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Research article

Comparative pharmacological study of Aerial parts and roots of ethanolic extract of Hibiscus micra thus Linn

Rekha Tripathi*

Department of Applied Sciences, Maharaja Surajmal Institute of Technology, New Delhi, India

Corresponding author: Rekha Tripathi, ✉

Department of Applied Sciences, Maharaja Surajmal Institute of Technology, New Delhi, India

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ABSTRACT

The present study deals with bioactivity studies of ethanolic extract of aerial parts and roots of H. micranthus Linn. (Family: Malvaceae). The ethanolic extracts (aerial parts and roots) were examined for antimicrobial studies by using Disc diffusion method, antiviral efficacy was undertaken by using plaque inhibition method and antineoplastic activity was tested by using Sarcoma 180A0 as the test system. The data of present study suggested that H. micrathus extract showed significant antifungal and antitumor activity.

Keywords: H. micranthus, Aerial parts, Roots, Ethanolic extract, Pharmacological studies.

INTRODUCTION

Medicinal plants are the nature's gift to human being to make disease free healthy life. In India different parts of medicinal plants have been used for curing various diseases from ancient time. The genus Hibiscus comprises about 46 species, 20 of which are found in India and some of these are found to be medicinally very important. H. micrathus is a herb growing in the hotter parts of India. It is used as a febrifuge. A survey of literature revealed that no work on the pharmacology is reported so far. Hence a systematic pharmacological examination of aerial parts and roots of this plant were undertaken [1, 2].

MATERIALS AND METHODLOGY

The plant material was collected from the campus of the University of Rajasthan, Jaipur and identified from the Herbarium. Department of Botany. The aerial parts and roots were dried under shade separately and then powdered with a mechanical grinder and stored in airtight container.

Bactericidal and fungicidal assay

Ethanolic extract of the powdered material was prepared by employing Soxhlet method. The extract of both parts was concentrated in vacuo and stored in dark colored bottles at 4^0C in a refrigerator separately. For both bactericidal and fungicidal assays Disc diffusion method[5] was adopted, because of reproductivity and precision. The different test organisms were preceded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whatman No. 1 paper (6 mm in diameter), which were containing 500 µg and 1000 µg of the test extracts and control amikacin (10 µg/ml) or mycostatin (100 units /ml) as reference drugs separately. These plates were initially placed at low temperature for 1 h, so as to allow the maximum diffusion of the compound from the discs into the agar plate and later, incubated at 37^0C for 24 h in case of bacteria and 48 h for fungi, after which the zones of inhibition could be easily observed [3].

Virucidal efficacy

50g of the fresh plant material (aerial parts and roots) were macerated in a waring blender with 150 ml of 80 % ethanol. The mixture was stirred at room temperature for 2 h, filtered (E1) and marc was percolated (E2) with 805 ethanol again to exhaustion. Both of filtrate (E1) and percolate (E2) were pooled and concentrated to a thick residue at a temperature, not to exceed 40^0C.Each of the residual mass was dissolved separately in sterile 0.01 M physiological tris buffer (8ml; pH 7.2) diluted with tissue culture medium (M-2; 128 ml) and adjusted to pH 7.2 with 1N NaOH. The diluted extract was filtered

through a Whatman GF-2 paper disc, followed by sterilisation (by filtration through a Millipore membrane filter,0.02 µ) and later, stored at ~ 300 °C, until tested.

The virucidal activity of plant extracts was tested by the plaque inhibition method. Confluent cultures of VERO cells were prepared in tissue culture dishes (50 mm diameter) and infected with 0.2 ml Poliomyelitis virus suspension in M-2 medium containing approx. 100PFU. After an absorption period of 1 h, the cells were washed to remove any unabsorbed virus and overlaid with 5 ml tissue culture medium (M-2) and 0.8% acetone purified agar at 42^0 C. After the solidification, a paper disc (6 mm diameter) impregnated with 0.2 ml of serial 2-fold dilution of plant extracts in M-2 medium was centrally placed on the surface of agar overlay and incubated at 37^0C for 2 days. A second agar overlay of the same composition supplement with 0.03% neutral red was added and incubation was continued for at least 2 days at 37^0C until the plaques were well developed. The discs were surrounded by a clear zone of toxic cells destruction and outside this, by plaque –free zone, where the zone of plaques were smaller than the normal, and later, the plaque-inhibition zones were measured in mm.

Antineoplastic efficacy

100g each of dried and powered plant material (aerial part and roots) were percolated at room temp. for 48 h and the remaining residue was re extracted using soxhlet apparatus for 16 h. Both these fractions were pooled together, dried under reduced pressure and stored at room temp, until used.

Total packed cell volume method was adopted for the antineoplastic screening. Five weeks old ICR albino mice (20± 3 g) implanted i.p. Sarcoma - 180Å (1x 10^6 cells/0.1 ml ascitic fluid) was used for experimentation in each of the mice. In each experiment, six animals per test group were used. Each of the test sample suspended in physiological saline solution mixed with 0.5

% carbon-methyl-cellulose (CMC) was given daily to the test animals at a dose of 100mg/kg/day(i.p.) for consecutive 5 days. A control set was also run parallel, where the animals were given only physiological saline solution. Each of the experimental animals were sacrificed after 7 days from ascites tumor implantation. The ascites of each animal was centrifuged (3000 rmp) for 5 min to isolate the tumor cells, the volume of the tumor cells (packed cell volume; PCV) and total volume of the ascites (total volume; TV) were determined in each case [4, 5].

RESULTS AND DISUSSION

In case of bactericidal activity against *S.aureus , E. coli, K. aerogenus, P. vulgaris* and *P.aeruginiosa* , both of extract failed to demonstrate any significant activity except trace activity against *S.aureus*.

In case of antifungal activity against *A. flavus, A. niger, F.moniliforme* and *R. bataticola* , the ethanolic extract (aerial parts and roots) was found to inhibit the growth of all four selected fungi. Aerial parts showed maximum activity against *F.moniliforme* (IZ= 13mm, 1000µg/disc), whereas the extract of roots exhibit maximum activity against *R. bataticola* (IZ= 26mm, 1000µg/disc).

In case of antiviral activity against the test viruses – *Semliki forest, Herpes simplex, Measles* and *Vesicular stomatitis*, ethanolic extract of aerial parts showed weak activity against *Semliki forest* and *Vesicular stomatitis* (R=10, 125µg/ml). Whereas the roots demonstrated good activity against *Semliki forest* (R=100, 250µg/ml and R=10, 125µg/ml) and weak activity against *Vesicular stomatitis* (R=10, 250 µg/ml).

In case of antitumor activity (using Sarcoma 180 Å) both the extracts exhibited significant degree of tumor inhibitory property (aerial parts = 16.4% , GR: ++ and roots= 12.1%, GR: ++)

On the basis of above results we can say that plant H. micranthus exhibited significant anti-fungal and antitumor activity, but on comparison between aerial parts and roots, it indicated that root extract demonstrated better efficacy in both the cases (antifungal and antitumor) and it also showed good activity against virus also [6].

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