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Chemical Investigations and Anti-inflammatory Activity of Fixed Oil of *Butea monosperma* Seeds

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Abstract – The fruit and seeds of *Butea monosperma* (Lam) Kuntze (Fabaceae) are useful in piles, anthelmintic, eye diseases, and inflammation in the Indian system of medicine. Hence, we have evaluated the anti-inflammatory activity of the fixed oil, mixed fatty acids, and unsaponifiable matter of *B. monosperma* against carrageenan-induced paw oedema and cotton pellet-induced granuloma in rats. The fixed oil, mixed fatty acids, and unsaponifiable matter of the oil exhibited significant anti-inflammatory activity on the tested experimental animal models. The unsaponifiable matter of the oil produced higher protection compared to fixed oil and mixed fatty acids. Phytochemical analysis of the fixed oil revealed the presence of steroids and terpenoids while unsaponifiable matter of the oil showed the presence of β-sitosterol. Also, four fatty acids were identified in the fixed oil by gas liquid chromatography. The anti-inflammatory activity of the fixed oil may be due to unsaponifiable matter or combination of unsaponifiable matter and mixed fatty acids.
Key words – *Butea monosperma*, Anti-inflammatory activity, Unsaponifiable matter, Mixed fatty acids

Introduction

Butea monosperma (Lam) Kuntze (Fabaceae) is a medicinal plant growing in Burma, India, Java and Sri Lanka. The bark of this plant is used in indigeneous medicine for the treatment of diabetes, diarrhoea, dysentry, ulcer, polypus in the nose, sore throat, and snake bite (Varier, 1993; Jayaweera, 1981). The flowers are tonic, astringent, aprodiasic, and diuretic. The roots are reported in the treatment of filariasis, night blindness, helmenthiasis, piles, ulcer, and tumours (Raj and Kurup, 1968). In the Indian system of medicine, fruit and seeds are bitter and oily; anthelmintic, and useful in piles, eye diseases, and inflammation (Kirtikar and Basu, 1989). The ethanolic extract of the seeds of *B. monosperma*, on oral administration showed antifertility activity in mice and rats (Razdan, *et al.*, 1969). Palasonin, an active principle isolated from *B. monosperma* seeds and its piperzaine salt exhibited good anthelmintic activity *in vitro* on *Ascaris lumbricoides* and *in vivo* on *Toxicara canis* (Raj and Kurup, 1968). The petroleum ether extract and triterpene isolated from flowers of *B. monosperma* exhibited anticonvulsant activity (Kasture *et al.*, 2000; Kasture, *et al.*, 2002). Thus phytochemical and pharmacological properties of flowers are available but little is known regarding the contribution of phytochemical constituents of fixed oil of *B. monosperma* seeds towards the pharmacological activities. Therefore, the present study was undertaken to report phytochemical analysis and anti-inflammatory activity of fixed oil and its fractions in experimental animals.

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Materials and Methods

Plant material – The seeds of *Butea monosperma* (Lam) Kuntze (Fabaceae) were collected from Valasa Malai, Dharmapuri District of Tamilnadu (India) in December 2002. The plant was identified by Dr. B. Velmurugan, Taxonomist, Sri Ramana Maharishi Natural Remedies Society, Thiruvannamalai. The voucher specimen was deposited in Herbarium Section of the Sri Ramana Maharishi Natural Remedies Society, Thiruvannamalai (Reg. No.: GPT/ 8-11/2002). The seeds were dried under shade, pulverized by a mechanical grinder and stored in closed container for further use.

Phytochemical study – The powdered seeds (850 g) were extracted in cold with petroleum ether (40-60°C) for

72 h at room temperature. The whole extract of seeds were collected and filtered. On evaporation of petroleum ether in vacuo, a greenish yellow coloured oil was obtained [yield - 26.66% (w/w) with respect to the dry starting material] and was stored in desiccator for further studies. Physico-chemcial characteristics of the oil was determined by conventional methods (I. P. 1985).

Separation of the unsaponifiable matter – About 50 g of fixed oil was subjected to saponification (A.O.A.C., 1965) to give unsaponifiable matter (1.5 g). The unsaponifiable matter (100 mg) was dissolved in 5 ml of absolute alcohol and kept over night at lower temperature. Colourless needles (m.p. 134-136°C) were obtained on recrystallization of this yellowish material. It gave the positive tests for sterols. It was identified as β-sitosterol by co-chromatography and measurement of mixed melting point.

Preparation of methyl esters of mixed fatty acids – The aqueous solution remaining after extraction of the unsaponifiable matter was acidified with dil. HCL (2M HCL), then extracted with ether (5×200 ml), washed with water, dried over anhydrous sodium sulphate and evaporated to leave the residue of fatty acids (13.4 g). The fatty acids were esterified with MeOH-H_2SO_4 (A.O.A.C., 1965). The methyl esters thus obtained were subjected to GLC analysis and identification of the resolved methyl esters were carried out by comparison of the retention times with authentic compounds. Quantitative estimation was carried out by peak area measurements and the results are presented in Table 2.

Anti-inflammatory Activity

Animals – Albino Wistar rats of either sex (weighing 210-260 gm) were housed in standard polypropylene cages and was provided with food and water *ad libitum*. The rats were allowed a week acclimatization period before the experimental session.

Carrageenan induced rat paw oedema – Pedal inflammation in rats was produced according to the method described by Winter *et al* (1962). The rats were divided into eight groups, each group consisting of six animals. The fixed oil (250 and 500 mg/kg), mixed fatty acids (100 and 200 mg/kg) and unsaponifiable matter (100 and 200 mg/kg) were given orally to six groups of rats. The seventh group of rats received indomethacine (10 mg/kg p.o). The eighth group served as control, received 0.5% carboxy methyl cellulose at a dose of 5 ml/kg. One hour later, an injection of 0.05 ml of 1% carrageenan (Sigma and Co., M.O.) was made into the right hind paw of each rat under the sub plantar region. Measurement of paw size was done by wrapping a piece of cotton thread round the rat paw and measuring the circumference on a meter rule (Bangbose and Noamesi., 1981). This was done before and at 3 h following carrageenan injection.

The inhibitory activity was calculated according to the formula.

$$\text{Percentage inhibition} = \frac{(C_t - C_o)\ \text{control} - (C_t - C_o)\ \text{treated} \times 100}{(C_t - C_o)\ \text{control}}$$

where,

C_t = Paw size 3h after carrageenan injection

C_o = Paw size before carrageenan injection

Cotton Pellet-induced Granuloma in rats – The rats were divided into eight groups with six animals in each group. After shaving off the fur, the rats were anaesthetized. Sterile preweighed cotton pellets (10 mg) were inserted one in each axilla region of each rat through a single needle incision (D'Arcy *et al.*, 1960). The first six groups of rats were treated with oral dose of 250 and 500 mg/kg of fixed oil, 100 and 200 mg/kg of mixed fatty acids and unsaponifiable matter. The seventh and eighth group of rats received 5 ml/kg carboxy methyl cellulose (or) 10 mg/kg indomethacin orally as vehicle and drug control respectively, for seven consecutive days from the day of cotton-pellet implantation. On the eighth day, the animals were anaesthetized, and the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C to constant weight. The increment in the dry weight of the pellets was taken as a measure of granuloma formation (Winter and Porter, 1975).

Statistical analysis – The results were analysed by Student's *t*-test and expressed as mean±S.E.M.

Results and Discussion

An extraction with petroleum ether, the seeds of *Butea monosperma* yielded a greenish yellow coloured oil (Table 1). The unsaponifiable matter was identified as β-sitosterol. The GLC analysis of fixed oil revealed the presence of mixture of oleic, palmitic, linoleic and linolenic acids (Table 2).

The anti-inflammatory activity of fixed oil of *B. monosperma* against carrageenan-induced rat paw oedema has been shown in Table 3. Results showed that the fixed oil at 250 and 500 mg/kg exhibited significant anti-inflammatory activity when compared with control group. The fixed oil at 500 mg/kg showed significant anti-inflammatory activity as compared to that of standard drug indomethacin (10

Table 1. Physico-chemical characteristics of fixed oil of *B. monosperma*

Yield	26.66 w/w with reference to dried seeds
Colour	Greenish Yellow
Consistency	Viscous liquid
Wt/ml (at 30°C)	0.98750 gm/cc
Acid value	7.3
Iodine value	70.2
Saponification value	153
Unsaponifiable matter	2.6%

Table 2. GLC analysis of mixed fatty acids methyl esters of *B. monosperma*

Name of mixed fatty acids	Percentage
Oleic acid	25.25
Palmitic acid	18.74
Linoleic acid	23.53
Linolenic acid	2.82

Table 3. Anti-inflammatory effect of *B. monosperma* on carrageenan induced rat paw oedema

Treatment	Paw Size (cm)	% inhibition
Control (CMC, 5 ml/kg)	3.32 ± 0.07	–
Indomethacin (10 mg/kg)	2.87 ± 0.08	70.10**
Fixed oil (250 mg/kg)	3.02 ± 0.13	41.23*
Fixed oil (500 mg/kg)	2.95 ± 0.10	61.85**
Mixed fatty acids (100 mg/kg)	3.12 ± 0.09	32.98*
Mixed fatty acids (200 mg/kg)	3.00 ± 0.06	51.54**
Unsaponifiable matter (100 mg/kg)	3.03 ± 0.05	52.57**
Unsaponifiable matter (200 mg/kg)	2.85 ± 0.03	75.25**

Values are mean ± S.E.M., $n = 6$, $P^* < 0.01$, $P^{**} < 0.001$ compared with control, student *t*-test.

mg/kg p.o.). Mixed fatty acid and unsaponifiable matter showed significant effect in carrageenan-induced rat paw oedema model. The maximum inhibition (61.85%) was observed with 500 mg/kg of fixed oil. On the other hand, mixed fatty acid and unsaponifiable matter at 200 mg/kg showed 52.5% and 75.25 oedema inhibition, respectively. The unsaponifiable matter showed maximum percentage inhibition in paw oedema method.

The results of the cotton-pellet granuloma in rats are shown in Table 4. The fixed oil as well as mixed fatty acids and unsaponifiable matter significantly inhibited the granuloma weight in a dose dependent manner. In this model, the fixed oil at 500 mg/kg produced a maximum inhibition of 39.85%, while mixed fatty acids at 200 mg/kg produced 47.93% inhibition in granuloma weight. The unsaponifiable matter (200 mg/kg) showed a maximum inhibition (57.90%) in granuloma weight, compared to indomethacin (59.60%).

The present study has established the physicochemical characteristics and the anti-inflammatory activity of fixed oil

Table 4. Anti-inflammatory effect of *B. monosperma* on cotton-pellet induced granuloma in rats

Treatment	Weight of granulation (mg)	% inhibition
Control (CMC, 5 ml/kg)	88.67 ± 2.92	-
Indomethacin (10 mg/kg)	35.83 ± 1.65*	59.60
Fixed oil (250 mg/kg)	65.50 ± 1.31*	26.13
Fixed oil (500 mg/kg)	53.33 ± 2.76*	39.85
Mixed fatty acids (100 mg/kg)	57.33 ± 1.76*	35.34
Mixed fatty acids (200 mg/kg)	46.17 ± 2.09*	47.93
Unsaponifiable matter (100 mg/kg)	51.67 ± 1.33*	41.72
Unsaponifiable matter (200 mg/kg)	37.33 ± 2.11*	57.90

Values are mean ± S.E.M., $n = 6$, $P^* < 0.001$ compared with control, student *t*-test.

as well as mixed fatty acids and unsaponifiable matter of the oil in the models used. Inhibition of carrageenin-induced rat paw oedema is the preliminary screening model for searching for potential anti-inflammatory compound from natural products (Della Loggia *et al.*, 1986; Alcaraz and Jimnez, 1988). The anti-inflammatory activity of fixed oil, mixed fatty acids and unsaponifiable matter showed significant activity in carrageenin-induced paw oedema. The unsaponifiable matter showed maximum percentage inhibition in paw oedema method. The cotton pellet granuloma in rats is frequently used as an experimental model to study the effect of drug on chronic inflammation. Swingle and Shideman (1972) demonstrated that there are three phases of inflammation after pellet implantation. The last phase is cell proliferation between third and sixth day; this phase can be inhibited by anti-inflammatory steroids such as dexamethasone and also by non-steroidal anti-inflammatory drugs. Hence, the present study was designed to investigate whether the fixed oil would modify the last phase of the cotton pellet granuloma. The fixed oil, mixed fatty acids and unsaponifiable matter significantly inhibited the proliferative phase of granuloma. This reflected its efficacy to a high extent to reduce an increase in the number of fibroblasts and the synthesis of collagen and mucopolysaccharides during granuloma tissue formation (Agrrigoni-Martellie, 1977).

Reports on the phytochemistry of *B. monosperma* seeds indicate the presence of compounds like mixed fatty acids and triglycerides (Sengupta and Basu, 1978), n-heneicosanoic acid δ-lactone (Bishnoi *et al*, 1979), monospermin (Mehta *et al*, 1981) and a-amyrin, β-sitosterol, β-sitosterol-β-D-glucoside and sucrose (Chandra *et al*, 1977). However, there is no scientific report on the chemical nature and pharmacological activity of *B. monosperma*. The present phytochemical study showed the presence of β-sitosterol and some fatty acids in the fixed oil of *B. monosperma* seeds. β-sitosterol is reported to have anti-inflammatory, anti-

pyretic (Gupta *et al*, 1996), anti-ulcer, anti-diabetic, and anti-cancerous activities, and is helpful in controlling rheumatoid arthritis (Pegel, 1985). Reports of anti-inflammatory effect of plant lipids are available. Lipids extracted from the seeds of evening primrose and borage plants have been reported to be anti-inflammatory (Ziboh and Chapikin, 1987) due to the presence of relatively large amount of gamma linolenic acid which is rapidly converted to dihomo gamma linolenic acid (DGLA). DGLA competes with arachidonate for oxidative enzymes and thereby reduces production of cyclo-oxygenase products derived from arachidonate. In addition, DGLA cannot be converted to inflammatory leukotrienes by 5-lipoxygenase. Instead it is converted to 15-hydroxy DGLA which can inhibit 5-lipoxygenase. GLA enriched diet has been claimed to suppress acute and chronic inflammation as well as joint tissue injury in several experimental animal studies (Tate *et al*, 1988).

The present study confirm the anti-inflammatory activity of fixed oil as well as mixed fatty acids, and unsaponifiable matter of oil, which may be due to presence of β-sitosterol and fatty acids.

The results obtained in this work clearly support the traditional use of *B. monosperma* in inflammatory diseases (Kritikar and Basu, 1989). However, it is necessary to identify the specific active principle(s) responsible for anti-inflammatory activity as well as to establish the mechanism of action of the fixed oil to come to a definite conclusion.

References

A. O. A. C., *Official Methods of Analysis*, 10th Ed., Washington D.C., 429, 434 (1965).

Alcaraz, M. J. and Jimenez, M. J., Flavonoids and anti-inflammatory agents. Fitoterapia. **59**, 25-38 (1988).

Arrigoni-Martellie, E., *Inflammatory and Antiinflammatory*. Spectrum Publication, Inc., New York, 1977, pp. 119-120.

Bishnoi, P. and Gupta, P. C., A new lactone from the seeds of *Butea frondosa*. *Planta. Med.* **35**, 286-288 (1979).

Chandra, S., Lal. J. and Shah, M., Phytochemical investigation of *Butea frondosa* seeds. *Indian J. Pharm.* **39**, 79-80 (1977).

D' Arcy, P. D., Howard, E. M., Muggelton, P. W. and Townsend, S. B., The anti-inflammatory action of griseoflavin in experimental animals. *J. Pharm. Pharmacol.* **12**, 659 (1960).

Della Loggia, A., Tubaro, A. P., Zilli, C. and Delnegra, P., The role of flavonoids in the anti-inflammatory activity of *Chamomilla recutita*. *Clin. Boil. Res.* **213**, 481-488 (1986).

Gupta, M. B., Nath, R., Srivastava, N., Shanker, K., Kishor, K. and Bhargava, K. P., Antiinflammatory and antipyretic activities of β-sitosterol. *Int. J. Immunopharmacol.* **18**, 693-700 (1996).

Jayaweera, D.M.A., *Medicinal Plants Used in Ceylon*, Part 3, Natl. Sci. Council, Sri Lanka, Colombo, 1981, p. 161.

Kasture, V. S., Chopde, C. T. and Deshmukh, V. K., Anticonvulsive activity of *Albizzia lebbeck*, *Hibiscus rosasinensis* and *Butea monosperma* in experimental animals. *J. Ethnopharmacol.* **71**, 65-75 (2000).

Kasture, V. S., Kasture, S. B. and Chopde, C. T., Anticonvulsive activity of *Butea monosperma* flowers in laboratory animals, *Pharmacol. Biochem. Behav.* **72**, 965-972 (2002).

Kirtikar, K. R. and Basu, B. D., *Indian Medicinal Plants*, Shiva Offset Press, Dehradun, 1989, pp. 785-788.

Mehta, B. K. and Bokadia, M. M., A new alkaloid of *Butea monosperma* seeds. *Chemy* and *ind.* **3**, 98 (1981).

Pegel, K. H., The importance of sitosterol and sitosterolin in human and animal nutrition. Planta Med. **39**, 157-163 (1980).

Pharmacopoeia of India, Third edition, Government of India, Ministry of Health and Family Welfare, Delhi, 1985, pp. A-71-A-73

Raj, R. K. and Kurup, P. A., Anthelmintic activity, toxicity and other pharmacological activities of palasonin, the active principles of seeds and its piperazine salt. *Indian J. Med. Res.* **56**, 13-18 (1968).

Razdan, M. K., Kapila, K. and Bhide, N. K., Antifertility effect and some pharmacological actions of *Butea frondosa* seed extracts. *Indian J. Physiol. Pharmacol.*, **13**, 239 (1969).

Sengupta, A. and Basu, S. P., The triglyceride composition of *Butea monosperma* seed oil. **85**, 533-535 (1978).

Swingle, K. F. and Shideman, F. E., Phase of the inflammatory response to subcutaneous implantation of cotton pellet and their modification by certain anti-inflammatory drugs. *J. Pharmacol. Exp. Ther.* **183**, 226-234 (1972).

Tate, G., Mandeli, B. F., Karmali, R. A., Laposata, M., Baker, D. G., Schumacher, R. Jr. and Zurier, R. B., Suppression of monosodium urate crystal-induced acute inflammation by diets enriched with gamma-linolenic acid and eicosapentaenoic acid. *Arthritis Rheum*, **31**, 1543 (1983).

Varier, S.P.V., *Indian Medicinal Plants*, Orient Longman Ltd., Madras, 1993, pp. 284-286.

Winter, C. A. and Porter, C. C., Effect of alteration in side chain upon anti-inflammatory and liver glycogen activities in hydrocortisone esters. *J. Am. Pharm. Sci. Ed.* **46**, 515 (1957).

Winter, C. A., Risley, E. A. and Ness, G. W., Carrageenan induced oedema in hind paw of rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.* **111**, 544-547 (1962).

Ziboh, V. A. and Chapikin, R. S., Biologica significance of polyunsaturated fatty acids in the skin. *Arch. Dermatol.* **123**, 1686 (1987).

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