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Physico-chemical and pharmacological characteristics of *Euphorbia rothiana* seed oil

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ABSTRACT

The minor seeds of Euphorbia rothiana plant belonging to the family Euphorbiaceae have been extracted with petroleum ether (40-60^{o}C). The seed oil obtained was evaluated for their physico-chemical characteristics as per standards of American oil chemical society (AOCS) procedures and analyzed by IR, GLC, NMR. The oil contains the saturated and unsaturated fatty acid viz. major quanties of linolenic rich (60.2%). The seed oil is nontoxic which exhibited significant anti-inflammatory (55.94%) and analgesic (66.20%) activities as compared with control and standard drug aspirin (91.10%) respectively.

Keywords: Seed oil, *Euphorbia rothiana*, physico-chemical, anti-inflammatory activity, analgesic activity.

INTRODUCTION

Inflammation is a defensive but exaggerated local tissue reaction in response to exogenous or endogenous insult. A large number of non-steroidal anti-inflammatory drugs (NSAIDs) are available clinically to treat inflammatory disorders. The most important mechanism of anti-inflammatory action of NSAIDs is considered to be primarily by inhibition of prostaglandin synthesis. The principal side effects associated with chronic use of non selective NSAIDs are gastrointestinal irritation, bleeding and formation of life threatening gastrointestinal ulcers [1-2].

Therefore, there is a need to develop new compounds with no side effects. Literature survey revealed that the seeds of *Euphorbia rothiana* is very bitter in taste, and possess excellent biological activities, the fruits are often laxative, strongly emetic and cathartic [3]. The seeds of different plants of Euphorbiaceae family contains unusual acids [4] and showed anti-infertility, anthelmintic, purgative activity [5] and anti acne, antifungal, antibacterial property [6-7].

In view of these facts and in continuation of our research program on physicochemical and pharmacological activity of miner seed oil of various plant family [8-9], now we are reporting the physico-chemical and pharmacological characteristics of *Euphorbia rothiana* seed oil which showed significant activity in acute anti-inflammatory model were further screened for analgesic activity.

EXPERIMENTAL SECTION

General Considerations

All research chemicals were purchased from Acros organics (NY, USA), Sigma-Aldrich (St.Loius, Missouri, USA), Lancaster Co. (Ward Hill, MA, USA). Solvents except laboratory reagent grade were dried and purified according to the literature whenever necessary. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel plates from E. Merck and Co. (Darmstadt, Germany).

Melting points of synthesized compounds were determined in Thermonik melting point apparatus (Chennai, India) and are uncorrected. The IR spectra were recorded on Thermo Nicolet FTIR -200 Spectrometer (Madison, WI, USA), using KBr pellets. The ^{1}H NMR were recorded on Bruker AVANCE II 400 (Bruker, Rheinstetten/ Karlsruhe, Germany) in $CDCl_3$ / DMSO-d_6 as solvent. Chemical shifts are reported in δ ppm units with respect to TMS. All the animal experiments approved by Institutional Animal Ethical Committee (IAEC).

Plant material:

Euphorbia rothiana (family Euphorbiaceae) is an annual erect, glabrous, profusely branched sub shrub of one-meter height and distributed in India (Karnataka, Maharastra and Tamilnadu). Fresh, unripe, green fruits of *Euphorbia rothiana* were collected from Belgaum district, Karnataka and were authenticated by head of botany department, Dr. Huddar, Kottambri science college, Hubli.

Preparation of extracts:

The air dried seeds shade at room temperature. The dried seeds were subjected to size reduction to course powder by using dry grinder and passed through sieve. This powered packed into soxhlet apparatus extracted successively with petroleum ether (b.r. 40-60^0 C). The extract were dried at 40-45^{0}C by using rota evaporator (Simadzo, Japan). The oil obtained was analyzed for their physico-chemical characteristics as per standard AOCS procedures [10].

The seed oil was examined by Halphen reagent [11], picric acid and 2,4-DNP TLC [12] test in addition to other chromatographic [direct, reversed–phase and silica gel silver nitrate TLC] techniques. [13, 14]

The methyl esters of the fatty acids were prepared by the method of Jamieson and Reid [13] using sulphuric acid as a catalyst. In most of the cases the crude methyl esters were purified by silica gel column chromatography prior to GLC analysis on a Perkin Elmer Sigma unit with DEGS column (Bruker, Germany).

Pharmacological screening:

Animals

Albino mice of either sex weighing between 20-25g were used for acute toxicity and anti-inflammatory studies. Healthy male albino adult rats weighing between 122-125g were used for analgesic activity. Animal ethical clearance was obtained from Ethics Committee of K.S. Hegde Medical Academy, Deralakatte, Mangalore, India (115/1999/CPCSEA). Animals were procured from K. S. Hegde Medical Academy, Deralakatte, Mangalore, India, and housed individually in polypropylene cages, maintained under standard conditions of alternating 12-hr light-and-dark cycles at a constant temperature (25 ± 2 ^{o}C and 35-60% relative humidity). Animals were fed with standard rat pellet diet, (Hindustan Lever Ltd., Mumbai, India) and water ad libitum.

Acute toxicity

The acute toxicity test was carried out according to OECD guidelines (OECD, 2000) [15] to establish the effective dose of the test compounds after obtaining ethical clearance from Ethics Committee of K.S. Hegde Medical Academy, Deralakatte, Mangalore, India. Albino mice of either sex weighing between 20-25 g were divided into six groups of 6 animals each. Animals were starved for 24 hr with water ad libitum prior to test. On the day of the experiment, animals were administered intraperitoneally with different compounds to different groups in an increasing dose of 10, 20, 100, 200, 1000 and 2000 mg/kg body weight. The animals were then observed continuously for 3 hr for general behavioral, neurological, autonomic profiles and then every 30 min for next 3 hr and finally for next 24 hr or till death.

From preliminary toxicity studies, it was observed that animals were found to be safe up to a maximum dose of 2000 mg/kg body weight. But there were few changes in the behavioral response like alertness, touch response, and restlessness. Therefore, 1/10th of the maximum tolerated dose, i.e. 200 mg/kg body weight (b.w.) was chosen for the studies.

Anti-inflammatory activity:

The albino mice of either sex were divided into single group with six animals each by the method of Brown and Robson [16]. Inflammation was induced by using xylol on left ear served as control and right ear as test compound (seed oil). The thickness was measured by using a micrometer screw gauge after a regular interval of time 0, 1, 3, 5 hr respectively. The results were shown in Table 3.

Analgesic activity:

About 24 albino rats of either sex weighing between 122-125g were used and randomly divided into three groups with six animals in each groups. First group served as control (1ml of gum acacia 2% per mg/kg body weight), second group serves as test compound (seed oil, 200 mg/kg body weight) and third group serves as standard (aspirin, 100 mg/kg body weight).This was done by caudal immersion technique by Bassat J. B. [17]. The tail was dipped into hot water bath

maintained at 55 ± 0.5^0 C and the time taken for withdrawal of the tail clearly out of water was taken as reaction time in second. The promising results at third hour are shown in Table 4.

RESULTS AND DISCUSSION

The seeds of *Euphorbia rothiana* yielded 58.1% of oil. The presence of unusual fatty acid was ruled out by Halphen test, Picric acid and 2, 4-DNP TLC tests. The analytical data seed oil of *Euphorbia rothiana* showed less acid value (5.9) indicates the safer for human consumption, higher iodine value (179.4) reveals higher degree of unsaturation and lower saponification value (288.3) accounted for presence long chain fatty acids. The results of analytical data of seeds are given in Table 1.

Table 1. Analytical data of seeds and seed oil of *Euphorbia rothiana*

Source	Seed analysis				Oil Characteristics				
	Oil %	Moisture %	Ash %	Protein %	Ref. Index at 40^0C	AV	IV	SV	UM %
Euphorbia rothiana	58.1	3.81	1.2	3.17	1.47	5.9	179.4	288.3	1.3

AV=Acid Value, IV=Iodine Value, SP=Saponification Value and UM=Unsaponifiable matter.

Argentation TLC [18] of the methyl esters indicated well defined spots for saturates monoenes and dienes comparative to those of authentic esters run along side.

The IR spectrum of compound showed characteristic absorption bands at 727 cm^{-1} due to cis double bond (-CH=CH-) and the absorption at range of 1739 cm^{-1} was attributed to C=O groups methyl ester of fatty acids respectively. In the ^{1}H NMR spectrum of methyl ester exhibited these compound characteristic signal at δ 3.6 which accounted for OCH_3 of methyl ester and characteristic singlet showed at δ 2.5 due to the presence of ($-CH_2-C=C-CH_2-$), at δ 1.2 [chain ($-CH_2$)] and at δ 0.89 (terminal $-CH_3$) of fatty acids respectively. It indicates the none of the oil and their esters contains either conjugation, trans unsaturated or any other functional groups. The structure of the compound was further supported by GLC analysis of the seed oil contains saturated fatty acid are palmitic acid (8.1%) and stearic acid (3.5%). The unsaturated fatty acids are oleic acid (10.4%), linoleic (21.6) and linolenic (60.2%). Therefore, the major component is linolenic acid rich, which is potential essential fatty acid. Results are tabulated in Table 2.

Table 2. Fatty acid composition of seed oil of seed oil of *Euphorbia rothiana*

Source	Methyl ester composition by GLC (wt %)				
	16:0(PA)	18:0(SA)	18:1(OA)	18:2(LA)	18:3(LLA)
Euphorbia rothiana	8.1	3.5	10.4	21.6	60.2

(PA): Palmitic acid, (SA): Strearic acid, (OA): Oleic acid, (LA): Linoleic acid, (LLA): Linolenic acid.

The test compound was found to be safe upto 2000 mg/kg body weight (b.w.). Therefore $1/10^{th}$ of the maximum tolerated dose, i.e. 200 mg/kg b.w. was used as therapeutic dose. Acute anti-inflammatory activity was carried out by the method of Brown and Robson. Application of xylol on left ear was used as control and application of seed oil of *Euphorbia rothiana* on right ear of albino mice serves as test compound. The anti-inflammatory activity results revealed that the test compound exhibited significant anti-inflammatory activity (up to 52.94 % edema inhibition), as

compared to the control after third hour. The anti-inflammatory activity of test compound may be attributed to the inhibition of cyclooxygenase enzyme, which plays vital role in the inflammation process. The results indicating the percentage inhibition of inflammation have been shown in Table 3.

Table 3. Anti-inflammatory activity of seed oil of *Euphorbia rothiana*

Ear thickness measured in time	Ear thickness in mm (mean)		Protection (%)
	Application of xylol*	*Euphorbia rothiana*#	
1 hr	0.16	0.07	43.75
3 hr	0.34	0.18	52.94
5 hr	0.37	0.15	40.50

**Serves as control (left ear thickness), # serves as test (right ear thickness)*

The analgesic activity was expressed as percentage of protection. The test compounds showed significant analgesic activity (up to 66.2 % protection) at dose of 200mg/kg body weight, as compared to the standard drug aspirin (91.1 %) at dose of 100mg/kg body weight after third hour. Results are shown in Table 4. Hence, it reveals that the test compounds showed significant anti-inflammatory and analgesic activities due presence of major unsaturated fatty acid viz. Linolenic acid (37.4%).

Table 4. Analgesic activity of seed oil of *Euphorbia rothiana*

Oil	Dose (mg/kg body weight)	Tail without response at 3rd hour (mean time in seconds)	Protection (%)
Control	1ml of gum acacia	3.20	-
Euphorbia rothiana	200mg	4.81	66.20
Aspirin	100mg	3.50	91.00

CONCLUSION

The present study revealed that the seed oil of *Euphorbia rothiana* was satisfactory yield. It was observed that major percentage of unsaturated fatty acid present in seed oil was found to have significant anti-inflammatory and analgesic activity. Further seed oil can be subjected for ulcerogenic activity. These can be regarded as strong candidates for future investigations.

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