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PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF ELAEOCARPUS TUBURCULATUS

Raju HV[1], Ganapathy S[2], Prasanna S Sutar[3*], Kishori P Sutar[4].

[1]Assistant Professor, Department of Pharmacognosy, Viveswarapura Institute of Pharmaceutical Sciences, Bangalore, Karnataka, India.

[2]Professor and Head of Department of Pharmacognosy, College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh.

[3]Assistant Professor, Department of Pharmacognosy, Maratha Mandal's College of Pharmacy, Belgaum, Karnataka, India.

[4]Lecturer, Department of Pharmaceutics, Rani Chennamma College of Pharmacy, Belgaum, Karnataka, India.

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***Correspondence for Author**
Dr. Prasanna S Sutar
Assistant Professor, Department of Pharmacognosy, Maratha Mandal's College of Pharmacy, Belgaum, Karnataka, India.

ABSTRACT

The present investigation revealed that the chloroform and methanolic extracts of *Elaeocarpus tuburculatus* bark have anti-inflammatory and antifungal activities. *Elaeocarpus* is a genus of tropical and subtropical evergreen trees and shrubs. About 60 percent of world's population uses traditional medicines for primary health care purpose. They are not only used just in rural areas of developing countries, but also in developed countries as well where modern medicines are predominantly used. India is the largest producer of medicinal herbs and is called the botanical garden of the world. Interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine.

KEYWORDS: *Elaeocarpus tuburculatus,* Methanolic extract, chloroform extract, anti-inflammatory activity, carrageenan, Ibuprofen.

INTRODUCTION

The use of plants as medicines goes back to early centuries. Certainly the great civilizations of the ancient Indians, Chinese and North Africans provided written evidence of man's

ingenuity in utilizing the plants for the treatment of wide variety of human diseases. It was not until the 19th century that man began to isolate the active principles of medicinal plants. One particular landmark was the discovery of Quinine from Cinchona bark by the French scientists Caventou and Pelletier. Such discoveries led to an interest in plants from the New World and expeditions scoured the almost impenetrable jungles and forests in the quest for new medicines. Traditional medicines are used by about 60 per cent of the world's population. These are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. While the traditional medicines are derived from medicinal plants, minerals, and organic matter, the herbal drugs are prepared from medicinal plants only.[1]

The present work was undertaken with an objective to study the pharmacological activities and chemical profile of *Elaeocarpus tuburculatus.* Many of the *Elaeocarpus* species are reported to be useful in indigenous system of medicine for a number of ailments such as treatment of inflammation, diabetic, malaria asthma, arthritis, cancer and liver diseases. Literature survey for *Elaeocarpus* species revealed that they are used for the treatment of inflammation. There is no scientific evidence that the barks of *Elaeocarpus tuburculatus* have been used for its anti-inflammatory activity.[2-4] Keeping this in view, the Chloroform and methanolic extracts of the barks of *Elaeocarpus tuburculatus* has carried out for anti-inflammatory activity.

MATERIALS

Elaeocarpus tuburculatus barks (1kg), Chloroform, Methanol (3 liters X 2 each), 1% Tween 80 solutions, Ibuprofen (10mg/kg).

All the solvents used were of Analytical grade. The materials used in this study are dried bark powder of *Elaeocarpus tuburculatus*, Chloroform, Methanol, Anesthetic ether, were obtained from S.D Fine Chemicals. Ibuprofen was procured from Vardhman Pharma distributors Pvt. Ltd. Bangalore.

ANIMALS USED

Wistar albino rats of either sex weighing between 200 – 250gms were used. Six rats were taken for each group. The rats were used after acclimatization to the laboratory environment. They were provided with food and water *ad libtum.*

METHODS

Elaeocarpus tuburculatus barks (1kg) were collected around the hilly area of Agumbe (Karnataka).[5] The same was authenticated by Regional Research Institute, Bangalore. Air dried drug was powdered in Willy mill and successively extracted with chloroform and methanol (3 liters x 2 each) and concentrated under vacuum to get corresponding residues (3 and 20 grams) respectively. Then the extracts were suspended in 1% Tween 80 solution and were used in three doses of 100, 300 and 500mg/kg body weight for Anti-inflammatory activity using Ibuprofen (10mg/kg) as reference standard by Carrageenan induced paw oedema method in rats.

PHYTOCHEMICAL EXAMINATION OF THE BARKS OF ELAEOCARPUS TUBURCULATUS

The barks of *Elaeocarpus tuburculatus* (2kg) were air dried, powdered in Willy mill and successively extracted with chloroform and methanol. The extracts were then concentrated under vacuum to get corresponding residues (10 and 40gms). These extracts were separately tested for identification of various chemical constituents like steroids, alkaloids, tannins, flavonoids, carbohydrates and glycosides.

To the Chloroform extract of *Elaeocarpus tuburculatus* add one to two drops of acetic anhydride. Also add one drop of concentrated sulphuric acid. The chloroform extract produced dark brown color, gave positive result for Lieberman Buchard reactions for Sterols (pink-blue-green) and tri-terpenoids and positive test for ferric chloride (olive green) and alkaloids.

The methanolic extract residue of *Elaeocarpus tuburculatus* was dark reddish in color, gave positive Lieberman Buchard reactions for sterols, tri-terpenoids, flavonoids and also alkaloids.[6-12]

Both Chloroform and Methanolic extracts were subjected for Thin layer chromatography showed same number of colored spots in chloroform and benzene (1:1) solvent system on spraying with 5% methanolic sulphuric acid followed by heating and same Rf values and hence both the extracts were mixed and separated for various chemical constituents by column chromatography over silica gel (100-200 mesh) and eluted with solvents like petroleum ether, benzene, chloroform and methanol and their mixtures in order of polarity,

the residue afforded seven compounds which were designated as ETB-01 ETB-02, ETB-03, ETB-04, ETB-05, ETB-06 and ETB-07.

Course of the Column chromatography of the chloroform and methanolic extracts of *Elaeocarpus tuburculatus* barks.

Weight of the extract: 50gm; Weight of silica gel: 900gm; Volume of each fraction: 250 ml.

Eluents	Fractions	Compounds
Pet ether	0-16	Waxy substance
Benzene: pet ether (10:90)	16-24	Waxy substance
Benzene: pet ether (20:80)	25-35	Waxy substance
Benzene: pet ether (30:70)	36-46	Intractable substance
Benzene: pet ether (40:60)	47-60	ETB-01**(β-Sitosterol)**
Benzene: pet ether (50:50)	61-75	Intractable substance
Benzene: pet ether (60:40)	76-84	ETB-02 **(Cucurbitacin D)**
Benzene: pet ether (70:30)	85-99	Intractable substance
Benzene: pet ether (80:20)	100-116	Intractable substance
Benzene: pet ether (90:10)	117-130	ETB-03 **(Cucurbitacin F)**
Benzene	131-140	Wax
Chloroform: benzene (10:90)	141-155	ETB-04 **(Kaempferol)**
Chloroform: benzene (20:80)	156-166	Intractable substance
Chloroform: benzene (30:70)	167-177	ETB-05 **(Quercetin)**
Chloroform: benzene (40:60)	178-200	ETB-06 **(Rutin)**
Chloroform: benzene (50:50)	201-210	Intractable substance
Chloroform: benzene (60:40)	211-221	ETB-07 **(Scopoletin)**
Chloroform: benzene (70:30)	222-235	Intractable substance
Chloroform: benzene (80:20)	236-249	Intractable substance
Chloroform: benzene (90:10)	250-260	Intractable substance
Chloroform	261-272	Intractable substance
Methanol: Chloroform (1:99)	273-280	Intractable substance
Methanol: Chloroform (2:98)	281-290	Intractable substance
Methanol: Chloroform (3:97)	291-300	Intractable substance
Methanol: Chloroform (4:96)	301-305	Intractable substance
Methanol: Chloroform (5:95)	306-310	Intractable substance

EXAMINATION OF THE ISOLATED COMPOUNDS

ETB-01: (β-Sitosterol)

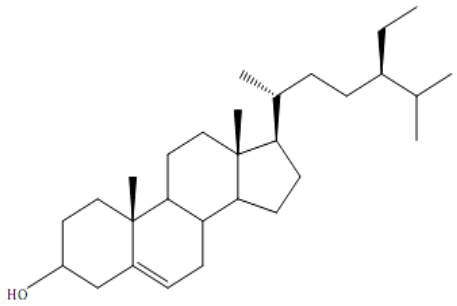
The fractions (47-60) were mixed as they were similar on TLC (Benzene: petroleum ether) (1:1) and were recrystallized from petroleum ether as colorless needles.

Melting point 134-140^0 C. Rf value 0.28.

It gave the following color reactions.

Salkowski test	: Deep red color layer.
Liebermann-Burchard Test	: Pink-violet-green.
Concentrated Sulphuric acid	: Yellow color.
$[\alpha]^{30}$ D-36^0	: (C,1.01 in $CHcl_3$).
Found	: C, 83.7%; H, 12.7%; O, 3.0%.
IR V_{max} cm^{-1} (KBr)	: 3440, 2970, 2880, 2050, 1470, 1385 and 1055.

The above data coincided well with that of β-sitosterol and the identity was confirmed by comparison with an authentic sample through melting point and Co-TLC.



(β-Sitosterol)

ETB – 02 (Cucurbitacin D)

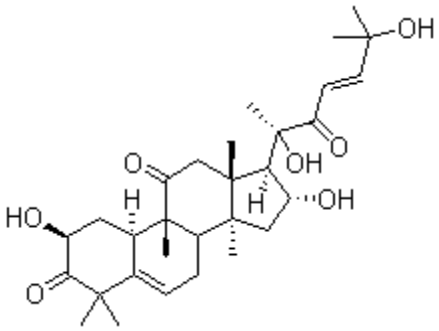
The fractions (76-84) were mixed as they were similar on TLC (Benzene: Chloroform) (1:1) and were crystallized from petroleum ether as white powder.

Melting point 151-153 ^{0}C. Rf value 0.49.

It gave the following color reactions.

Liebermann-Burchard Test	: Pinkish brown
Salkowski test	: Deep red color layer.
$[\alpha]^{30}$ D+48^0	: ($CHCl_3$,C,1.01)
λ_{max} (MeOH) nm	: 230 nm
IR V_{max} (KBR)	: 1710, 3600, 3470 cm^{-1}
^{1}H NMR ($CDCl_3$ 400 MHz)	: δ 0.69,0.108,1.30,1.37 and 1.43 assigned to tertiary methyl groups and another singlet at δ 2.20 (3H) was due to methyl ketone.

The above data coincided well with that of Cucurbitacin D and the identity was confirmed by comparison with literature data by melting point and Co-TLC.



Cucurbitacin D

ETB-03: (Cucurbitacin-F)

The fractions (117-30) were mixed as they were similar on TLC (Benzene: petroleum ether) (1:1) and were crystallized from petroleum ether as white powder.

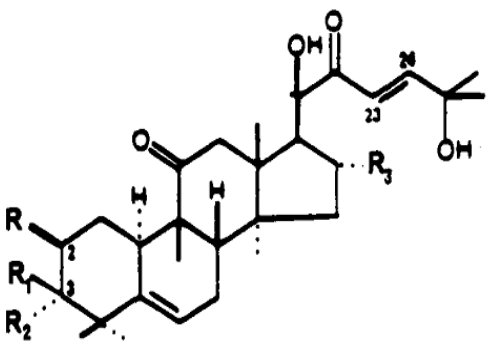
Melting point 249-253^0 C. Rf value 0.52.

It gave the following color reactions.

Salkowski test	: Deep red color layer.
Liebermann-Burchard Test	: Pinkish brown.
[α] ^{30} D-59 ^{0}	: (C, 0.7 in chloroform).
UV λ_{max} (MeOH)	: 234, 267 nm.

: 3437, 2973, 2952, 2919, 2879, 2850, 1687, 1633, 1390,1375, 1366, 1285, 1167, 1090, 1056, and 103.

The above data coincided well with that of Cucurbitacin-F and the identity was confirmed by comparison with a literature data by melting point and Co-TLC.



R	R_1	R_2	R_3
OH	H	OH	OH

ETB-04: (Kaempferol)

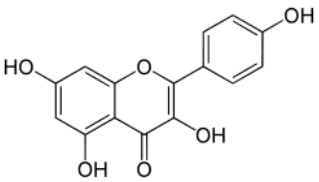
The fractions (141-155) were mixed as they were similar on TLC (Benzene: Chloroform) (1:1) and were crystallized from chloroform as yellow crystalline needles.

Melting point 278-279 ^{0}C. Rf value 0.68.

It gave the following color reactions.

Ferric chloride	: Green colour.
Lead acetate	: Orange precipitate.
Shinoda	: Magenta.
Wilson's boric and citric acid	: Yellow.
Found	: C, 62.8; H, 3.64%.
$C_{15}H_{10}O_6$ requires	: C, 62.9, H, 3.49%.
λ_{max} (MeOH) nm	: 253 h, 265, 294 s h, 332 s h, 365.
+Nao AC	: 275, 385.
+$Alcl_3$/Hcl	: 275, 420.
+NaoAc / H_3BO_3	: 257,420.

The above data coincided with that of kaempferol and its further identity was confirmed by comparison with an authentic sample through melting point and co-TLC.



Kaempferol

ETB-05: (Quercetin)

The fractions 167-177 were mixed as they were similar in Paper chromatography,

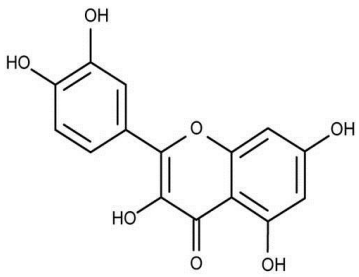
TLC (Benzene: Chloroform) (3:7) and on crystallization from chloroform as a yellow crystalline solid was obtained.

Melting point 312-314^{0}C. Rf value 0.34.

It gave the following color reactions.

Shinoda test	: Magenta colour.
Neutral lead acetate	: Bright orange precipitate.
Ferric chloride	: Green colour.
Aqueous sodium hydroxide	: Deep yellow colorsolution.
Wilson's boric –citric reagent	: Yellow solution.
$[\alpha]^{30}$ D+28 ^{0}	: (C, 0.27 in methanol).
Found	: C, 59.6%; H.3.33%, O 37.06%.
$C_{15}H_{10}O_7$ requires	: C, 59.6 %:H.3.3%.
UV λ_{max} nm (MeOH)	: 257,267 s h, 301 s h 370.
+Nao Me	: 247s h, 321(dec).
+$Alcl_3$/Hcl	: 265,301 s h, 359,425.
+NaoAc	: 275,329,390.
+NaoAc / H_3BO_3	: 257 s h, 274,329,385.

The above data coincided with that of Quercetin and identity was confirmed by comparison with an authentic sample through melting point and co-TLC.



Quercetin

ETB-06: (Rutin)

The fractions (178-200) were mixed as they were similar on TLC (Benzene: Chloroform) (2:8) and were crystallized from chloroform as a yellow crystalline needles.

Melting point 208-210 ^{0}C. Rf value 0.45.

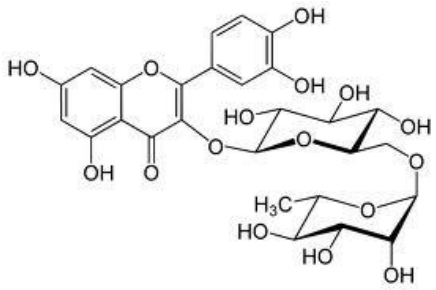
It gave the following color reactions.

Ferric chloride	: Green colour.

Lead acetate	: Orange precipitate.
Shinoda	: Magenta.
Wilson's boric and citric acid	: Yellow.
$[\alpha]^{23}D+13.82^{0}$	: (C, 0.27 in methanol).
Found	: C 53.12%,H 4.95%,O 41.93%.
λ_{max} (MeOH) nm	: 371,301 s.h,268 s.h,255 232 nm.

^{1}H NMR (500 MHz; MeOH-d_3): δ 7.66 (1H, *d*, *J* = 2.2 Hz, H-2′), 7.63 (1H, *dd*, *J* = 8.4, 2.2 Hz, H-6′), 6.85 (1H, *d*, *J* = 8.4 Hz, H-5), 6.28 (1H, *d*, *J* = 2.0 Hz, H-8), 6.11 (1H, *d*, *J* = 2.0 Hz, H-6), 5.05 (1H, *d*, *J* = 7.8 Hz, H-1), 4.52 (1H, *d*, *J* = 1.6 Hz, H-1), 3.83–3.35 (10H, H-2–H-6 and H-2–H-5), 1.13 (3H, *d*, *J* = 6.2 Hz, H-6).

The above data were identical to those in literature of Rutin.[13] and the further identity was made by comparison with an authentic sample through co-TLC and melting point.



Rutin

ETB-07 :(Scopoletin)

The fractions (211-221) were mixed as they were similar on TLC (Methanol: Chloroform) (1:9) were recrystallized from Chloroform: Benzene as yellow crystalline needles.

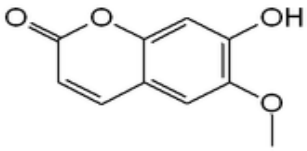
Melting point 208-210 ^{0}C.

Rf value 0.21.

It gave the following color reaction.

Ferric chloride	: Green color.
λ_{max} (MeOH) nm	: 230,254,260,298,346 nm.
IR V_{max} cm^{-1} (KBr)	: 3396 (OH), 1713(CO δ-lactone),1611(CH=CH 1565 and 1514 (aromatic benzene ring).

The 1H NMR spectra of it showed two doublets with coupling constant of 9.15 Hz at δ 6.27 and 7.60 ppm, which were assigned as H-3. The IR spectrum of ETB-07 showed absorption bands at 3396 cm^{-1}, due to a hydroxyl group, 1713 cm-1, corresponding to carbonyl group (δ-lactone), 1611 cm-1, corresponding to CH=CH group; 1565 and 1514 cm-1, corresponding to aromatic benzene ring. The ^{1}H NMR spectrum of ETB-07 showed a methoxyl group singlet at δ 3.94 ppm and two aromatic proton singlets at δ 6.91 and 6.84 ppm., indicating that the isolated compound had a scopoletin and the further identity was made by comparison with an authentic sample through melting point and co-TLC.



Scopoletin

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF ELAEOCARPUS TUBURCULATUS

In this method, 0.1 ml of 1% Carrageenan in 0.9% saline was injected into sub-plantar area of right hind paw of rat subcutaneously to induce oedema. Paw thickness was measured before Carrageenan was injected. At hourly intervals up to 5 hrs thickness of paw was measured using plethysmometer.

Procedure

The Albino rats weighing between 200-250g were selected and housed in laboratory temperature. They are divided into 06 groups each group consisting of six animals respectively. Group I was given Normal saline (5ml/kg, p.o) which served as Control group, Group II was administered with Ibuprofen (10 mg/kg) which served as Standard group and remaining groups were administered with 100, 300 and 500 mgs/kg body weight of methanolic and chloroform extracts of *Elaeocarpus tuburculatus*.

0.1ml of carragenan (1%) in Normal saline (0.9%) was injected into sub-plantar area of right hind paw for all the groups. All the drugs were given orally one hour prior to carragenan injection. The paw volume of rats was measured at 0 hr and hourly intervals up to 5^{th} hour

after the administration of carragenan, by volume displacement method, using plethysmometer.[14-17]

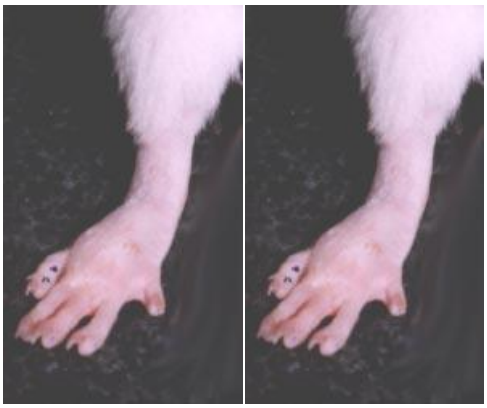
The percentage inhibition of oedema was calculated for each group with respect to the vehicle treated control group by using the formula mentioned below.

% Inhibition of oedema = 100[1-(Vt/Vc)].

Where Vt and Vc are volume of carragenan-injected paws of drug treated group and control group respectively.

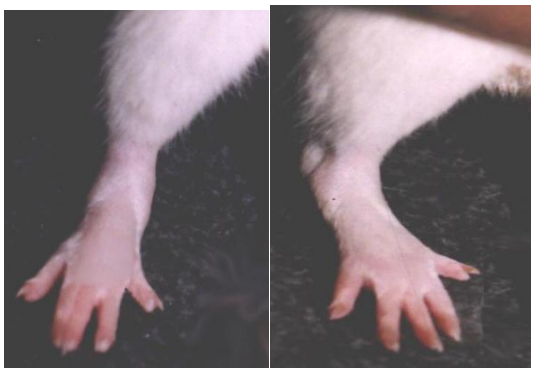
The results were tabulated and data is statistically analyzed using one-way ANOVA followed by Dunnet's test & results were presented in the Table no 1 and 2.

Photograph showing rat paw oedema of control, standard, test groups before and after treatment



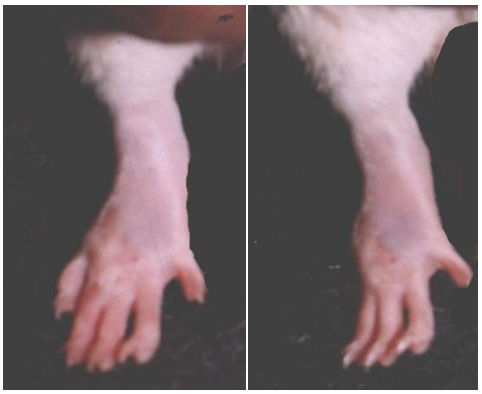
Control group

Photograph showing paw oedema at 0 hr and also observed oedema at 5th hr



Standard Group Ibuprofentreated10mg/kg)

Photograph showing paw oedema at 0 hr and decrease in paw oedema is observed at 5th hr.



Test group

Methonolic extract of *Elaeocarpus tuburculatus* 300mg /kg Photograph showing paw oedema at 0 hr and decrease in oedema observed at 5th hr.

Table 1-Anti inflammatory activity of *Elaeocarpus tuburculatus* bark extracts on carragenan induced paw oedema in rats.

Sl No	Treatment	Dose	Volume of mercury displaced in ml at various time intervals in hours.					
			0	1	2	3	4	5
1	Control	0.1ml 1% W/V	0.40 ± 0.025	0.50± 0.057	0.63± 0.057	0.66± 0.057	0.68± 0.057	0.70± 0.058
2	Ibuprofen	10 mg/ kg	0.26± 0.005	0.30±*** 0.005	0.34±*** 0.002	0.26±*** 0.003	0.26±*** 0.003	0.20±*** 0.04
3	Chloroform Ext. of *E.T.*	100 mg/ kg	0.38± 0.01	0.49±^{NS} 0.01	0.60±^{NS} 0.02	0.62±^{NS} 0.01	0.63±^{NS} 0.01	0.63±^{NS} 0.01
4	Chloroform Ext. of E.T.	300 mg/ kg	0.39± 0.01	0.47±^{NS} 0.03	0.59±^{NS} 0.05	0.60±^{NS} 0.04	0.60±^{NS} 0.03	0.62±^{NS} 0.02
5	Chloroform Ext of *E.T*	500 mg/ kg	0.40± 0.01	0.48±^{NS} 0.01	0.59±^{NS} 0.02	0.60±^{NS} 0.01	0.60±^{NS} 0.03	0.62±^{NS} 0.04
6	Methanolic Ext of *E.T*	100 mg/ kg	0.35± 0.01	0.38±** 0.02	0.40±*** 0.05	0.30±*** 0.03	0.31±*** 0.01	0.29±*** 0.04
7	Methanolic Ext of *E.T*	300 mg/ kg	0.32± 0.01	0.42±** 0.02	0.48±** 0.05	0.42±** 0.0 3	0.42±** 0.01	0.40±*** 0.04
8	Methanolic Ext of *E.T*	500 mg/kg	0.39± 0.01	0.48±^{NS} 0.02	0.60±^{NS} 0.05	0.58±^{NS} 0.03	0.58±* 0.01	0.56±* 0.04

Significance of difference in control and extracts treated groups were determined by one – way analysis of variance (ANOVA) *** P<0.001 **P<0.01, * P<0.05 are significant and NS P> 0.05, are not significant. All values are means of individual data obtained from six rats (n=6).

Histogram showing the anti-inflammatory activity of various extracts of *Elaeocarpus tuburculatus.*

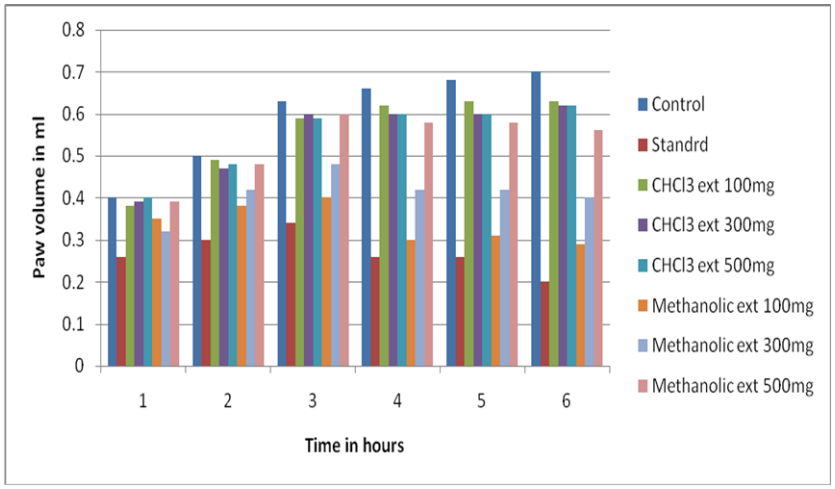


Table 2-Percentage inhibition of *Elaeocarpus tuburculatus* bark extracts on carragenan induced Paw edema in rats.

Sl no	Treatment	Dose	% Inhibition of paw volume at various time intervals					
			0	1	2	3	4	5
1	Control	0.1ml1% W/V	00	00	00	00	00	00
2	Ibuprofen	10 mg/ kg	00	40	46.03	60.60	61.76	71.42
3	Methanolic extract of *Elaeocarpus tuburculatus*	100 mg/ kg	00	24	36.5	54.14	54.41	58.57
4	Methanolic extract of *Elaeocarpus tuburculatus*	300 mg/ kg	00	20	23.8	36.36	38.2	42.85
5	Methanolic extract of *Elaeocarpus tuburculatus*	500 mg/ kg	00	4	4.7	12.12	14.7	20
6	Chloroform extract. of *Elaeocarpus tuburculatus*	100 mg/ kg	00	2	6.3	6.06	7.3	10
7	Chloroform extract of *Elaeocarpus tuburculatus*	300 mg/ kg	00	6	6.3	9.1	11	11.4
8	Chloroform extract of *Elaeocarpus tuburculatus*	500 mg/ kg	00	4	6.3	9	11	11.4

RESULTS AND DISCUSSION

The anti-inflammatory activity of chloroform and methanolic extracts of the barks of *Elaeocarpus tuburculatus* against carragenan induced paw oedema has been shown in table 1 and 2. Chloroform extract showed mild activity, methanolic extract showed significant activity to that of reference standard Ibuprofen and the activity was dosage dependent.

CONCLUSION

It was demonstrated by animal model studies that methanolic and chloroform extracts of *Elaeocarpus tuburculatus* reduced inflammation at various doses of different body weight. Further this study indicates chloroform and methanolic extracts showed significant anti-inflammatory activity comparable to that of reference standard.

ACKNOWLEDGEMENT

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