Investigations on the essential oil of leaves of *Uvaria narum wall*

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The essential oil of the leaves of *Uvaria narum wall* (Annonaceae) was obtained by steam distillation. Out of the 80 components detected in the oil, 8 were identified by GC-MS analysis. The oil showed good antibacterial and anthelmintic activity.

UVARIA NARUM wall (Annonanceae) is a small shrub or a woody climber growing in the Western Ghats of India. In previous studies, the root bark essential oil of this plant was reported to possess antibacterial, antifungal, anthelimintic and CNS depressant activities[1] and 22 components of this oil have been detected[2]. The present study was undertaken to determine the composition of the essential oil from the leaves of this plant and to carry out its antimicrobial and anthelmintic studies *in vitro.*

Fresh leaves of *Uvaria narum* wall, were collected from Thiruvananthapuram, Kerala, in January, 1994. Essential oil was obtained by steam distillation in a Neo-Clevenger's apparatus (0.035% v/w). GC-MC analysis of the volatile oil was recorded on a 5971 series II H-GC-MS.

Antibacterial activity was tested by disc diffusion technique[3] and antifungal activity by the poisoned food technique[4]. Bacteria used were *Staphylococcus pyogenes, Bacillus brevis, B. circulans, B. licheniformis, Salmonella typhi, Escherichia coli, Klebsiella aerogenes, Pseudomonas pyocyaneae and Proteus vulgaris.* Fungi included *Candida albicans, Aspergillus niger, Penicillium notatum, Trichophyton mentagrophytes, Microsporum gypseum and Epidermophyton floccosum.*

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For the anthelmintc study, the method of Gaind et al[5] was used with the cattle parasite *Haemonchus contortus*, obtained from the Department of Animal Husbandry, Government of Kerala. An emulsion of the oil was prepared using 4% gum acacia and dilution with saline to give different concentrations. Mebendazole was used as standard.

About 80 components were detected in the essential oil, of which 8 were identified by comparison of their mass spectral data with reference spectra from DATA BASE.NBS 54 K.L. These were caryophyllene, 4, 7, 10-cycloundecatriene, 1, 1, 4, 8-tetramethyl cyclohexane-4-methylene-(1-methyl ethenyl) cyclohexane-1,2- benzene dicarboxylic acid , 3-nitro cyclopentadecanone-2-hydroxy octadecanoic acid butylester and 1,5-hepatodiene-2,5-dimethyl-3- methylene. Among these, caryophyllene has been reported from the root bark essential of the plant[1,2].

The oil showed good activity against a range of bacterial species except *Proteus vulgaris* and totally inhibited the fungi *Penicillium* and *Aspergillus.* The oil also showed good anthelmintic activity. The volatile oil from the roots of the plant showed significant antimicrobial action against *B. megathecium, E. Coli* and *Candida albicans*[1].

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Colorimetric determination of *Thiamine Hydrochloride* using *Alzarin Briliant Violet R.*

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A new colorimetric method for the determination of thiamine hydrochloride is developed. It is based on the formation of coloured ion-pair complex between thiamine hydrochloride and alizarin brilliant violet R in the aqueous phase at pH 4.5 extractable into chloroform which obeyes Beer's law in the range of 2-16 mcg/ml.

SEVERAL colorimetric procedures are reported in literature[1-10] for the determination of thiamine hydrochloride. The present method is based on the formation of coloured ion pair complex between thiamine hydrochloride and alizarin brilliant violet R in the aqueous phase at pH 4.5 extractable into chloroform in which it is stable for 4 hours and is measured at 575 nm.

Chemito 2500 U.V-Visible scanning spectrophotometer, aqueous solution of alizarin brilliant violet R (0.2% w/v) and potassium hydrogen phthalate-sodium hydroxide solution (pH 4.5) were used.

About 10 mg. of thiamine hydrochloride (pure or formulations) was accurately weighed and dissolved in distilled water to make 100 ml. which gave a concentration of 100 mcg/ml of thiamine hydrochloride.

In a 100 ml separating funnel containing 25 ml of chloroform, aqueous solutions of 0.5 ml - 4 ml of thiamine hydrochloride (50 mcg - 400 mcg), 2 ml of alizarin brilliant violet R (0.2%), 6 ml of potasium hydrogen phthalate-sodium hydroxide buffer (pH 4.5) and appropriate volume of water to bring the total volume to 10 ml were added. The seperator was shaken for 2 min and set aside for separation. The chloroform layer was separated and the absorbance of the blue coloured chloroform extract was measured at 575 nm against a reagent blank. The standard graph was plotted using absorbance versus concentration.

The sample solutions were also treated in a similar manner as in the standard curve. The amount

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