

The purpose of this study is to evaluate the chemical constituents from *A. triplinervis* essential oil by GC-MS and analyse its antimicrobial and anti-inflammatory activities. The antimicrobial activity has been performed by well-diffusion method and anti-inflammatory activity has been measured by using complete Freund's adjuvant (CFA) model.

Materials and Methods

The chemicals and reagents viz. Dimethylsulfoxide (DMSO), Tween 80 and sodium chloride were used in this study of Analytical reagent grade (Sigma-Aldrich). The Nutrient broth, Agar-agar powder and Potato dextrose agar (Hi-media) were used for microbial culture. The selected two bacterial strains viz. *Escherichia coli* MTCC 452 (*E. coli*; gram-negative), *Staphylococcus aureus* MTCC 737 (*S. aureus*; gram-positive) and one fungal strain viz. *Aspergillus niger* MTCC 1344 (*A. niger*) were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC, IMTECH), Chandigarh, India. The standard drugs, ciprofloxacin (for antibacterial activity) and fluconazole (for antifungal activity) were purchased from Local retail pharmacy shop. CFA was purchased from Sigma chemicals (St Louis, USA) and methotrexate from MacLeod's Lab (Mumbai, India).

Plant materials

The whole plant of *A. triplinervis* was collected from high-altitude forest of Parashar Lake (2730 m above sea level; Latitude: 31°45'15.7176"N Longitude: 77°6'4.1148"E and elevation: ~2730 m), Himachal Pradesh, India during the flowering period in August 2019. The specimen of whole plant was prepared. The botanical identification and authentication of plant specimen has been done by S. K. Singh (Scientist E & HOD), Botanical Survey of India in Dehradun, Uttarakhand, India. A voucher specimen number-BSI/326 has been preserved for further verification and voucher sample has been deposited at the Herbarium of Botanical Survey of India, Dehradun, Uttarakhand, India. The whole plant material was air-dried in a shady room until the weight was stable then grounded into a fine powder which was kept in an airtight container for subsequent use.

Extraction of essential oil

The whole plant of *A. triplinervis* (500 g) was used to obtain the essential oil using Clevenger apparatus for 6h by the hydro-distillation method following the Polish Pharmacopeia VIII (Warszawa, 2008). The essential oil was collected and added anhydrous sodium sulphate to remove water contents. The oil was preserved in a sealed vial at 4°C for further analysis and bioassays. The yield of essential oil was found to be about 0.38% based on the dry weight of plant material.

GC and GC-MS analysis

The GC analysis was performed from Shimadzu GC-2010 (Shimadzu, 2010, Tokyo, Japan) instrument equipped with HP-5MS (30 m, 0.25 mm i.d., 0.25 µm df) fused silica capillary column and FID. Nitrogen was used as a carrier gas with 1.05 mL/min flow rate. The oven was programmed follow: 70°C (hold 3 min) at a rate of 4°C/min to 220°C (hold 5 min). The sample was injected using split (1:10) ratio technique using 1.0 µL. The Injector and detector were set at 240°C respectively.

The GC-MS was performed using a Shimadzu QP2010 system with AOC5000 auto-injector, Software XCalibur 2.2SP1 with foundation 2.0SP1. An HP-5MS (30 m, 0.25 mm i.d., 0.25 µm df) was used with helium at 1.05 mL/min as a carrier gas. The oven programme was kept 70°C (hold 3 min) at a rate of 4°C/min to 220°C (hold 5 min). The split ratio was adjusted to 1:10, the injection volume was 1.0 µL and the injection temperature was 240°C. Mass range was m/z 40-800 amu respectively.

Identification of chemical constituents

The chemical constituents were identified by matching their mass spectroscopic data and retention indices with those recorded in NIST 11 library and comparison of retention indices and mass spectroscopic data with literature values. The n-alkane (C₈-C₂₄) mixture was used for retention indices calculated (Adams, 2012; Joulain & Koenig, 1998; ESO, 2000; Kaur et al., 2020).

Antimicrobial activity

Test Microorganisms

The antimicrobial activity of essential oil of *A. triplinervis* was studied against selected two bacterial strains viz. *Escherichia coli*, *Staphylococcus aureus* and one fungal strain viz. *Aspergillus niger*. All the stock culture were obtained from The Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

Culture media and inoculums preparation

For the culturing of bacterial strains, the Nutrient broth was used as media. Loops full of all the bacterial cultures were inoculated on nutrient at 37°C for 24-48 h. The potato dextrose agar was used as the media for the culturing of fungal strain and the loops full of fungus culture was inoculated at 27°C for 48-72 h.

Antibacterial assay

The antibacterial activities of essential oil of *A. triplinervis* were performed by adopting well-diffusion method (Ajiboye & Olawoyin, 2020). Through the serial dilution method, different concentrations (100, 200, 400 ppm) of essential oil sample were prepared in DMSO and activities were compared with standard antibiotic ciprofloxacin (100 ppm). The respective solvent (sterile DMSO) was used as a negative control. The freshly prepared inoculums (10^8 CFU/ml) of each test bacterium spread on the sterile nutrient agar Petri dishes. The dishes were allowed to dry then four wells were bored having 7 mm diameter using sterile cork-borer and were labelled properly (Toit & Rautenbach, 2000). Subsequently, 40 µL of each dilution of essential oil was added in three different wells using microtiter-pipette and dishes were allowed to stand at least 1 h for diffusion to take place. The dishes were then incubated in the upright position at 37°C for 24 h. The result was evaluated by measuring the width of the zone of inhibition of growth against the selected organisms in comparison with ciprofloxacin and mean values were tabulated.

Antifungal assay

The essential oil of *A. triplinervis* was also screened for the antifungal activity in comparison with standard antibiotic (fluconazole (100 ppm)) by well-diffusion method (Ajiboye & Olawoyin, 2020). The culture was prepared using the test organism on potato dextrose agar. The different concentrations (100, 200, 400 ppm) of the essential oil was prepared in DMSO using serial dilution method respectively and DMSO was used as a negative control. The dishes were allowed to dry for a few minutes. The four wells were bored (7 mm diameter) using sterile cork-borer at the required distance. Using sterile micropipette, 40 µL of each dilution of essential oil was added into the three different wells. The dishes with fungi was incubated at 27°C for 72 h. The activity was determined by measuring the diameter of zone of inhibition against the selected organisms and compared with fluconazole as a standard and mean values were tabulated.

Anti-inflammatory activity

Animals

Wistar rats of both sexes (weighing 200-250 g) were used after obtaining the approval of the Institute Animal Ethics Committee (MMCP-IAEC-84). The animals were housed under standard conditions of temperature ($25\pm 2^\circ\text{C}$) and relative humidity (60-70 %) with a 12 h light/dark circulation environment. The standard pellet diet (Lipton India, Ltd.) and water *ad libitum* were provided during the study period.

Preparation of test samples

The essential oil was administered orally in different doses (50 mg/kg and 200 mg/kg, dissolved in Tween 80). The standard methotrexate (0.5 mg/kg) was used to cure inflammation and orally administered in the form of suspension.

Acute oral toxicity study

The acute oral toxicity was tested according to the OECD guideline 425 (OECD 2002). The animals were fasted 16 h preceding to the experiment with *ad libitum* access to water. The essential oil was administered orally to five groups of rats and each group consists of six rats (n=6) at doses of 50 mg/kg, 500 mg/kg and 2000 mg/kg. Rats were continuously observed for 3 h to detect changes in behaviour or autonomic responses and following 48 h then for 7 days. The essential oil had not shown any significant toxic sign up to 2000 mg/kg. Hence, the present study was carried at the doses of 50 mg/kg and 200 mg/kg dose level.

Experimental design

Animals were divided randomly into five groups and each group consisted of six rats. Group I: Normal control treated with 0.9% normal saline, orally; Group II: Positive control treated with CFA 0.1 ml, subcutaneous route; Standard i.e., Group III treated with methotrexate 0.5 mg/kg, orally; Group IV treated with 50 mg/kg of essential oil orally; and Group V treated with 200 mg/kg dose of essential oil, orally.

Evaluation of anti-inflammation activity-CFA induced

Preceding experiment, paw volume of each rats were measured at 0th day. The complete freund's adjuvant (suspension of heat-killed *Mycobacterium tuberculosis* bacteria homogenized in liquid paraffin at 10 mg/ml) was used to induce arthritis in rats of all groups (except normal control group). The rats were anesthetized by using thiopentone sodium (40 mg/kg) intraperitoneal injection. The arthritis was induced through CFA by subcutaneous route (0.1 ml) into the left hind paw. No treatment was not given to normal control group. Drug treatment was started from 1st day and continued till the 21st day. The paw volume was monitored on 0, 1st, 7th, 14th and 21st days by using digital plethysmometer (Model 7140, UGO Basile, Italy). Increase in the size of oedema of the tissues had shown the cause of inflammation. The standard drug i.e., methotrexate (0.5 mg/kg) was used for comparison. The body weight was observed on alternate days of protocol (0, 1st, 3rd, 5th, 7th, 9th, 11th, 13th, 15th, 17th, 19th, 21st days) and the ankle-joint diameter was measured on 0, 1st, 6th, 11th, 16th and 21st days using Vernier calliper instrument (Tripathy et al., 2009).

Haematological study

At the end of the study, the blood samples were drawn from each rat through the retro-orbital plexus puncture, then it was collected into vials containing EDTA, and used for haematological parameters such as the Haemoglobin (Hb), Red blood cells (RBC), White blood cells (WBC), Erythrocyte sedimentation rate (ESR) count (Parasuraman et al., 2010).

Statistical analysis

The data presented in mean ± standard deviation (SD). The statistical difference between the mean was analysed using ANOVA and Tukey's multiple comparison test. The $P < 0.001$ were considered as significant.

Results and Discussion

Chemical compositions

Essential oil was attained from whole plant of *A. triplinervis* through hydro-distillation and was analysed by GC-MS. A total of nineteen components were identified, representing 93.2% of the total oil. The constituents identified by GC and GC-MS analysis are shown in **Table 1**. The constituents of essential oil was separated into seven classes, which includes sixsesquiterpene hydrocarbons, six oxygenated sesquiterpenes, three fatty acids, one fatty acids methyl esters, one diterpene, and one monoterpene, one phthalate contamination. The major components of the oil were n-hexadecanoic acid (33.7%), cis-β-farnesene (17.1%), phytone (7.1%), tetradecanoic acid (4.4%), α-humulene (3.8%), elemol (3.6%), caryophyllene oxide (3.4%), δ-cadinene (3.3%), β- caryophyllene (3%), valerenyl acetate (2.8%), phytol (2.7%) and others components were present in amounts less than 2%. This study have indicated the presence of a high percentage of fatty acids (40%) of which the main constituents were n-hexadecanoic acid and tetradecanoic acid. Sesquiterpenes hydrocarbons (29%) and oxygenated sesquiterpenes (19.7%) were present in fairly good amount in which the major constituents of sesquiterpenes hydrocarbons were cis-β-farnesene, α-humulene, δ-cadinene, β-caryophyllene whilemajor constituents oxygenated sesquiterpenes were phytone, elemol, caryophyllene oxide, valerenyl acetate in the oil. Other fatty acids methyl esters (1.1%), phthalate contamination (0.6%), diterpene (2.7%) and remaining percentage (0.8%) consists of monoterpene were also present in essential oil with a minor amount.

Table 1. Chemical constituents of *Anaphalis triplinervis* essential oil

RT	Identified Constituents	RI ^a	RI ^b	Area %	Identified method
7.8	m-Cymene	1031	1025	0.8	MS ^c , RI ^d
21.6	β-Caryophyllene	1411	1418	3.0	MS ^c , RI ^d
22.6	cis-β-Farnesene	1442	1458	17.1	MS ^c , RI ^d
22.8	α-Humulene	1453	1454	3.8	MS ^c , RI ^d
23.4	γ-Cadinene	1515	1513	1.0	MS ^c , RI ^d
24.1	δ-Cadinene	1527	1524	3.3	MS ^c , RI ^d
24.8	α-Cadinene	1537	1538	0.5	MS ^c , RI ^d
26.1	Elemol	1551	1537	3.6	MS ^c , RI ^d
26.8	Caryophyllene oxide	1586	1581	3.4	MS ^c , RI ^d
27.7	Humulene epoxide II	1619	1614	1.8	MS ^c , RI ^d
29.1	Nerolidyl acetate	1687	1735	0.8	MS ^c , RI ^d
30.3	Valerenyl acetate	1728	1785	2.8	MS ^c , RI ^d
32.1	Tetradecanoic acid	1766	1760	4.4	MS ^c , RI ^d
34.4	Phytone	1846	1843	7.1	MS ^c , RI ^d
34.6	dibutyl phthalate	1873	1855	0.6	MS ^c , RI ^d
36.4	Methyl palmitate	1927	1920	1.1	MS ^c , RI ^d

37.5	n-Hexadecanoic acid	1984	1977	33.7	MS ^c , RI ^d
41.6	Linoleic acid	2047	2055	1.9	MS ^c , RI ^d
42.0	Phytol	2109	2122	2.7	MS ^c , RI ^d
				Fatty acids	40
				Sesquiterpene hydrocarbons	29
				Oxygenated sesquiterpenes	19
				Diterpene	2.7
				Fatty acids methyl esters	1.1
				Monoterpene	0.8
				Phthalate contamination	0.6
Total				93.2	

RT: Retention time

RI^a: Retention indices on HP-5MS capillary column

RI^b: Literature indices

MS^c: Identified base on mass spectra data

RI^d: Identification based on NIST11 library and comparison with literature

It has been observed from literature survey that out of nineteen chemical constituents reported in this study, eight constituents viz. n-hexadecanoic acid, β-caryophyllene, α-humulene, α-cadinene, γ-cadinene, δ-cadinene, caryophyllene oxide, humulene epoxide II match with other different studies carried out on essential oil of various *Anaphalis* species/ *Anaphalis triplinervis* (Joshi et al., 2009; Joshi, 2013; Rawat et al., 2017; Sharma et al., 2019). The detection differences in chemical constituents of essential oil contents of *A. triplinervis* might be due to climatic and soil variation (Viuda-Martos et al., 2007). Therefore, our results support the fact that plant species from the same genus may differ due to geographical region (Verma et al., 2010).

Rest eleven constituents found in this essential oil have been reported as the part of essential oil extracted from different plants. Components, m-cymene is found in essential oil of *L. meyeri* (Collin et al., 2010), cis-β-farnesene is identified in the essential oil of *M. chamomile* (Alireza, 2012), nerolidyl acetate is isolated from *I. viscosa* essential oil, linoleic are identified in the essential oil of *M. communis* (Messaoud & Boussaid M, 2011), elemol, valerenyl acetate are found from the essential oil of plants *D. floribunda* and *D. composita* (Odimegwu et al., 2013), cis-β-farnesene, tetradecanoic acid, methyl palmitate, linoleic acid is identified from *F. chrysanthemi*, *E. equisetina* and *B. chinese* essential oils (Zhao et al., 2009), phytone is isolated from the essential oil of *S. orientalis* (Gao et al., 2018), dibutyl phthalate is found in *Citrus* essential oil (Di Bella et al., 1999; Manayi et al., 2014) and phytol is found in essential oil of *P. rhodantha* and *P. peucedanifolia* (Tabanca et al., 2006).

Antimicrobial activity

In the present study, antimicrobial activity of the essential oil of whole plant of *A. triplinervis* was performed by well-diffusion method against *E. coli* (gram-negative), *S. aureus* (gram-positive) bacterial strains and *A. niger* a fungal strain. The essential oil inhibited the growth of all tested microorganisms with zone of inhibition range from 3±0.2 to 10±0.2 mm for bacterial strains and for fungus strain the range of zone of inhibition was 0.5±0.1 to 3.5±0.2 mm. The activities has shown effective results against selected bacterial and fungal strains (Table 2 and Figure 1). The oil exhibited effective results against *S. aureus* as compared to *E. coli* bacterial strain and the zone of inhibition value was found maximum for *S. aureus* (11±0.2 mm) at 400 ppm concentration. For *A. niger* the maximum zone of inhibition (3.5±0.2 mm) has been observed at 400 ppm concentration.

Table 2. Zone of inhibition of essential oil against different antimicrobial organisms

Essential oil	Bacterial strains (Zone of inhibition in mm)						Fungal strain (Zone of inhibition in mm)		
	<i>E. coli</i>			<i>S. aureus</i>			<i>A. niger</i>		
Dosages	100 ppm	200 ppm	400 ppm	100 ppm	200 ppm	400 ppm	100 ppm	200 ppm	400 ppm
Zone of inhibition	3±0.2	5.5±0.2	10±0.2	4±0.2	7±0.2	11±0.2	0.5±0.1	2±0.1	3.5±0.2
Standards	100 ppm (ciprofloxacin)			100 ppm (ciprofloxacin)			100 ppm (fluconazole)		
	13±0.3			15±0.3			4±0.2		
Control (DMSO)	Not observed			Not observed			Not observed		

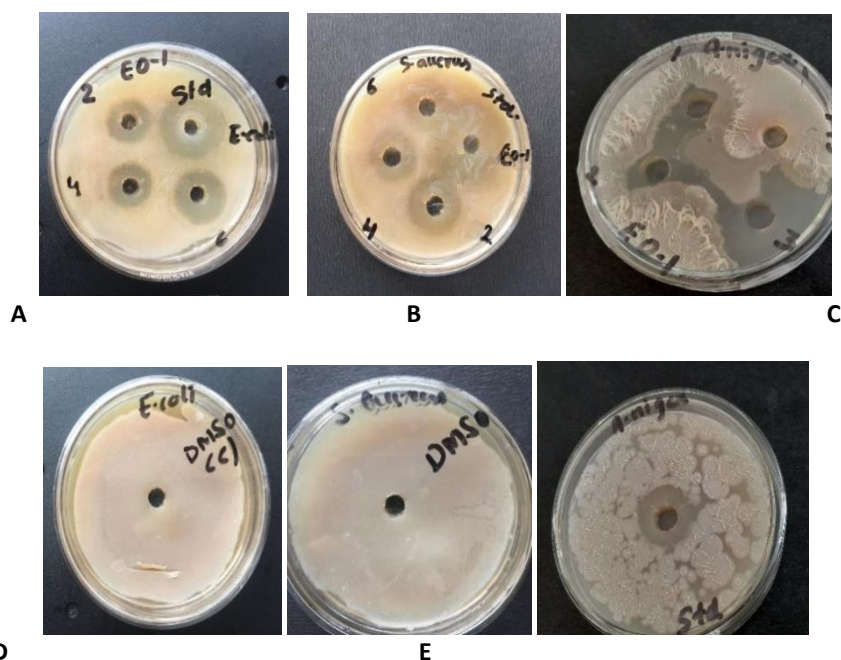


Figure 1: Antibacterial activity of essential oil; A) antibacterial activity against *E. coli*. B) Effect of antibacterial activity against *S. aureus*. C) antifungal activity against *A. niger*. D) antibacterial activity against *E. coli* with C E) Effect of antibacterial activity against *S. aureus* with C F) Effect of antifungal activity against *A. niger* with Std. (Std. = Standard; C = Control; 2 = 100 ppm; 4 = 200 ppm; 6 = 400 ppm)

The components n-hexadecanoic acid, cis- β -farnesene, α -humulene and caryophyllene oxide result to the cause of antimicrobial activity (Ali et al., 2017; Chehregani et al., 2010; Chandrasekaran et al., 2011; Satyal et al., 2015; Abubakar & Majinda, 2016; Saravanakumar et al., 2018). The constituents n-hexadecanoic acid, α -humulene, caryophyllene, δ -cadinene, β -caryophyllene, linoleic acid, oleic acid, methyl palmitate and γ -cadinene inhibit the number of bacteria (*E. coli*, *S. aureus*, *P. aeruginosa*, *B. cereus*, *B. subtilis*, *K. pneumoniae*, *P. Aueruginosa*) due to their structure (Bettarini et al., 1993; Dilika et al., 2000; Vukovic et al., 2007; Chandrasekaran et al., 2011; Gonzalez et al., 2012; Ali et al., 2017; Saravanakumar et al., 2018).

Anti-inflammation activity

In chronic CFA model, it was perceived that the swelling and redness observed over 24 h in the injected paw. The MTX treated group showed reduction in paw volume from 7th day of treated drug to till the end of the study. Both doses 50 mg/kg and 200 mg/kg of essential oil have decreased the paw volume throughout the 21st day of the study. The 200 mg/kg dose of the essential oil showed prominent results in paw volume as compared to 50 mg/kg dose. The percentage inhibition of both doses showed results almost similar to the results of methotrexate. The CFA treated group of rats has shown gradual increase in paw volume from 1st day to till the end of the study (Table 3 and Figure 2).

Table 3. Effect of essential oil on CFA model (paw volume by plethysmometer)

Group (n=6)	0 day	1 st day	7 th day	14 th day	21 st day
Normal control	1.18±0.105	1.16±0.173*** (58.12%)	1.18±0.361*** (63.23%)	1.18±0.076*** (69.66%)	1.16±0.064*** (72.18%)
Positive control	1.21±0.052	2.77±0.088	3.21±0.095	3.89±0.037	4.17±0.055
Standard	1.21±0.027	2.74±0.152 (1.08%)	2.18±0.078 (32.08%)	1.82±0.046*** (53.21%)	1.33±0.056*** (68.10%)
Essential oil (50 mg/kg)	1.14±0.052	2.85±0.116 (2.88%)	2.70±0.043*** (15.88%)	2.52±0.053*** (35.21%)	2.11±0.045*** (49.40%)
Essential oil (200 mg/kg)	1.20±0.027	2.84±0.086 (2.52%)	2.41±0.090*** (24.92%)	2.09±0.034*** (46.27%)	1.88±0.090*** (54.91%)

Values was plotted as the mean \pm SD, n=6 rats in each group, significant change was analysed by using ANOVA and Tukey’s multiple comparison test, ***P < 0.001, compared with positive control group, Percentage (%) inhibition of paw volume

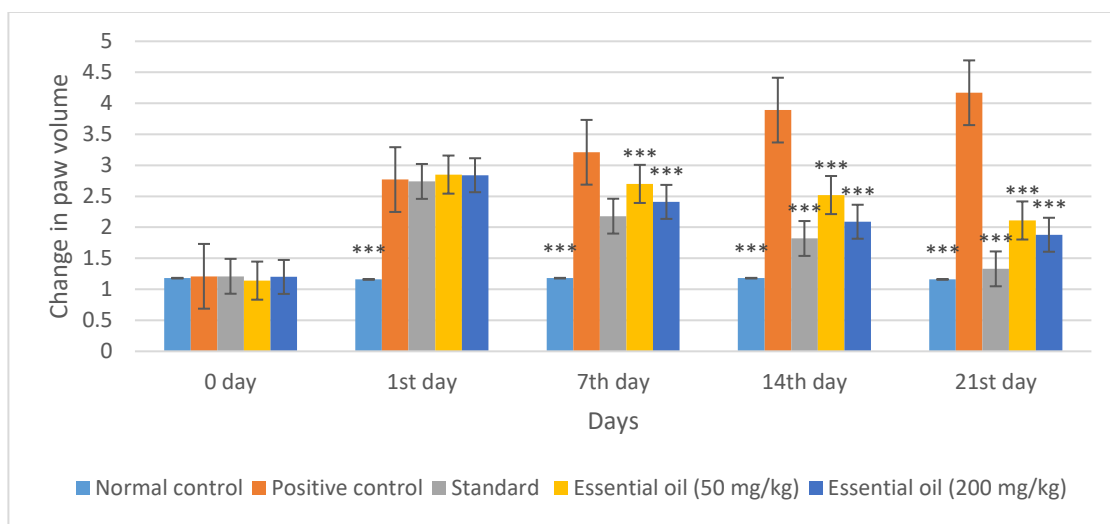


Figure 2. Effect of essential oil on CFA model (paw volume by plethysmometer)

Body weight and ankle-joint diameter

There was no significant change in body weight of normal control group throughout the 21st day of the study. The reduction in body weight was observed in the induced arthritis group as compared to normal group. Both doses of essential oil (50 and 200 mg/kg) and MTX (0.5 mg/kg) treated groups have been shown to restore body weight (Table 4 and Figure 3).

Table 4. Effect of essential oil on body weight

Group (n=6)	0 day	1 st day	3 rd day	5 th day	7 th day	9 th day	11 th day	13 th day	15 th day	17 th day	19 th day	21 st day
Normal control	220.83 ±5.833	221.66 ±5.833	222.5 ±3.354	221.66±2.78 8	222.5±2.81	222.5±2.81	223.33±3.57 4	224.1 6±3.2 15	224.166± 3.515	225±3 .651	225±3 .651	228.33±2. 108
Positive control	225.83 ±6.759	221.66 ±6.666	217.5± 6.677	214.16±4.72 8	211.66±4.409	209.16±4.36	207.5±3.818	206.6 6±4.5 94	204.166± 2.236	200.8 3±4.1 66	199.1 6±3.7 45	196.66±2. 788
Standard	229.16 ±5.833	223.33 ±6.666	225±5	226.66±4.95 8	227.5±5.833	228.33±4.21	227.5±2.5	229.1 6±2.0 06	229.166± 2.472	230±2 .581	232.5 ±2.14 0	234.16±2. 713
Essential oil (50 mg/kg)	222.5± 6.291	220.83 ±5.387	221.66 ±4.944	222.5±2.813	223.33±2.108	224.16±2.38 6	223.33±3.07 3	224.1 6±2.7 13	225±2.23 6	226.6 6±2.1 08	227.5 ±2.5 72	228.33±2. 72
Essential oil (200 mg/kg)	221.66 ±5.577	219.16 ±3.51	220±2. 581	224.16±3.00 4	225.83±2.386	227.5±2.5	226.66±2.10 8	227.5 ±2.14 0	228.33±2 .47	228.3 3±2.4 7	229.1 6±2.0 06	230.83±3. 004

Values are plotted as the mean ± SD, n=6 rats in each group

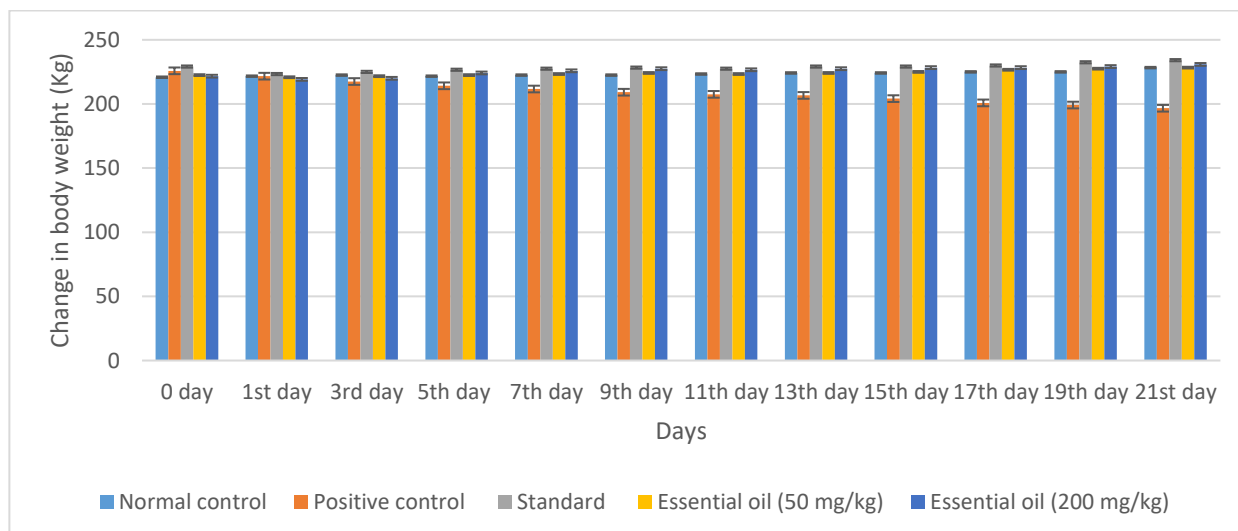


Figure 3. Effect of essential oil on body weight

The ankle-joint diameter of essential oil treated groups (50 and 200 mg/kg) have showed that the non-significant reduction in swelling on ankle-joint and attaining the normal position as comparable with positive control group from 16th to 21st day (Table 5 and Figure 4).

Table 5. Effect of essential oil on ankle-joint diameter

Group (n=6)	0 day	1 st day	6 th day	11 th day	16 th day	21 st day
Normal control	0.43±0.081	0.43±0.081	0.43±0.081	0.43±0.081	0.43±0.081	0.43±0.081
Positive control	0.41±0.098	0.78±0.075	0.85±0.054	0.9±0.089	0.96±0.081	1.08±0.075
Standard	0.45±0.054	0.75±0.104	0.7±0.089	0.66±0.081	0.58±0.075	0.48±0.075
Essential oil (50 mg/kg)	0.38±0.075	0.75±0.104	0.75±0.054	0.73±0.081	0.7±0.063	0.65±0.054
Essential oil (200 mg/kg)	0.43±0.081	0.76±0.081	0.75±0.054	0.71±0.075	0.66±0.081	0.58±0.081

Values was plotted as the mean ± SD, n=6 rats in each group

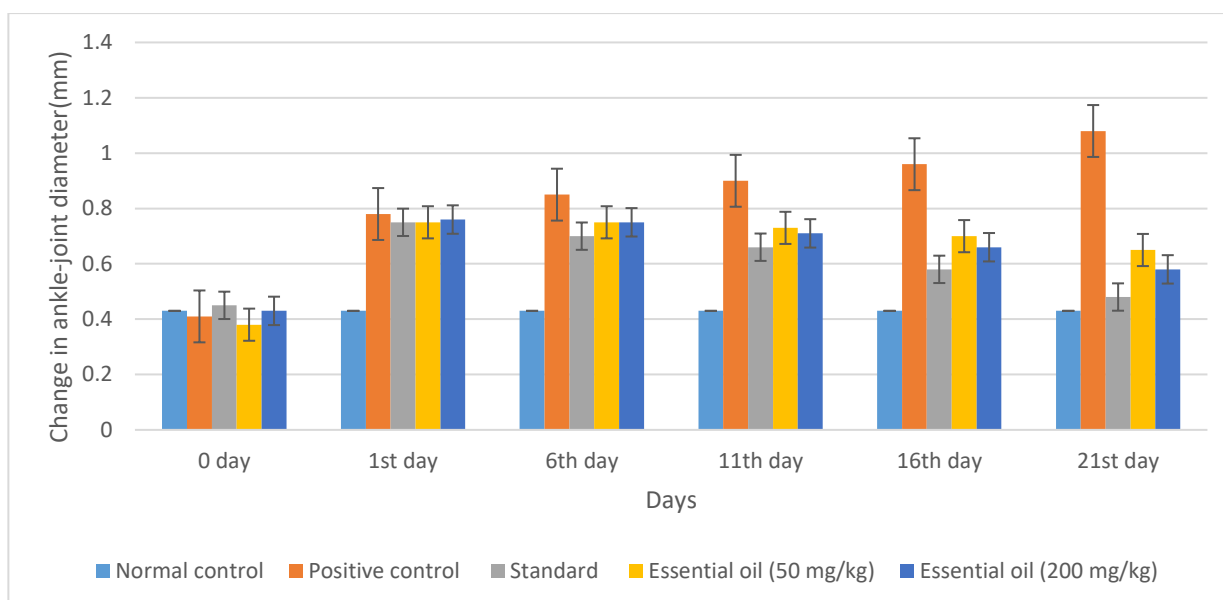


Figure 4. Effect of essential oil on ankle-joint diameter

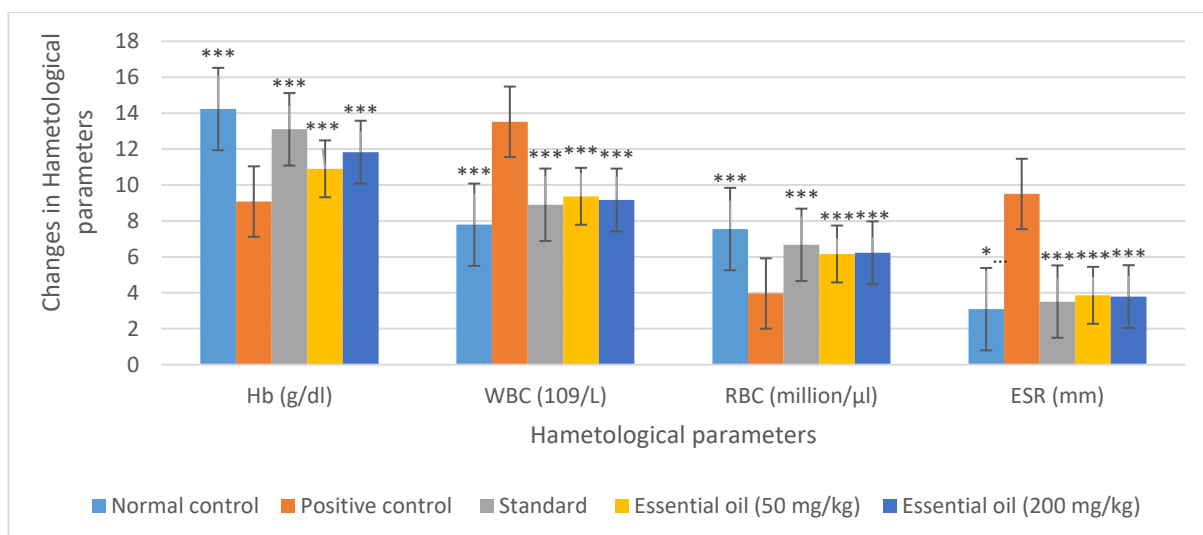
Haematological parameters

The haematological parameters like Hb, RBC, WBC, and ESR were observed. In induced CFA group, the blood WBC, ESR content increased with decreased Hb, RBC content. Both doses of essential oil (50 and 200 mg/kg) showed significant result in haematological analysis. The essential oil 200 mg/kg had shown effective result with decrease of WBC, ESR content and increase the Hb, RBC content (Table 6).

Table 6. Effect of essential oil on haematological parameters

Parameters	Hb (g/dl)	WBC (10 ⁹ /L)	RBC (million/ μ l)	ESR (mm)
Normal control	14.23±0.326***	7.79±0.365***	7.55±0.218***	3.09±0.071***
Positive control	9.08±0.466	13.52±0.257	3.96±0.074	9.50±0.064
Standard	13.1±0.447***	8.90±0.538***	6.67±0.246***	3.51±0.058***
Essential oil (50 mg/kg)	10.9±0.414***	9.37±0.389***	6.16±0.386***	3.86±0.019***
Essential oil (200 mg/kg)	11.83±0.436***	9.165±0.514***	6.23±0.538***	3.79±0.083***

Values are plotted as the mean ± SD, n=6 rats in each group, significant change was analysed by using ANOVA and Tukey's multiple comparison test, ***P < 0.05, compared with positive control group



Chronic inflammation is a dysregulated form of inflammation, prolonged reactions to pathogens or certain endogenous or exogenous substances. Earliest times, inflammation disorders were treated with several plants extracts or derived compounds from plants (Gupta et al., 2011). In this study, our objective was to evaluate the scientific base for traditional use of *A. triplinervis* with CFA-induced arthritis model. CFA-induced arthritis is a chronic inflammation disease considered by percolation of the synovial membrane and associated with destruction of the joints resembling closely to the human rheumatoid arthritis (Barsante et al., 2005; Lin et al., 2014). The paw volume, body weight, ankle-joint diameter, and haematological parameters have been investigated for anti-inflammation activity of *A. triplinervis* essential oil at the 50 and 200 mg/kg doses and compared to positive control group. The redness and swelling has been developed over 24 h in the CFA injected paw. The inflammatory response has been slightly decreased through the 7th day of study and increased at the time when arthritis spread (Kumar et al., 2013). At dose level of 200 mg/kg, the rats have shown the reduction in paw volume and arthritis spread as compared to positive control group. The significant weight loss from the day following the injection of CFA was observed, but after the doses of essential oil and methotrexate there was again weight gain in the CFA-induced rats. These results show the relationship between inflammation and loss of body weight (Chitme & Patel, 2009). The ankle-joint parameters clearly showed the reduction in swelling on ankle-joint diameter and recurring to the normal position in both doses of (50 and 200 mg/kg) in essential oil treated groups. Haematological parameters have shown the mild to moderate rise in WBC and ESR count due to release of IL-1 β (Patil et al., 2011). Other haematological parameters such as Hb and RBC count decreased due to abnormal storage of iron in synovial tissue and reticuloendothelial system (Suresh et al., 2012). Both the doses (50 and 200 mg/kg) of essential oil have revealed the normalization of WBC, Hb, ESR, RBC counts in the 21st day of protocol.

The chemical constituent, n-hexadecanoic acid is the major constituents present in essential oil results into anti-inflammatory effect by inhibition of inflammation mediators such as phospholipase A₂, prostaglandins E₂, IL-6, IL-1 β , TNF α , and nitric oxide synthase (Aparna et al., 2012; Guerrero et al., 2017; Godara et al., 2019; Joshua et al., 2020).

Researchers have also reported that the sesquiterpenes contents (cis- β -farnesene, β -caryophyllene, α -humulene β -caryophyllene oxide) also play an important role to inhibit the inflammatory mediators (Sharma et al., 2009). Sesquiterpenes (cis- β -farnesene) inhibit the prostaglandin and nitric oxide synthase and cure swelling (Afulous et al., 2013). Constituents, β -caryophyllene, α -humulene β -caryophyllene oxide present in oil have inhibit cytochrome P1A2, P3A/2B and cure inflammation (Gao et al., 2018; Chehregani et al., 2010; Bhargava et al., 1970; Wu et al., 2013; Rahman et al., 2016). In summary, the results suggest that the essential oil of *A. triplinervis* give effective results against antimicrobial and anti-inflammation activities.

Conclusions

The essential oil of *A. triplinervis* possess a total of 19 phytochemicals which includes 06 Sesquiterpene hydrocarbons, 06 Oxygenated sesquiterpenes, 03 fatty acids, 01 Fatty acids methyl esters, 01 diterpene, 01 monoterpene and 01 benzenoid. Its major components are n-hexadecanoic acid, cis- β -farnesene, phytone, tetradecanoic acid, elemol, caryophyllene oxide, α -humulene, δ -cadinene and β -caryophyllene. The essential oil has shown effective results against *S. aureus*, *E. coli* and *A. niger* at 400 ppm concentration. The essential oil exhibit significant anti-inflammatory ability to CFA-induced rat adjuvant arthritis. The oil has prevented the volume of paw oedema, ankle-joint diameter,

and haematological parameters in inflammatory rats. The results witnessed that the *A. triplinervis* essential oil at the 200 mg/kg dose showed effective results as compared to 50 mg/kg dose and positive control group.

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CONFLICTS OF INTEREST

The authors declare that they have read policy and guidelines of the journal and there is no conflict of interest.

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