

in tablets and capsules also did not interfere in the estimation of 82/437 by the proposed methods.

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Chemical Constituents and Bioactivity Studies of *Hibiscus micranthus* Linn.

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Petroleum ether and benzene extract of leaves and stem of *Hibiscus micranthus* afforded long chain alkanes, alcohols, an acid, a ketone and β -sitosterol and the ether extract of aerial parts furnished phenolic acids. The ethanolic extract of aerial parts of roots demonstrated significant antifungal and anticancer activity.

ALTHOUGH a number of plants of the genus *Hibiscus* (Fam. Malvaceae) have been widely studied¹⁻⁴, a survey of literature revealed no reports on the chemical investigation of *H. micranthus*. Hence, a systematic chemical and pharmacological examination of this plant was undertaken. Herein, we report the isolation of some alkanes, alcohols, an acid, a ketone, β -sitosterol and some phenolic acids from pet. ether and benzene extract of leaves and stem separately and from ether extract of the aerial parts alongwith the antimicrobial, antiviral and antitumor efficacy of ethanolic extract of aerial parts and roots.

The plant material was collected from the campus of the University of Rajasthan, Jaipur and identified from the Herbarium, Department of Botany. The dried ethanolic extract of leaves and stem after reextraction with pet. ether and benzene separately and the dried ethanolic extract of the aerial part after reextraction with ether were taken up for phytochemical studies.

The TLC behaviour of the pet. ether and benzene extract of leaves were same, so these were mixed together and subjected to column chromatography over silica gel. Elution of the column with pet. ether afforded pentacosane⁵ (m.p. 55-57°), nonadecanone-10 (m.p. 64-65°) and docosyl alcohol⁵

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(m.p. 66-68°). Nonadecanone-10 is an unusual compound which gave positive 2,4-dinitrophenyl hydrazine test for ketones, it exhibited ν_{\max} 1720 (C=O), 730, 720 cm^{-1} (CH_2)_n in IR spectrum, in its ¹H NMR spectrum two terminal methyl groups (δ 0.72, s), two methylene groups adjacent to the carbonyl function (δ 2.10, t) and methylene groups (δ 1.26, m) were observed. The mass spectrum displayed M⁺ at m/z 282 corresponding to the molecular formula C₁₉H₃₈O. The presence of abundant ions at m/z 155, 127 (α -fission) and 170, 112 (β -fission) involving McLafferty rearrangement⁶ in addition to other peaks with uniform loss of 14 mu indicated the position of carbonyl group at C-10. The absence of (M-Me)⁺ peak indicated the straight chain nature of the ketone. Pet. ether : benzene (3:1) on elution furnished tricosyl alcohol⁵ (m.p. 70-71°). Elution with pet. ether: benzene (1:1) afforded pentacosyl alcohol⁵ (m.p. 76-78°) and myristic acid⁵ (m.p. 57-58°). Elution with pet. ether : benzene (1:3) afforded β -sitosterol⁵ (m.p. 136-137°).

Combined pet. ether and benzene extract of stem was subjected to column chromatography (Si-gel). Elution of column with pet. ether afforded tetracosane⁵ (m.p. 54-56°), octacosane⁵ (m.p. 60-62°) and eicosyl alcohol⁵ (m.p. 65-66°); tetracosanol⁵ (m.p. 70-74°) and β -sitosterol⁵ eluted with pet. ether : benzene (3:1). Elution of the column with pet. ether : benzene (1:1) afforded an ester of β -sitosterol⁵ (m.p. 70°) which on hydrolysis afforded β -sitosterol and an unidentified aliphatic acid.

The ether extract of aerial parts was extracted with 10% solution of NaHCO₃. The alkaline portion was separated, acidified with 5% HCl solution and reextracted with ether. The ether layer was separated, which on evaporation yielded a light brown residue, which was subjected to column chromatography to afford vanillic acid⁵ (m.p. 208-10°) eluted with benzene : ethyl acetate (2:3) and protocatechuic acid⁵ (m.p. 197-98°) eluted with benzene : ethyl acetate (1:4). The ether layer left after the extraction with NaHCO₃ was extracted with 4% NaOH solution,

the alkaline portion was acidified with 10% HCl and reextracted with ether. This, on evaporation provided an orange-brown residue, which on column chromatography afforded naringenin⁵ (m.p. 245°) eluted with chloroform : methanol (4:1), kaempferol⁵ (m.p. 282°) eluted with CHCl₃: MeOH (4:1) and quercetin⁵ (m.p. 309-10°) eluted with CHCl₃: MeOH (3:2).

Biologically, the antifungal and antibacterial activity using disc diffusion method⁷ of the ethanolic extract of the aerial parts and roots and the antiviral (by using plaque inhibition method)⁸ and antitumor (using Sarcoma 180 A in mice)⁹ activities of the aerial parts were determined.

In case of antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Pseudomonas aeruginosa*, both the extracts failed to demonstrate any significant activity except trace activity against *S. aureus*.

In antifungal efficacy, both the extracts were found to inhibit the growth of all four selected fungi (*Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme* and *Rhizoctonia bataticola*). The ethanolic extract of aerial parts showed maximum activity against *F. moniliforme* (IZ = 13 mm, 1000 $\mu\text{g}/\text{disc}$), while the extract of roots demonstrated maximum inhibition against *R. bataticola* (IZ = 26 mm, 1000 $\mu\text{g}/\text{disc}$).

Ethanolic extract of aerial parts failed to demonstrate any antiviral activity against the test viruses - *Poliomyelitis*, *Coxsackie*, *Semliki forest*, *Herpes simplex*, *Measles* and *Vesicular stomatitis*.

In case of antitumor activity (using Sarcoma 180 Å) the ethanolic extract of aerial parts demonstrated significant degree of tumor inhibitory property (16.4%, GR : ++)⁹.

On the basis of above mentioned results we can say that the plant *H. micranthus* exhibited significant antifungal and antitumor efficacy.

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Three simple Spectrophotometric Methods for the Assay of Ketotifen in Pharmaceutical Formulations

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Three simple and sensitive spectrophotometric methods for the determination of ketotifen based on the formation of a charge-transfer complex between ketotifen and chloranilic acid (method A, λ_{max} : 550nm), by the inner molecular complex with sodium nitroprusside (method B, λ_{max} : 770 nm) or oxidation with excess potassium permanganate and the determination of unconsumed permanganate using Fast Green FCF (method C, λ_{max} : 625 nm) have been developed.

A survey of literature revealed only a few reported methods which include a single titrimetric¹, three UV¹⁻³ and two visible^{4,5} spectrophotometric and three HPLC⁶⁻⁸. This paper describes three visible spectrophotometric methods for the determination of Ketotifen by exploiting char-

acteristic properties due to the presence of tertiary amino group (formation of charge-transfer complex⁹ with chloranilic acid, method A or inner molecular complex¹⁰ with sodium nitroprusside, method B) and unsaturation¹¹ (oxidation with excess potassium permanganate and the determination of unconsumed permanganate using Fast Green FCF, method C).

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