ANTI-TUMOUR AND PHARMACOLOGICAL EFFECTS OF THE OIL FROM *SEMECARPUS ANACARDIUM* LINN. F.

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Summary : *Semecarpus anacardium* Linn.f. nuts were extracted by using non-polar and polar organic solvents. Hot methanol extract and a resinous fraction, isolated from it, showed antitumour activity against P388 lymphocytic leukaemia in BDF_1 mice as judged by their median survival time. Petroleum ether extract and its chromatographically isolated fraction were obtained. The latter fraction was distilled under reduced pressure to get an orange-coloured oil, (b.p. 200–20°/2-3 mm). Both had antitumour activity. The orange-coloured oil, on further distillation under reduced pressure, yielded Bhilawanol. An acetyl derivative of the oil was also obtained. The latter two also had antitumour activity.

Key words : *semecarpus anacardium* oil; derivatives of *S. anacardium* oil; pharmacology of S. anacardium oil; effects against murine tumours

INTRODUCTION

Semecarpus anacardium Linn.f. (Family : *Anacardiaceae)* is a deciduous tree distributed in the sub-Himalayan tract and in hotter parts of India (13). In vernacular language, it is known as Bibba (Marathi), Bhilawa (Hindi) and Bhallataka (Sanskrit). The fruit is known as the marking nut, since the juice of pericarp is used for marking cotton clothes. The nut of *S. anacardium* is reported useful, particularly in the treatment of lepra nodules, warts and rheumatism (3).

S. anacardium has been under investigation for its antitumour properties and certain extracts of *S. anacardium* (whole fruit) have exhibited promising antitumour

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properties (2,8,9,14).It has been observed that chloroform extract of nut (pericarp and seed), designated as "SAN-AB", gives symptomatic relief to patients suffering from oesophageal cancer and myeloid leukemia (19). In view of such observations, fractionation studies on *S. anacardium* (whole fruits-nut with thalamus) were undertaken with a view to isolating and characterizing the active principle(s) in the plant. This paper reports observations on fractionation studies, antitumour activity and pharmacological effects of some promosing *S. anacardium* fractions.

MATERIALS AND METHODS

Preparation of the extracts : Authentic samples of *S. anacardium* (whole fruit) were procured from the local market. The extracts of freshly crushed material (1.0 *kg*/ batch) were obtained by employing two methods. In method-I, the crushed material was extracted with methanol in a Soxhlet apparatus for 32 hr. The solvent from the extract was removed under reduced pressure (10 *mm*). The methanol extract (200 g) was transferred to a separating funnel and partitioned between chloroform (2 lit) and water (2 lit containing 200 *ml* methanol). The contents were shaken vigorously for 20 min, then occasionally and kept overnight. The chloroform layer (2 lit) was separated, concentrated and again partitioned between petroleum ether (2 lit) and methanol (2 lit containing 200 *ml* water).

The methanol-partitioned fraction (Method-I) was subjected to chromatography on Silica gel (60-120 mesh, BDH) and the column was eluted with different solvents. The benzene-eluted fraction yielded a brown-coloured resinous material (yield, 0.5%).

Method-II consisted of initial cold extraction of fruits with petroleum ether (60-80°) by percolation, followed by Soxhlet extractions of the residue with petroleum ether (60-80°), benzene, chloroform, ether, ethyl acetate and methanol in succession. The solvents from these extracts were removed under reduced pressure (10 mm) to get the corresponding extracts.

Isolation of oil, (b.p. 200-20°/2-3mm) : Petroleum ether extract obtained by cold percolation (Method-II), (67 g) was transferred to petroleum ether (60-80°) and chromatographed on silica gel (60-120 mesh, BDH). The major benzene eluate was collected and concentrated under reduced pressure (10 mm), when an oily material was obtained (yield, 34 g). On distillation under reduced pressure, this benzene eluate yielded an orange-coloured oil, (b.p. 200-20/2-3 mm) (yield, 17 g). It gave characteristic ferric chloride test indicating the presence of a phenolic hydrpxyl group in the oil.

Its spectral data showed UV : λ_{max}^{MeOH} 280 and 210 nm, IR : ν_{max}^{Neat} ~ 3450 (OH stretching, Catechol), ~ 2900 (CH_3 and CH_2), 2850 (CH_2 stretching), 1625 (C=C), 1595 (aromatic), 1480 (CH_2) and 1280 cm^{-1} (benzene, aromatic). NMR (CCl_4, δppm) showed singlet at 6.6 (aromatic 3H), broad singlet at 6.0 (hydroxy protons 2H), triplet at 5.35 (olefinic protons, 4H) and a 4-peak broad multiplet from 2.9 to 0.9 accounting for 23 protons. These data suggest that oil contains pentadecyl catechol (Bhilawanol) (16).

Preparation of the derivatives of S. anacardium oil : Acetyl, methyl and benzoyl derivatives of the oil (b.p. 200-20°/2-3 *mm)* were prepared as soon as the oil was freshly obtained (yield, 17 g). Bhilawanol (16) was obtained by redistillation of oil under reduced pressure when a light yellow oil (b.p. 215-17°/3*mm)* was obtained (yield, 13 g). Its diacetate and dimethylate were obtained following the usual procedures (6). A compound, m.p. 70-72°, was obtained by hydrogenating acetylated Bhilawanol in the presence of freshly prepared Raney nickel as catalyst. The derivatives were charaterized by qualitative tests and physico-chemical methods.

Preparation of test material for antitumour activity : The test material was dissolved in refined ground nut oil (Postman Brand) so as to make a concentration of 40 *mg/ml.* Different doses (400, 200 and 100 *mg/kg/day)* were tried intraperitoneally to ascertain the toxicity of the material. The tolerable single dose was administered intraperitoneally to BDF_1 mice, 24 hr after the tumour transplantation and treatment was given for 9 days. Different routes and schedules of administration were tried in other sets of experiments. The animals were kept on normal diet and their body weights were recorded on days 1 and 5. Deaths were recorded daily.

Anti-tumour activity evaluation : The maintenance of P388 lymphocytic leukaemia in DBA/2 mice was carried out because this strain is the inbred strain in which the tumour was originally induced. For the purpose of drug testing, this tumour is transplanted into F_1 hybrid (DBA/2♂♂ x C57BL/6 ♀♀) as the number of off-springs in F_1 hybrid is larger. Mice of either sex (6 weeks old and weighing between 18-20 g) were used for experiments.

The inoculum of cells (10^6/mouse) from a seven-day-old P388 lymphocytic leukemia was given intraperitoneally in BDF_1 mice which caused 100% mortality between 8 and 11 days. Control and experimental mice were given inoculum of cells as described above. Six mice of either sex were used to evaluate antitumour activity at each dose

level. The median survival time (MST) for P388 was determined and the results are expressed as percent survival time of treated animals versus that of control animals as described by National Cancer Institute Drug Screening Protocol (7). In general, percent T/C values equal to or greater than 125 indicate significant antitumour activity.

Some promising fractions of *S. anacardium* were also tested on L1210 lymphoid leukaemia and Sarcoma 180 (solid) according to NCI Drug Screening Protocol (7), but they did not produce activity (Unpublished data).

The pharmacodynamic (1,4,18,20,21), antibacterial and antifungal (5) activities were screened using the active and well-tolerated fraction of *S. anacardium*, an orange coloured oil (b.p. 200-20°/2-3 mm) on standard animal models and bacterial and fungal organisms *in vitro*.

RESULTS

It can be seen from Table I that the methanol extract and the resinous material from it produced anti-tumour activity. Attempts to purify the resinous material were, however, unsuccessful. Several fractions obtained by partitioning the methanol extract in appropriate polar and non-polar solvents and subsequent chromatography over silica gel yielded sub-fractions possessing anti-tumour activity. However, the TLC pattern and spectral data of these fractions were identical.

Petroleum ether extracts (cold/hot) also produced anti-tumour activity (Table I); while other successive extracts did not demonstrate any promising activity. The fractionation of petroleum-ether extract, under high vacuum, yielded an orange-coloured oil, (b.p. 200-20°/2-3 mm) which also produced good antitumour activity against P388 tumour (Table I). In order to increase the therapeutic efficacy of oil, various dose levels, routes and schedules were tried. The oil has produced the maximum antitumour activity at 200 *mg/kg* when given intraperitoneally (Table I).

As regards the routes of administration, only intraperitoneal administration produced good antitumour activity (Table II). High doses and oral route of administration did not enhance antitumour activity. There were variations in antitumour activity when different administration schedules were followed (Table III). A single dose of oil (400 *mg/kg)* compared fairly well with the dose of 200 *mg/kg* given for nine days.

Apart from the ground nut oil which was usually used as a vehicle, Tween 80 and saline emulsion were tried, but there was loss in antitumour activity. Other vehicles such as ethyl alcohol, dimethyl sulphoxide and propylene glycol could not be used due to the

TABLE I : Anti-tumour activity of extracts and oil of *S. anacardium* against P388 tumour.

	Material	*Dose mg/kg x 9 (IP)*	*Survivors on day 5**	*Av. body wt. diff***	*Survival ratio****	*T/C%*
(a)	*Extracts*					
	Hot methanol extract.	400	3/6	—0.4	—	Toxic
	(yield. 35.4%)	100	6/6	—2.3	11/10	110
		100	5/6	+0.2	14/10	140
	Resinous material	400	0/6	—	—	Toxic
	(methanol extract)	200	6/6	- 1.7	17/9	188
	(yield. 0.5%)	90	6/6	- 2.0	16/10	160
		60	6/6	- 1.2	13.5/10	135
	Petroleum ether extract	400	3/6	—	—	Toxic
	(cold percolation)	200	5/6	- 6.6	9/11	82
	(yield. 28.0%)	100	6/6	+0.1	15/12	125
	Petroleum ether extract	400	6/6	- 1.4	13.5/9.5	142
	(hot successive)	200	6/6	- 1.5	13.5/10	135
	(yield. 1.59%)					
	Benzene eluate	400	5/6	- 6.3	—	Toxic
	(Petroleum ether	200	5/6	- 5.0	10/11	91
	extract) (yield. 14.2%)	100	6/6	- 2.0	15/10.5	143
(b)	*S. anacardium* oil	400	6/6	- 0.3	14/11	127
		200	6/6	+0.1	15.5/11	141
		100	6/6	+0.1	14.5/11	132
		50	6/6	- 0.1	11.5/10	115
		25	6/6	+0.9	11.5/10	115

* $\frac{\text{No. of animals surviving in the treated group.}}{\text{No. of animals surviving in the control group}}$

** Difference (g) between average change in body weight in the treated animals and average change in body weight in control animals on day 5.

*** $\frac{\text{Median survival time (days) of treated animals (T)}}{\text{Median survival time (days) of control animals (C)}}$

solubility and toxicity problems. The nut oil on keeping outside for one year, showed decreased antitumour activity as compared to the freshly distilled sample. This is probably due to air-oxidation which is apparent from the change in its colour from orange to black.

None of the extracts, obtained by preparative TLC of the oil, possessed anritumour activity against P388. Of the six bands, only the major one appeared to get air-oxidised. Hence, suitable derivatives of the oil were prepared as soon as it was freshly distilled. Acetyl and methyl derivatives of the oil and Bhilawanol showed activity against P388 tumour (Table IV).

There was no effect on the cardiovascular system in the anaesthetized cat when the *S. anacardium* oil (5 *mg/ml* in propylene glycol) was administered intravenously. There was no effect on CNS in mice, isolated guinea-pig ileum and chemically induced acute inflammatory reaction in rats. The oil did not possess significant antibacterial or antifungal activity.

TABLE II : Antitumour activity of oil given by different routes of administration.

Route	*Dose mg/kg x 9*	*Survivors on day 5**	*Av. body wt. diff.***	*Survival ratio****	*T/C%*
Intraperitonerl	400	6/6	− 0.3	14/11	127
	200	6/6	+0.1	15.5/11	141
Peroral	400	6/6	− 2.1	10.5/10	105
	200	6/6	− 0.5	11.5/10	115
Subcutaneous	400	6/6	+0.5	12/10.5	114
	200	6/6	+1.2	10.5/10.5	100
Intramuscular	400	6/6	+0.2	9/9.5	95
	200	6/6	− 0.8	9/9.5	95

*No. of animals surviving in the treated group / No. of animals surviving in the control group

**Difference (g) between average change in body weight in the treated animals andaverage change in body weight in control animals on day 5.

***Median survival time (days) of treated animals (T) / Median survival time (days) of control animals (C)

TABLE III : Variations of anti-tumour activity of oil given by different administration schedules.

Schedule (days)	*Dose mg/kg (IP)*	*Surrivors on days 5**	*Ar. body wt. diff***	*Surviva ratio****	*T/C%*
1	400	6/6	− 1.9	14/9.5	147
	200	6/6	− 0.9	12/9.5	126
1–4	400	6/6	− 1.8	8.5/11	77
	200	6/6	− 1.3	14/11	127
5–9	200	6/6	− 1.8	12/11	109
1,3,5,7,9,11	200	6/6	− 1.7	14.5/11	132
1,5,9,13	400	6/6	− 1.9	11.5/11	105
	200	6/6	− 1.3	14/11	127
1,5,9@	33.3	6/6	− 0.5	14/11	127

*No. of animals surviving in the treated group / No. of animals surviving in the control group

**Difference (g) between average change in body weight in the treated animals and average change in body weight in control animals on day 5.

***Median survival time(days) of treated animals (T) / Median survival time (days) of control animals (C)

@On days 1,5,9 six injections were given on each day at the interval of four hr.

TABLE IV : Antitumour activity of the derivatives of oil against P388 tumour.

Derivative	*Dose mg/kg x 9 (IP)*	*Survivors on day 5**	*Av. body wt. diff.***	*Survival ratio****	*T/C %*
Methylated oil	400	6/6	− 0.5	10.5/10.5	100
	200	6/6	+0.9	13.5/9.5	142
Acetylated oil	400	6/6	− 0.1	13/10.5	124
	200	6/6	− 0.6	14/10	140
Benzoylated oil	200	6/6	− 0.4	12/10.5	114
Bhilawanol	400	6/6	− 1.3	12.5/9.5	132
	200	6/6	− 0.7	12.5/9.5	132
Bhilawanol diacetate	400	6/6	+0.8	13/12	108
Bhilawanol dimethylate	400	6/6	− 0.4	13/11	118
Compound (I) m.p. 70-72°	200	6/6	− 0.5	11/11	100

*No. of animals surviving in the treated group / No. of animals surviving in the control group

**Difference (g) between average change in body weight in the treated animals and average change in body weight in control animals on day 5.

***Median survival time (days) of treated animals (T) / Median survival time (days) of control animals (C)

DISCUSSION

In view of the reported (sic) antitumour properties of *S. anacardium*, fractionation studies were undertaken to isolate and characterize the active principle(s) in the nuts. The response of P388 lymphocytic leukaemia to standard anticancer drugs has been studied by several investigators (7) and this tumour has been found to be a useful model for detecting active principle(s) which may ultimately be useful clinically.

Since the crude oil, expressed from *S. anacardium* nuts, is a powerful vesicant, its antitumour activity was not determined. The present study has indicated that a resinous material from methanol extract and an orange-coloured oil (b.p. 200-20°/2-3 *mm)* from petroleum ether extract of *S. anarcardium* have been found to possess antitumour properties. Although resinous material could not be further purified, the orange-coloured oil (b.p. 200-20°/2-3 *mm)* yielded Bhilawanol, a phenolic liquid containing long-chain alkenyl catechols. Qualitative tests, spectral data and preparation of certain derivatives were useful in the identification of Bhilawanol.

The derivatives were mainly prepared since the original oil was found to be unstable due to air oxidation. The extracts were also tested for *in vitro* cytotoxic activity, wherein small amounts of the material are required. Our preliminary studies indicate that *S. anacardium* oil and its acetyl derivative possessed the most significant growth-inhibitory activity against KB cell line *in vitro* (7). It has also been observed that the *in vitro* exposure of P388 cells to different concentrations of *S. anacardium* oil and its acetyl derivative are cytotoxic in nature; while acetyl derivative inhibits the macromolecular biosynthesis *in vitro* (15). Hembree *et al.* (11) have shown that one of the fractions isolated on the basis of cytotoxicity studies on Eagles 9 KB tumour cell culture contains a mixture of closely related unsaturated pentadecyl catechols which are cytotoxic. The present investigations on *S. anacardium* have indicated that the resinous material from methanol extract possesses maximum antitumour activity. It is possible that in this instance, the activity results from the blocking of an oxidized group present in the oil.

The experiments have been in progress to prepare more derivatives of the oil and also to stabilize and increase its anti-tumour activity using antioxidants. Since, there were no effects on any major physiological functions and also any antibacterial or antifungal activity was lacking, it could be presumed that the *S. anacardium* oil produces its anti-tumour effects through a mechanism which is not reflected in any acute physiological disturbance.

A review of literature shows that *S. anacardium* nut contains about 30% oil, Bhilawanol (6,10,16). Flavonoid compounds (12,17), sterols and their glycosides (14) have been reported in *S. anacardium*. Although, the principle anti-tumour constituent has not yet been reported, the present findings have given encouraging leads to the investigations on *S. anacardium*.

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