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EXAMINATION OF *GIRARDINIA DIVERSIFOLIA'S* PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES (URTICACEAE)

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Abstract: Stem and root extracts from Girardinia diversifolia showed variable levels of efficacy against the following microorganisms: Saccharomyces cerevisiae, Aspergillus niger, Bacillus pumilus, Staphylococcus aureus, and Escherichia coli. The petroleum ether root extract yielded three distinct compounds: 3-hydroxystigmast-5-en-7-one, 7-hydroxysitosterol, and β-sitosterol. Girardinia diversifolia has been traditionally used to treat microbial infections; this study provides scientific validation for this practice.

INTRODUCTION

Another name for Girardinia diversifolia (Link) Friis (Urticaceae) is huge nettle. It is an upright annual plant that can grow to a height of 75–300 cm, depending on rainfall and soil conditions. Its leaves are big, up to 8 cm broad, alternating, lobed, and coarsely dentate. The base of the stem can enlarge to 4 cm in diameter. The roots are fibrous, and the flowers are either monoecious or dioecious, appearing in dense axillary inflorescences. Long, thin, stinging trichomes cover the leaves, stems, and flowers.(1)

G. diversifolia leaves are applied externally to cure scrofula and as an astringent . Constipation, stomach issues, and malignant boils are treated with an oral infusion of the roots and basal stems. The root is administered externally to alleviate headaches, aching joints, and cuts and wounds. The plant's ashes are applied externally to treat ringworms and dermatitis, a decoction of the plant is used to treat fevers. (2)

EXPERIMENTAL :

Plant collection and identification:

In September 2006, the roots and stems of G. diversifolia were harvested from the Kamweti Location, which is close to Mount Kenya Forest in the Kirinyaga District of Kenya. A taxonomist identified and verified the plant on the spot. A specimen of the plant (voucher specimen number SoP/06/001) is kept in the herbarium of the University of Nairobi's Department of Botany. The gathered roots and stems were allowed to dry on the ground and at room temperature.(3)

PREPARATION OF EXTRACTS *:*

The powdered root and stem were subjected to a series of Soxhlet extractions using petroleum ether (60-80 °C), chloroform, and methanol. The extracted materials were then dried in a vacuum. The ethyl acetate extract was produced by dividing the methanol extract between water and ethyl acetate.(4)

Isolation of compounds from the petroleum ether root extract :

Eleven fractions were obtained from the petroleum ether root extract by gradient elution from benzene to chloroform on silica gel. Colorless plates (I), colorless needles (II), and tiny colorless plates (III) were produced by the recrystallization of fractions F8, F9, and F10 in acetone, respectively.(5)

Screening for antibacterial and antifungal activities of *Girardinia diversifolia:*

The method employed was the agar diffusion assay. To obtain working cultures, the test microorganisms were subcultured in their recommended nutritional media for 18 hours over night. Each extract was made into a 50 mg/ml solution in dimethyl sulfoxide.(6)

The Hewitt and Vincent methodology was followed to prepare test solutions of standard antibiotics, which were gentamicin 10 µg/ml, benzylpenicillin 1 IU/ml, and nystatin 150 µg/ml.

(7)The manufacturer's instructions were followed in the preparation of the nutrient media for the test microorganisms, which were then autoclaved at 125°C and cooled to 50°C. Every cultivated bacterium was suspended in 5 milliliters of distilled water that had been sterilized, and then around 5 milliliters of this suspension were added to the corresponding growth medium to create agar that had roughly 1×106 colony forming units per milliliter. Next, the inoculated nutrient media were quickly but carefully poured into petridishes so that each petridish had 20 ml of agar with a consistent thickness of 3 mm. (8) After cooling, the stacked agar solidified into a firm gel that could be used for the plating-out process.

The nutritive medium for the test microorganisms was prepared in accordance with the manufacturer's instructions, and it was thereafter autoclaved at 125°C and cooled to 50°C. To make agar with approximately 1×106 colony forming units per milliliter,(9) 5 milliliters of sterilized distilled water were used to suspend each grown bacterium. This suspension was then put to the corresponding growth medium. Subsequently, the nutritional media that had been infected were swiftly yet carefully filled into petridishes, ensuring that each one had 20 milliliters of agar that was consistently 3 mm thick. (10) The stacked agar cooled and formed into a solid gel that was suitable for plating out.

Before the petridishes were incubated for 18 hours at 37 °C, a pre-diffusion time of 1 hour was permitted to aid in the diffusion of the applied solutions into the inoculation media. The zones of inhibition were measured and photographed to maintain their sizes.(11)

B. pumilus and S. cerevisiae, the test microorganisms, were cultured at 37 °C for eighteen hours.

Bioautography (12)

The preparation of Sabouraud's dextrose agar nutrient medium and tryptone soy agar followed the manufacturer's recommendations. By dissolving 100 mg of petroleum ether, chloroform, and ethyl acetate extracts in 5 ml of chloroform and the methanol extracts in 5 ml of methanol, a 20 mg/ml solution of each extract was created. The two glass-backed TLC plates were then spot-spotted with a 100 µl aliquot of each solution, and the plates were developed using a CHCl3:MeOH, 90:10% v/v mobile phase. One plate received a 100 µl aliquot of 10 µg/ml gentamicin solution, and the other received a 100 µl aliquot of 150 µg/ml nystatin solution. (13)

The produced tryptone soy agar was homogeneously inoculated with a standardized B. pumilus inoculum at 50 °C. The inoculated agar was then quickly but carefully spread over the formed TLC plate to create a uniform 1 mm thick agar layer. The nystatin-containing TLC plate and Sabouraud's dextrose agar inoculated with S. cerevisiae were used to repeat this process. Before incubating at 37 °C for 18 hours, the plates were allowed to stand for 1 hour at room temperature to allow for a pre-diffusion interval. (14)(15)

To destroy the microorganisms, the bioautograms were sprayed with a methylthiazolyl tetrazolium bromide solution, incubated for an additional 4 hours at 37 °C, and then sprayed with 100% ethanol. The bioautograms were photographed and examined for bioactive components determined by their Rf values.(16)

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