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ISOLATION OF CHEMICAL CONSTITUENTS FROM *MIMUSOPS ELENGI* BARK AND EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

The ethanolic extract of *Mimusops elengi* bark used for the isolation of chemical constituents and evaluation of anti-inflammatory activity. To identify the anti-inflammatory component of this drug, we adopted an activity directed fractionation approach. The active fraction of the ethanolic extract of *Mimusops elengi bark* was subjected to silica gel and column chromatography to yield a single compound and was identified as triterpenoid. The triterpenoid is β-amyrincaprylate and ethanolic extract was tested for anti-inflammatory effect by using carrageenan induced paw oedema served as acute models and formation granulation tissues by cotton pellets served as a chronic model in rats. The effect was compared with Indomethacin used as standard drug. Pretreatment with ethanolic extract of *Mimusops elengi* bark (MEB) was administered orally 200, 400mg/kg(p.o) and isolated compound (5mg/kg) exhibited significant anti-inflammatory activity in acute and chronic models. These results indicate that ethanolic extract and β-amyrincaprylate contributes to the anti-inflammatory action of *Mimusops elengi bark.*

Key words: *Mimusops elengi;* isolation; anti-inflammatory.

INTRODUCTION

Mimusops elengi belongs to the family sapotaceae and medium-sized evergreen tree found in tropical forests in South Asia, Southeast Asia, and Northern Australia. Commercially known as spenish cherry [1]. It is indigenous to tropical countries and is considered as an important folk medicine. In the traditional system of medicine, The bark, flowers, fruits and seeds are astringent, cooling, anthelmintic, tonic, and febrifuge. It is mainly used in dental ailments like bleeding gums, pyorrhea, dental caries and loose teeth. The major components have been identified in the *Mimusops elengi* plant which includes penta hydroxyl flavones,triterpenoid, saponins and essential oil [2]. TheThe current study was undertaken to isolate the β-amyrincaprylate and examined by anti-inflammatory activity of ethanolic extract of *Mimusops elengi* bark by carrageenan-induced paw odema and cotton pellet granuloma model [3].

MATERIALS AND METHODS

Instruments

Melting points were determined on a scientific model of melting point apparatus. A UV spectrum was recorded on a Double Beam UV-VIS Spectrophotometer and IR spectra on a Shimadzu 8900 FT-IR spectrometer. The NMR spectra (δppm, *j*, in Hz) were recorded in $CDCL_3$ using AM-500 spectrometer (500MHz) with tetra methylsilane (TMS) as an internal standard. A mass spectrum was recorded on QTOF micro mass UK electron spray Ionization mass spectrometer sample purity was checked by TLC (silica gel, precoated plates Merck, PF_{254} 10×5 cm, 0.25mm). The biochemical parameters of anti-inflammatory the rat paw odema were done by Plethysmometer.

Plant material

The *Mimusops elengi* stem barks (1.2kg) were collected in the month of July 2010, from Vandakottai, pudukottai District, Tamil Nadu, India. The plant material was taxonomically identified by Dr.D.Stephen Department of Botany, The American College, Madurai, Tamil Nadu, India. The bark of the plant materials were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no 40 and stored in an airtight container for further use.

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Preparation of extract

The dried powdered barks (1kg) were extracted with 80% ethanol (2.5 liter) in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semisolid mass was obtained (yield 14.2%). The ethanolic extract was concentrated to dryness in *vaccuo*at 35°C. Active constituents from the dried extract were separated by column chromatography with different solvent ratio.

Preliminary Phytochemical Test

The extract was subjected to preliminary screening for various active phytochemical constituents such as, steroids, triterpenoid, and flavonoids

Isolation

Dried barks (1kg dry wt) of *Mimusops elengi*was extracted with 80% ethanol in Soxhlet apparatus after extraction solvent was distilled and concentrated to dryness in *vaccuo* at 35°C, with the concentrate (20g) was then partitioned between water and ethyl acetate with the aqueous layer was basified with ammonium hydroxide (pH 9) and extracted repeatedly with chloroform. The combined chloroform layer was concentrated to dryness in *vaccuo* with the concentrate (12g) separated by column chromatography in to various fractions of hexane-chloroform(95:05) increasing polarity gradient elution. Thehexane-$CHCl_3$ (80:20)fraction was concentrated to dryness in *vaccuo* at 35°C. The combined fractions were identified by TLC and recrystallized with methanol. These compound were identified as triterpenoid respectively by chemical test and spectral datas.

Vehicle

The extract and isolated compound at the different doses of 200, 400mg/kg and 5mg/kg was suspended in aqueous Tween 80 solution (1%) and Indomethacin (10mg/kg Torrent, Bombay) in saline were used for the present study.

Animals and Sample Preparation

Adult Wister albino rats of either sex weighing between 120-150gm were purchased from Experimental animal house BBS College of Pharmacy in Greater Noida, India. Animals were housed at a temperature 25±2°C and relatively humidity of 45-55% a 12:12 light; day cycle was followed. All animal were fed with standard pellet diet supplied by Amrut-rat mice feed, Mumbai. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and werein accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment Government of India. The animal Ethical Committee experimental protocol No. is1346/C/10/CPCSEA.

The test samples (*i.e.* EtOH extract and isolated compound) were first dissolved in 10% Tween 80 and diluted with 1% saline before being orally administered. The same volume of solvent was administered to control rats. Extracts were administered at 200, 400mg/kg once a day week and isolated compound also given by orally administered at 5mg/kg for the same period.

Acute Toxicity Studies

Acute toxicity study was performed for the extracts ascertain safe dose by acute oral toxic class method of organization of Economic Co-operation and Development as per 423 guidelines (OECD) 12. A single administration of starting dose of 2000mg/kg body weight/p.o of the MEB was administered to 3 female mice and observed for 3 days. There was no considerable change in body weight before and after treatment and no sign of toxicity were observed. When the experimental was repeated again with same dose level 2000mg/kg body weight/p.o of the MEB for 7 more days and observed for fourteen days no change was observed from the experiments.

Anti-inflammatory activity

Carrageenan-Induced Paw Oedema

The rats were divided into 5 groups (n=3). The extract, isolated compound and standard drug used for this study were prepared in the same manner as mentioned earlier. Animals were deprived of food and water for 18 hours before the experiment. They were marked and numbered for identification. Paw oedema was induced by sub plantar injection into the rat right hind paw of 0.1ml sterile saline containing 1% carrageenan (control group). A group of rats were treated with MEB extract, isolated compound and standard drugs were administered orally concomitantly with carrageenan injection. Control group of animals received the same volume of vehicle instead of the tested agents. The volume of the paw was measured by a Plethysmometer immediately after the injection as previously described. The increase in paw volume was taken as oedema volume. The percentage of inhibition of inflammation was calculated for comparison. The ratio of the anti-inflammatory effect of MEB extract and isolated compound was calculated by the following equation anti-inflammatory activity (%) (1-D/C)× 100, where D represents the percentage difference in paw volume after extract and compound was administered to the rats, and C represents the percentage difference of volume in the control groups.

Cotton Pellet Granuloma Model

In cotton pellet the animals were divided into five groups as described in the carrageenan induced paw oedema model. The animals were anaesthetized with Diethyl ether. The back skin was shaved and disinfected with 70% ethanol. An incision was made in the lumbar region. Subcutaneous tunnels were formed by a blunted forceps and a sterilized, pre weighed cotton pellet was placed on both sides in the scapular region. The animals were treated with Indomethacin, extract and isolated compound of *Mimusops elengi* bark for 7 days. Then the pellets were dissected out and dried until the weight remains constant. The net dry weights, i.e. after subtracting the weight of the cotton pellet were determined.

Statistical Analysis

All the results were expressed as mean ± standard error (S.E.M). Data were analyzed using one-way ANOVA followed by Dunnett's*t*-test. $p<0.05$ were considered as statistically significant.

RESULT

Preliminary Chemical Tests

The extract showed positive test for steroids, flavonoid and triterpenoid

Isolation of β-amyrincaprylate

The isolated compound is white color powder, is an β-amyrincaprylate (proved by Libermann-burchard chemical test), mp90-93°C; UV λ_{max} MeOH nm (log ε): 226.4 IR λ_{max} (KBr) cm^{-1}2920,2850,1735,1637,1461,1377,1080,885 and EI-MS *m/z* **(**rel.int.%**)** 552**,**408(144), base peak 333,218and 190 (333-143) (calcd. For C_{38} O_2 H_{64}). ^{1}H-NMR and ^{13}C-NMR and DEPT-135see the Table-1.and Table-2

Anti-inflammatory activity

Carrageenan-Induced Paw Oedema

The anti-inflammatory effect of the MEB extract and isolated compound and standard on the carrageenan-induced hind paw oedema mode. Standard drug Indomethacin (10mg/kg,p.o) produced a significant reduction in paw oedema volume (69.44%). Treatment with MEB extract an isolated compound reduced the carrageenan-induced paw oedema volume in a dose dependant manner. MEB extract and isolated compound showed maximum ($p<0.01$) inhibition of 59.5%, and 47.2% at the dose of 400mg/kg and 5mg/kg after 4 h of treatment of carrageenan-induced paw oedema. Similarly the inhibition were 55.5% ($p<0.01$) at the dose of 200mg/kg, p.o in pretreated rats.

Table 1. ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$, ^{1}H: 300 MHz; ^{13}C: 75MHz) spectral data of isolated compound

C/H#	δ_H (MHz)	δ_C
1	-	38.077
2	1.33	27.34
3	4.56	77.39
4	-	39.53
5	-	54.07
6	1.253	17.94
7	1.322	32.67
8	-	39.89
9	2.375	47.8
10	-	37.03
11	-	25.05
12	4.943	109.34
13	-	150.7
14	-	40.68
15	1.175	26.5
16	1.679	27.02
17	-	31.86
18	2.489	47.87
19	1.253	47.25
20	-	29.97
21	1.253	34.07
22	1.253	36.79
23	0.850	29.30
24	0.850	15.71
25	1.139	15.71
26	0.929	15.907
27	1.332	27.02
28	0.953	29.30
29	0.929	33.4
30	0.929	22.63
1'	-	161.0
2'	2.155	34.07
3'	1.679	25.04
4'	1.175	29.64
5'	1.175	29.738
6'	1.175	31.86
7'	1.175	22.632
8'	0.874	14.07

Table 2. DEPT 135 SPECTRUM OF ISOLATED COMPOUND

SIGNAL ASSIGNMENT	CH SIGNALS	CH_2SIGNALS	CH_3 SIGNALS
5CH	54.76	-	-
9CH	47.62	-	-
12CH	109.31	-	-
18CH	47.82	-	-
$1CH_2$	-	-	-
$2CH_2$	27.28	38.01	-
$6CH_2$	-	-	-
$7CH_2$	33.42	17.90	-
$11CH_2$	-	-	-
$15CH_2$	-	25.01	-
$16CH_2$	-	27.28	-
$19CH_2$	-	27.28	-
$21CH_2$	-	48.10	-
$22CH_2$	-	33.42	-
$2'CH_2$	-	35.38	-
$3'CH_2$	-	34.04	-
$4'CH_2$	-	25.01	-
$5'CH_2$	- 29.65	29.26	-
$6'CH_2$	-	-	-
$7'CH_2$	-	31.86	-
$23CH_3$	-	22.63	29.60
$.24CH_3$	-	-	19.52
$25CH_3$	-	-	19.52
$26CH_3$	-	-	19.57
$27CH_3$	-	-	27.31
$28CH_3$	-	-	29.60
$29CH_3$	-	-	34.04
$30CH_3$	-	-	22.60
$8'CH_3$	-	-	14.04
		-	

Table 3. Effect of ethanolic extract and isolated compound of *Mimusops elengi* on Carrageenan induced paw oedema in rats

Groups	Dose	paw oedema (mm)			Inhibition (%)	
		0 hr	2 h	4 h	2 h	4h
Vehicle		2.2 ± 0.01	4.0 ± 0.02	3.6 ± 0.01	-	-
MEB Extract	200 mg/kg	1.6 ± 0.03	2.3 ± 0.03*	1.6 ± 0.01	42.5	55.55
MEB Extract	400 mg/kg	1.4 ± 0.02	1.7 ± 0.011	1.6 ± 0.05**	56.4	59.5
Indomethacin (STD)	10 mg/kg	1.2 ± 0.00	1.3 ± 0.01**	1.1 ± 0.01**	65	69.44
ISC-	5mg/kg	1.5± 0.01	1.8± 0.01*	1.9 ± 0.01**	42.6	47.2

n=3 per group, Values are mean ± SEM **p<0.01, compared with control.

Table 4. Effect of ethanolic extract and isolated compound of *Mimusops elengi* on cotton pellet granuloma in rats

Group	Weight of Granuloma (mg)		% inhibition	
	Wet weight	Dry weight	For wet weight	For dry weight
Control	147 ± 3.22	49.33 ± 2.19	-	-
Indomethacin 10mg/kg	68.66 ± 3.18	23 ± 2.52	53.29%	53.37%
MEB Extract-200mg/kg	92.67 ± 2.33	37 ± 2.65	42.4%	36.8%
MEB Extract-400mg/kg	73 ± 4.16	27.67 ± 2.33	50.2%	44.6%
ISC 5mg/kg	91.33 ± 2.33	34.33 ± 0.88	39.8%	35.4%

n=3 per group, Values are mean ± SEM **p<0.01, compared with control.

Fig 1.Structure of isolated compound is β-amyrincaprylate

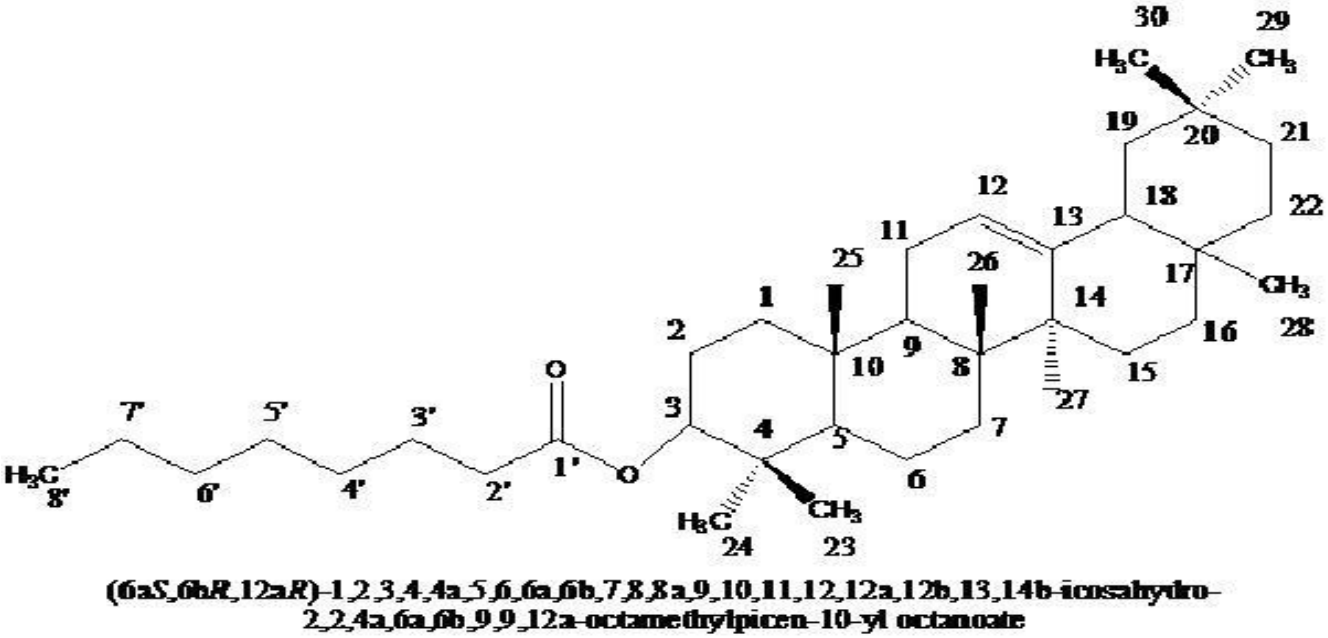
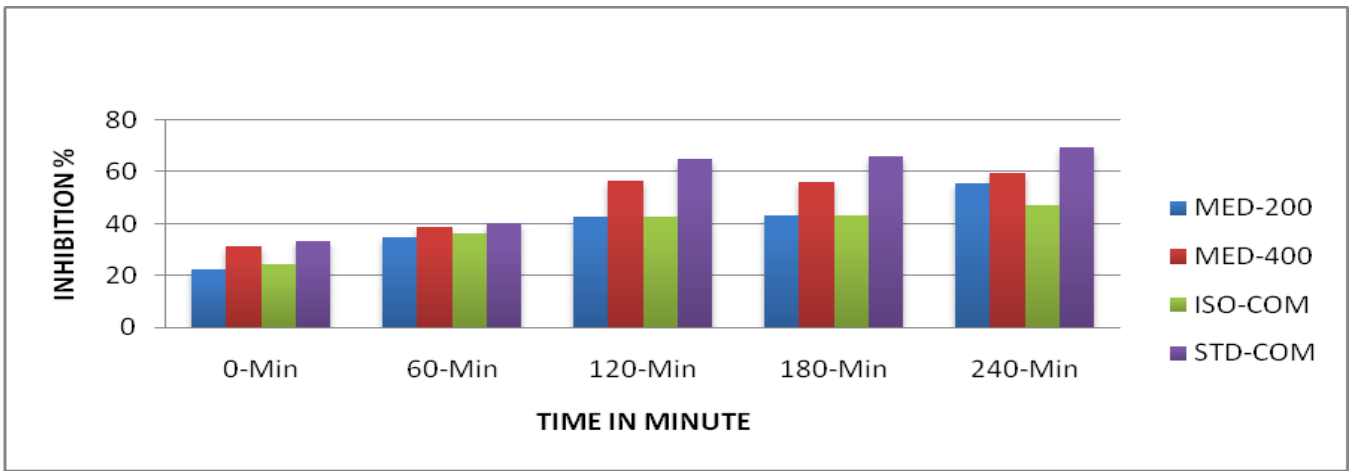


Fig 2. Effect of ethanolic extract and isolated compound of *Mimusops elengi* on Carrageenan induced paw oedema in rats



Cotton Pellet Granuloma Model

The effect of MEB extract and isolated compound on cotton pellet induced granuloma in rats. In this the mean weight of the cotton pellets was determiner treatment with the MEB extract and isolated compound significantly decreased the granuloma weight 36.8%, 44.6% and 35.4% at the dose level of 200, 400mg/kg and 5mg/kg, p.o respectively. Similarly 53.3% decreased was found in Indomethacin (10mg/kg, p.o) treated rats.

DISCUSSION AND CONCLUSION

The shade dried stem bark of *Mimusops elengi* (sapotaceae) was extracted with 80% ethanol. Thus the phytochemical investigation shows the presence of steroids, flavonoid and triterpenoid compounds. The residue of the ethanolic extract on column chromatography yielded the isolated compound. In column chromatography a single compound was isolated in the elution of (n-hexane 80%:$CHCl_3$20 %). The isolated compound yellow amorphous powder. The isolated compound gave positive test for triterpenoid.

β-amyrincaprylate was obtained as a white colour powder. Based on the molecular ion peak EI-MS at *m/z*552 suggested its molecular formula as$C_{38}H_{62}O_2$. The IR spectrum of 1 showed characteristic absorptions for ester (1735 cm^{-1}) group , CH stretching (2920,2850 cm^{-1}),CH bending(1377 cm^{-1})[4]. The UV spectrum showed absorption at nm (log ε): 226.4 suggestive of a triterpenoid skeleton [5]. The ^{1}H-NMR spectrum displayed a methyl group by a signal at δppm 0.850(23-H), 0.850(24-H) which has connected carbon at δ ppm 39.53(C-4) in the HMQC plot and presence of ester carbon at δ 161.8 (C-3) in the spectrum. Presence of methyl group was evident from a signal at δ (30H, 29 H) which had its connectivity with a carbon at δ 29.97 (C-20) in the spectrum. The

assignments of ^{1}H and ^{13}C-NMR shifts are comparing well with the values of related partial structures reported in literature [6-8].Fragmentation pattern in the mass similar to those of the Δ_{12}-oleane series of triterpinoid molecular ion peak at m/z552 underwent loss at 144(capyrlic acid) mass unit to give a peak at m/z 408 .while the peak at 333 and 218 (base peak) were due to loss of retro-diels alder fragmentation. The peak of 190 (333-143) was due to loss of an ester moiety from the left half showing that the ester grouping is at the C-3 position. These data led to elucidate the structure of β-amyrincaprylate [9,10].

The anti-inflammatory effects of ethanolic extract and isolated compound of MEB in experimental animal models. The potential of the MEB for its anti-inflammatory effect and short term toxicity was investigated. The effect of MEB ethanolic extract and isolated compound at the test dose of 200mg, 400mg/kg and 5mg/kg showed significant anti-inflammatory activity. Significant anti-inflammatory was observed in carrageenan-induced oedema and also the chronic model.

It is well known that carrageenan induced paw oedema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role while in second phase (2-4 h after carrageenan injection) Kinin and prostaglandins are involved [11]. Our results revealed that administration of extract and isolated β-amyrincaprylate compound inhibited the oedema starting from the first hour and during all phases of inflammation. This is probably inhibition of different aspects and chemical mediators of inflammation. In cotton pellet induced grauloma the extract and isolated β-amyrincaprylate fraction produced significant anti-inflammatory activity at the dose of 200, 400mg/kg and 5mg/kg. The literature survey of plant reported the presence of β-amyrincaprylateand phytochemical screening of ethanolic extract revealed that ethanolic extract of leaves of *Mimusops elengi* contains various classes of phytoconstituents such as flavonoids, sterols and triterpenoids. Several triterpenoids isolated from the medicinal plant have been discovered to possess significant anti-inflammatory activity [12].

This study confirmed that ethanolic extract and isolated triterpenoids fraction from ethanolic extract of bark of *Mimusops elengi*are responsible for its anti-inflammatory activity and the effects observed are attributing due to the presence of β-amyrincaprylatein the plant. The toxicity studies of the plant suggest that it has reasonable safety margin justifying its wide application in various communities and lack of any reported side effects with traditional use of this plant.

In conclusion the result of the study supports the traditional use of this plant in some painful inflammatory conditions. Further studies are currently in fact underway to characterize the active principles responsible for its anti-inflammatory activity.

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