

Chemical Characterization and Pharmacological Screening of Some *Annona* Seed Oils

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Seed oils from the indigenous species, viz., *Annona squamosa*, *Annona muricata* and *Annona reticulata* belonging to *Annonaceae* family have been analyzed for their physico-chemical properties. Major fatty acids identified from *Annona squamosa* seed oil are palmitic acid (8.9%), linoleic acid (11%), stearic acid (1.5%), caproic acid (0.4%) and traces of caprylic acid, while in *Annona muricata* seed oil, caprylic acid (76%), capric acid (13%), stearic acid (10%) and traces of capric acid are present. The fatty acid constituents of *Annona reticulata* seed oil are linoleic acid (13%), caprylic acid (4.2%), oleic acid (0.7%), capric acid (0.26%) and traces of caproic acid. The *Annona* seed oils are individually screened for the acute toxicity studies and found that all the three seed oils are non-toxic. They exhibited significant anti-inflammatory effects against carageenan induced paw oedema in albino rats. This is the first report of the study of chemical constituents as well as anti-inflammatory activity of annonaceous seed oils.

Key Words: *Annona squamosa*, *Annona muricata*, *Annona reticulata*, Seed oil, Chemical composition, Anti-inflammatory activity.

INTRODUCTION

Annona squamosa (*Annonaceae*) is a small, more or less evergreen tree, occurs wild and is cultivated all over India¹. The unripe seed, leaves and root are considered medicinal and used for destroying insects and lice². Several *Annona* species were found to be rich in lipids and fatty acids³. The important fatty acids identified from the seed oils of *Annonaceae* were oleic acid, linoleic acid, myristic acid, palmitic acid, stearic acid, arachidic acid and unsaturated acids like palmitoleic acid and linolenic acids etc.^{3–7}.

There is no report on the comparison of chemical constituents and pharmacological screening of these oils and hence the study is relevant.

EXPERIMENTAL

The fruits of the three *Annona* species: *Annona muricata*, *Annona squamosa* and *Annona reticulata* were collected during April 2001 from a tree near the campus of Regional Research Laboratory, Trivandrum and their seeds were separated, shade dried, powdered and extracted with hexane in a soxhlet apparatus. The hexane extract was concentrated in a rotary evaporator under

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reduced pressure to get the seed oils. The oil content, specific gravity, refractive index, acid value, saponification value and iodine value were determined as per AOCS procedures⁸.

Methyl esters were obtained by refluxing oil (350 mg) in methanolic NaOH solution (2%) for 1 h and BF_3 in methanol (12%, 10 mL) was added from the top of the condenser and boiling continued for few minutes. The reaction mixture was cooled, saturated sodium carbonate solution was added and the hexane layer was washed with water several times and dried over anhydrous sodium sulphate. Gas chromatographic analysis was carried out in Hewlett Packard 5840A using the column (10% FFAP + 10% H_3PO_4) on Chromosorb WHP (100–120) under temperature programming at the rate of 5°C/minute having initial temperature 100°C and final temperature 190°C for 20 minutes, with nitrogen as the carrier gas at the rate of 20 mL/min. with injection port temperature 250°C and FID detector temperature 300°C. The fatty acid compositions and the physical constants of the *Annona* seed oils were listed in Table-1 and in Table-2 respectively. By comparing the acid values of the seed oils, it was clear that the presence of free fatty acids is more in the case of *Annona squamosa* oil than the other two. Annonaceous oils have higher specific gravity; similar refractive indices and the degree of unsaturation of the oils are close to that of sunflower oil in comparison with coconut oil, palm kernel oil and sunflower oil.

TABLE-1
FATTY ACID COMPOSITION OF *ANNONA* SEED OILS

Serial No.	Fatty acids	<i>Annona squamosa</i> (% composition)	<i>Annona reticulata</i> (% composition)	<i>Annona muricata</i> (% composition)
1.	Caproic acid	0.40	Traces	Traces
2.	Caprylic acid	0.11	4.20	76.00
3.	Unknown	—	—	0.30
4.	Capric acid	—	0.26	13.00
5.	Unknown	—	—	0.40
6.	Palmitic acid	8.90	—	—
7.	Stearic acid	1.50	—	10.23
8.	Linoleic acid	11.00	12.60	—
9.	Oleic acid	—	0.70	—

TABLE-2
CHARACTERISATION OF *ANNONA* SEEDS

Serial No.	Seed analysis	<i>Annona squamosa</i>	<i>Annona reticulata</i>	<i>Annona muricata</i>
1.	Oil content (%)	22	53.5	24
2.	Specific gravity	0.929	0.914	0.932
3.	Refractive index	1.466	1.468	1.465
4.	Acid value	5.971	3.66	2.846
5.	Saponification value	180.34	159.31	186.11
6.	Iodine value	104.5	95.91	99.53

Pharmacological screening

Acute toxicity studies of all the three *annonaceous* seed oils were carried out using albino rats of either sex weighing 150–250 g by the method of Miller and Tainter⁹. Antiinflammatory studies were carried out in albino rats against carrageenan induced paw oedema. The acute toxicity studies of the seed oils were performed up to a dose level of 2000 mg/kg of the body weight and showed no mortality. The antiinflammatory studies revealed that all the three seed oils were antiinflammatory, compared with the standard drug indomethacin. It was found that the seed oil of *Annona reticulata* exhibited 80–86% inhibition of oedema in rats followed by 40–48% for *Annona muricata* and 27–39% for *Annona squamosa* oil at a dose level of 500 mg/kg body weight. The antiinflammatory activity was not dose dependent in the case of *Annona muricata* and *Annona squamosa* oils, while in the case of *Annona reticulata* the activity was dose dependent. The results are summarized in Table-3.

TABLE-3
ANTIINFLAMMATORY EFFECT OF SEED OILS OF *A. SQUAMOSA*,
A. RETICULATA AND *A. MURICATA*

Groups	Dose	Mean increase in paw thickness (in mm)	Reduction in the paw thickness (%)	P value
1	Control 5% Acacia solution	1.4960 ± 10.1270	—	—
2	<i>A. squamosa</i> seed oil 250 mg/kg	1.098 ± 0.0393	26.60	< 0.01
3	<i>A. squamosa</i> seed oil 500 mg/kg	0.9160 ± 0.0351	38.77	< 0.001
4	<i>A. reticulata</i> seed oil 250 mg/kg	0.3526 ± 0.0169	80.35	< 0.001
5	<i>A. reticulata</i> seed oil 250 mg/kg	0.2336 ± 0.1190	86.98	< 0.001
6	<i>A. muricata</i> seed oil 250 mg/kg	0.7640 ± 0.1189	48.93	< 0.001
7	<i>A. muricata</i> seed oil 500 mg/kg	0.8860 ± 0.2176	40.78	< 0.001
8	Indomethacin 20 mg/kg	0.5188 ± 0.0504	71.07	< 0.001

F = 66.340, df = 40, values are mean ± SEM.

P < 0.01/0.001 in comparison to control.

Statistical Analysis

The data were calculated per group as mean ± SEM. The significance was calculated by ANOVA. The minimum level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

The gas chromatographic analysis indicated that the chemical composition of the three *annonaceous* seed oils is different. In *Annona muricata* seed oil, 76% of the total composition is caprylic acid, 13% is capric acid and 10% stearic acid. In *Annona reticulata* the major fatty acid constituents are caprylic acid (5%), linoleic acid (13%), oleic acid (1.2%) and traces of caproic acid. In the case of

Annona squamosa the major fatty acids identified are linoleic acid (11%), palmitic acid (8.9%), stearic acid (1.5%) and traces of caproic acid and caprylic acid. More than 70% of the fatty acids, possibly higher unsaturated acids, could not be identified by GLC in the case of *Annona squamosa* and *Annona reticulata*. The study revealed that *Annonaceous* oils were rich in unsaturated fatty acid contents except in *Annona muricata*. The % oil content is very high in *Annona reticulata* (53.4) and moderate in *Annona squamosa* (22) and *Annona muricata* (24). Refining and partial hydrogenation would lead to more acceptable edible oils from *Annonaceous* seed oils. It was clear from the study that these seeds are of high oil content and could be used as lubricating oils or even as edible oil after some processing. Thus waste utilization can be effectively taken up.

As in the case of chemical composition, the anti-inflammatory activity also varied in the three *annonaceous* oils. The acute toxicity studies revealed that all the three seed oils are harmless up to a level of 2000 mg/kg body weight of the albino rats. Anti-inflammatory studies of the three seed oils revealed the significance of using these oils as anti-inflammatory agents. The seed oil of *Annona reticulata* showed significant inhibition of oedema when compared to indomethacin, the standard drug imparting the activity. *Annona reticulata* seed oil showed 80–86% inhibition of oedema and 40–48% was exhibited by *Annona muricata* seed oil. *Annona squamosa* seed oil showed 27–39% inhibition, being the least. In the case of *Annona muricata* seed oil the activity was not dose dependent. But in *Annona reticulata* seed oil, the effect was more dose dependent. A detailed pharmacological study was under progress for correlating the chemical composition and the anti-inflammatory activities of these oils.

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REFERENCE

1. The Wealth of India: Raw materials, C.S.I.R., New Delhi, Vol. 1, p. 234 (1962).
2. M. Loebouef, A. Cave, P.K. Bhaumik, B. Mukherjee and R. Mukherji, *Phytochemistry*, **21**, 2783 (1982).
3. C.D. Hufford, W.L. Lasswell (Jr.), *Lloydia*, **41**, 151 (1978).
4. ———, *Lloydia*, **41**, 156 (1978).
5. ———, *J. Org. Chem.*, **41**, 1297 (1977).
6. C.D. Hufford and B.O. Oguntimein, *Phytochemistry*, **19**, 2036 (1980).
7. C.D. Hufford and W.L. Lasswell (Jr.), *J. Org. Chem.*, **41**, 1297 (1976).
8. W.E. Link, Official and Tentative Methods of the American Oil Chemists Society, 3rd Edn., p. 11 (1973).
9. M.B. Miller, M.L. Tainter and R.A. Turner, Screening Methods in Pharmacology, Academic Press, New York, p. 61 (1965).