with 10% HCl for 2 h, cooled and filtered. The residue was washed first with water and then with 0.1 N NaOH and finally with water to neutrality. The residue was then dried at 60 C, powdered and soxhlet-extracted with $CHCl_3$ for 48 h. The $CHCl_3$ extract was evaporated and the diosgenin and bound sterols were quantified by comparing O.D. values with the standard curve constructed by preparative TLC method[6].

Exposure of *D. deltoidea* callus to any kind of light, i.e. blue, red or white was superior to dark incubated cultures for growth of tissues and production of diosgenin and sterols on culture basis (Table 1). Cultures grown under blue or red light alone reached higher growth values than those grown under white light. Continuous blue light was beneficial for diosgenin production and did not affect sterol content markedly over the control (treatment 3). Increase in production of diosgenin over the control under blue light was by a factor of 1.17 and 1.5 on percentage and absolute basis respectively. Red light inhibited diosgenin and sterol percentage but on absolute basis the production was at par with control (treatment 4).

Similar stimulatory effect of blue light on anthocyanin synthesis in *Haplopapus gracilis* was reported by Reinert *et al.*[7] They also found red light to have no effect on anthocyanin synthesis. It is interesting to note that in the treatments with blue light (Table 1) at second phase (i.e. treatments 5,7) or blue light followed by dark phase (treatment 10) there was increase in diosgenin percentage by 1.1- 1.75-, 1.28-folds respectively. Similarly, percentage of sterols increased in these treatment over the control. It was clear from treatment 5 that blue light could reverse the inhibitory effect of red light on diosgenin and sterol production. Kadkade and Andrade[8] showed that fluorescent light influences diosgenin synthesis in *Dioscorea* spp. over the cultures grown in dark. Both enhancement[9] and inhibitive[10] effects of red light on growth have been reported from *Pelargonium zenale* and *Daucus carota* respectively. It is clear from this study that quality of light influences the diosgenin production and blue light influences diosgenin synthesis more markedly than red light.

This study establishes that enhancement of growth of *D. deltoidea* and diosgenin as well as sterol production is possible by exposing culture to blue light which can be adopted in large scale production systems.

1. Holden, P. R., Holden, M. A. and Yeoman, M. M., in *Manipulating Secondary Metabolism in Culture* (eds Robins R. J and Rhodes, J. C.), University Press, Cambridge, 1988, p. 15.
2. Fontanel, A. and Tabata, M., in *Nestle Research News* (ed. Horisberger, M), Nestec Ltd, Switzerland, 1987, p. 93.
3. Seibert, M. and Kadkade, P. G., in *Plant Tissue Culture as a Source of Biochemicals* (ed. Staba, E. J.), CRC Press, Boca Raton, 1982, p. 123.
4. Kaul, B. and Staba, E. J., *Lloydia*, 1968, **31**, 171.
5. Heble, M. R. and Staba, E. J., *Planta Med Suppl.*, 1980, 124.
6. Sanchez, G. L., Acevedo, J. C. H. and Sota, R. R., *Analyst*, 1972, **97**, 973.
7. Reinert, J., Glauss, H. and Von Ardenne, R. *Naturwissenschaften*, 1964, **51**, 87.
8. Kadkade, P. G. and Andrade, J. E., in *Informe de Actividades Programa Multinacional de Extractos Vegetales*, (ed Rolz, C.), Instituto Centro Americano Investigacion. Technologia Industrial, Guatemala City, 1971.
9. Ward, H. B. and Vance, B. D., *J. Exp. Biol.*, 1968, **19**, 119.
10. Polevaya, V. S., *Fiziol. Rast.*, 1967, **14**, 48.

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Cardol: The antifilarial principle from *Anacardium occidentale*

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Cardol, a phenolic compound isolated from cashew nut shell, showed pronounced antifilarial activity. The hydroxyl groups and the alkyl side chain were found to be necessary for activity. Compared to diethylcarbamazine that was active at a concentration of 350 ppm(LC_{100}), cardol was active at as low a concentration as 3.5 ppm (LC_{100}). Cardol given orally was tolerated up to a concentration of 5 g/kg body weight in laboratory rats.

FILARIASIS is a major tropical disease. More than 400 million people in the world are infected by filarial parasites. *Wuchereria bancrofti* and *Brugia malayi*, the two common filarial parasites causing filariasis in the Asiatic region are estimated to infect around 800 million people[1]. Although the mainstay of therapy and control of filariasis is the drug 1-diethylcarbamyl-4-methyl-piperazine (DEC), by itself it does not provide a complete cure and is also reported to have harmful side effects[2]. Consequently considerable research has been taken up to develop an alternative to DEC[3–5]. The anthelmintic activities of many indigenous materials have already been reported[6–8]. In a recent study, *Anacardium occidentale* was found to be the most active among 28 medicinal plants screened for antifilarial activity. Among the compounds reported from cashew nut shell liquid, our studies showed the antifilarial activity to cardol. Investigations on the isolation, detection of activity and certain structural studies form the subject matter of this paper.

The filarial parasite of cattle was used as the test organism. This was specially selected for the *in vitro*

Table 1. List of plants screened for antifilarial activity.

Plant	Part used	Activity
Achyranthes aspera	Leaf	–
Adhatoda vasika	Leaf	–
Allium cepa	Bulb	+
Allium sativum	Bulb	+
Alstonic scholaris	Leaf	–
Anacardium occidentale	Seed shell	+
Ananas sativus	Leaf	+
Andrographis paniculata	Leaf	+
Bambusa arundinancea	Leaf	–
Calotropis gigantea	Leaf	+
Cuscuta reflexa	Stem	–
Cymbopogon citratus	Leaf	+
Datura fastuosa	Fruit	+
Guasuma tomentosa	Leaf and fruit	–
Hemidesmus indicus	Root	+
Hydrocotyle asiatica	Leaf	–
Ichnocarpus fruitescens	Leaf	–
Lichin odoriferous	Leaf	–
Melia azadirachta	Leaf	+
Ocimum sanctum	Leaf	–
Piper nigrum	Leaf	+
Ricinus communis	Leaf and seed	–
Sida cordifolia	Root	+
Tabernaemontana dichotoma	Fruit	+
Trachyspermum ammi	Seed	+
Trigonella foenum-graeceum	Seed	–
Vitex negundo	Leaf	+
Vitis vinifera	Leaf and stem	–

+ Indicates antifilarial activity; – indicates no activity.

study because of its established similarity to the human filarial parasite *W. bancrofti* and *B. malayi* in anatomical and biochemical features and the extreme difficulty of getting the adult human parasites. *Setaria digitata* was collected from the local slaughter house in Tyrode solution (composition w/v: sodium chloride 0.8%, potassium chloride 0.02%, calcium chloride 0.02%, magnesium chloride 0.01%, sodium bicarbonate 0.015%, sodium dihydrogen phosphate 0.05% and glucose 0.5%).

The list of medicinal plants selected was prepared from the *Indian Materia Medica*[7] and from discussions we had with local Ayurvedic (Indian System of Medicine) physicians. Plants were collected and identified according to Ganble[9].

The antifilarial activity was carried out by the *in vitro* technique. Three worms in 100 ml Tyrode solution were used in all the experiments. The anthelmintic studies were conducted by crushing known quantities of the fresh plant preparations in Tyrode solution in preliminary trials. Those that showed activity against the parasite were investigated further and the concentrations required to get complete mortality in 6 h was determined.

The cashew nut shell was extracted with petroleum ether, solvent ether and ethanol successively. The activity against the parasite was determined as the concentrations required to produce complete mortality (LC_{100}) in 6 h.

The solvent ether extract found to be the most active was further fractionated. The extract was dried, suspended in water, neutralized using sodium bicarbonate and exhaustively extracted with ether. The ether extract after removal of the solvent gave the phenolic fraction. The mother liquor was then acidified with dilute HCl and exhaustively extracted with ether. The ether was then removed which gave the acid fraction. Activity studies were carried out with these two fractions. The phenolic fraction, on passing through alumina column, gave a major compound with some minor ingredients. The major compound was identified to be cardol. The benzoyl derivative of cardol was prepared by standard methods. Controls were maintained for all the experiments by suspending the worm in drug-free medium. The chemicals and reagents, used were of highest quality available. DEC was purchased from Sigma Chemical Co, USA.

Among the 28 medicinal plants screened, antifilarial activity was detected only in 15 (Table 1). The active plants were further investigated and the minimum concentration required for eliciting action against *S. digitata* was determined. The results are shown in Table 2. *A. occidentale* was found to be the most active one with LC_{100} of 25 ppm. Systematic study located the activity to the solvent ether fraction. When further fractionated into bicarbonate soluble and insoluble fraction, most of the activity was located in the insoluble fraction. The insoluble fraction that proved to contain phenols was then separated by column chromatography in which the major and most active component turned out to be similar in properties to cardol, a phenolic compound reported from cashew nut shell liquid[10]. Spectroscopic studies confirmed the structure of the active antifilarial component to be cardol. The bicarbonate soluble

Table 2. Concentration of fresh plant materials for complete mortality of the parasite in 6 h.

Plant	Part used	Concentration (ppm)
Anacardium occidentale	Seed shell	2.5×10^1
Allium sativum	Bulb	6.0×10^2
Trachyspermum ammi	Seed	4.3×10^3
Vitex negundo	Leaf	4.8×10^3
Ananas sativus	Leaf	5.2×10^3
Allium cepa	Bulb	7.0×10^3
Cymbopogon citratus	Leaf	7.5×10^3
Melia azadirachta	Leaf	8.2×10^3
Piper nigrum	Leaf	1.2×10^4
Hemidesums indicus	Root	1.5×10^4
Tabernaemontana dichotoma	Fruit	2.0×10^4
Calotropis gigantea	Leaf	2.7×10^4
Sida cordifolia	Leaf	3.1×10^4
Andrographis paniculata	Leaf	4.6×10^4
Datura fastuosa	Fruit	5.0×10^4

Average of six experiments.

Table 3. Concentration of various compounds required for complete mortality of the parasite in 6 h.

Compound	Concentration (ppm)
Anacardic acid	1000
Cardol	3.5
Benzoyl derivative of cardol	100
Resorcinol	250
DEC	350

Average of six experiments.

fraction, which turned out to be anacardic acid was active at a concentration of 1000 ppm while cardol was active at comparatively very low concentration of 3.5 ppm. The concentration worked out for resorcinol was 250 ppm and that for the benzoyl derivative of cardol, 100 ppm. The above experiment was repeated with the standard drug DEC and the comparative results are given in Table 3.

Cashew nut shell liquid was reported[11] to contain mainly anacardic acid (82%), cardol and 2-methyl cardol (16.5%). The structures of anacardic acid and cardol are given in Figure 1*a* and *b* respectively. It is clear that both the compounds have hydroxyl group in position 1 and alkyl side chain in position 3 of the benzene ring. The only difference is that anacardic acid has a carboxylic group in position 2 while cardol has a hydroxyl group in position 5. Lot of studies have been carried out on the biological activities of *A. occidentale*[12-15]. While anacardic was reported to possess powerful biological properties[16-18], cardol was reported to be a toxic material[19]. Our studies showed cardol to be a very powerful antifilarial agent while anacardic acid has only very weak antifilarial activity. Preliminary studies on laboratory rats fed with cardol indicate that cardol was tolerated without any visible untoward symptoms up to a concentration of 5 g/kg body weight. Hence the statement that cardol is toxic warrants further detailed study.

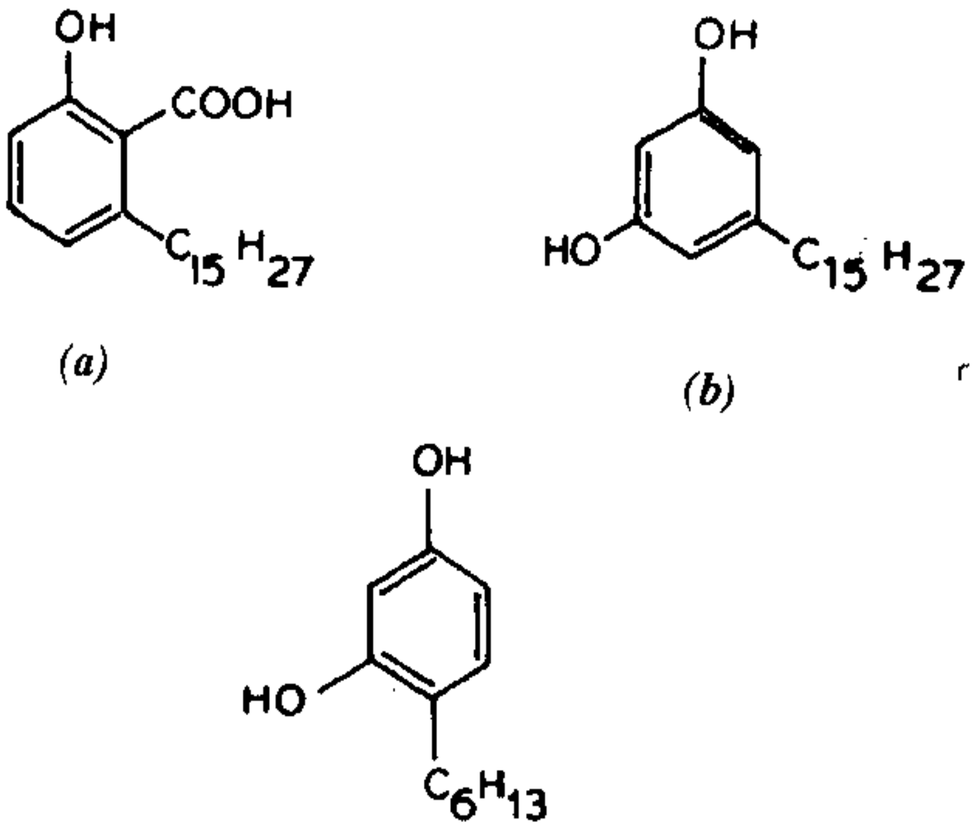

Figure 1. Structure of *a*, anacardic acid; *b*, cardol; *c*, hexyl resorcinol.

It was found that both the hydroxyl groups and the unsaturated alkyl side chain of cardol are required for antifilarial activity. When the activity was studied with resorcinol lacking the alkyl side chain and with benzoyl derivative of cardol lacking the two hydroxyl groups, a decrease in activity was observed and this is clear from Table 3.

Many phenolic compounds have been tested and used in treating many worm infections. 4-*n*-hexyl resorcinol (Figure 1*c*) possess excellent anthelmentic properties and because of its low toxicity is recommended as a desirable nematocide and taenicide[20]. Cardol differs from hexyl resorcinol only in the alkyl side chain. It is speculated that the observation of the antifilarial activity of cardol will open up a new era in the development of a series of new antifilarial agents.

1. WHO Tech. Rep. Ser., No. 702, 1984, World Health Organization: Fourth report of an expert committee on Filariasis.
2. Hawking, F., *Diethylcarbamazine*, WHO/ONCHO/ 78, 142.
3. Shukla, U. K. et *al.*, *Indian J. Chem.*, 1983, **B22**, 664.
4. Xi, *et al.*, *Acta Pharmacol. Sin.*, 1984, **5**, 207.
5. Angelo, *et al.*, *J. Med. Chem.*, 1983, **26**, 1250.
6. Chopra, R. N., Nayar, S. L. and Chopra, I. C., *Glossary of Indian Medicinal Plants*, Council of Scientific and Industrial Research, New Delhi, 1956, pp. 1-262.
7. Nadkarni, K. M., *Indian Materia Medica* (eds. Purenik, M. V. and Bhatkal, G. R.), Dhoota Papeswar Prakashan Ltd, Panvel Kolaba and The Popular Book Depot, Bombay, 1954, pp. 1-1318.
8. Kaleysa Raj, R., Ph. D. thesis, University of Kerala, Trivandrum, 1966.
9. Ganble, J. S., *Flora of Presidency of Madras, 2 and 3* (ed. Guha Ray). Sree Saraswathi Press Ltd, Calcutta, 1967.
10. Backer, H. J. and Baack, N. H., *Recl. Trav. Chim. Pays-Bas*, 1941, **60**, 661.
11. Tyman, J. H., *Anal. Chem.*, 1976, **48**, 30.
12. Kubo, I., *Sci. Today*, 1985, **2**, 69.
13. Kubo, I., Komatsu, S. and Ochi, M., *J. Agric. Food Chem.*, 1986, **34**, 970.
14. Dhar, M. L., Dhar, M. M., Dhawan, B. N., Mahrotra, B. N. and Ray, C., *Indian J. Exp. Biol.*, 1968, **6**, 232.
15. Gedam, P. H. and Sampathkumaran, P. S., *Prog. Org. Coat.*, 1986, **14**, 115.
16. Lloyd, H. A., Denny, C. and Krishna, G., *J. Lig. Chromatogr.*, 1980, **3**, 1497.
17. Sullivan, John, T., Richards, Charles, S., Lloyd, Helen, A. and Gopal, Krishna, *Planta Med.*, 1982, **44**, 175.
18. Yoshiro Yamagiwa, *Tetrahedron*, 1987, **43**, 3387.
19. Wasserman, D. and Dawson, C. R., *J. Am. Chem. Soc.*, 1948, **70**, 3675.
20. Back, Jr. F. L. and Kushner, *Medicinal Chemistry* (ed. Alfred Burger), Interscience Publishers, New York, 1960, pp. 1061–1063.

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