

Antibacterial behaviour of root extracts of *Tragia involucrata* L. in gradient extraction

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ABSTRACT - *Tragia involucrata* has been widely used for treating different diseases in ancient systems of medication. Tribal people use the plant root in controlling skin infections and asthma. In this study, roots of *Tragia involucrata* checked for antibacterial nature and the occupancy of phytochemical constituents in discrete solvent extracts in accelerating polarity with respect to disease producing bacterial species implicated in skin diseases in human beings. Antibacterial activity tested by disc diffusion method. The results pointed out that the roots showed antibacterial power mainly in acetone extract. Petroleum ether and water extracts could not present a little antibacterial ability towards all of the tested organisms. As the extraction proceeded from non-polar to polar solvents, gradual reduction in activity was noticed. Phytochemical analysis indicated that phenolics and flavonoids were predominant in acetone extracts. Presence of phenols and flavonoids in acetone decoction can be considered as one of the judgements for antibacterial potential of roots *Tragia involucrata*. Acetone extract of roots of the plant manifested minimum inhibitory concentration around 50mg/ml. Acetone extract of roots of the plant demonstrated minimum bactericidal concentration at the rate of 100mg/ml with respect to *Pseudomonas aeruginosa*.

Keywords: *Tragia involucrata*; Euphorbiaceae; Antibacterial; disc diffusion; MIC

INTRODUCTION

Tragia involucrata L. (Euphorbiaceae), commonly known as kodithoova or choriyanam (Malayalam) and Indian stinging nettle (English) [1] was selected as the experimental material. The plant is a perennial evergreen climbing herb with scattered stinging hair, stem slender, elongate. Twining leaves are simple alternate, leaves not cordate at base, acute in uppermost, stipulate oblong lanceolate to broadly ovate, serrate base, flowers shortly pedicellate and leaf opposed racemes, male flowers many in the upper part, female flowers few in the lower part. Ovary 3 celled, ovules 1 in each cell, fruit capsulate, and 3 lobed, seeds globose and smooth [2]. *Tragia involucrata* has been reported to have hypoglycaemic activity diabetic rats, brought about with alloxan [3]. Roots of *Tragia involucrata* extracted in methanol extract manifested significant wound healing effect towards *Staphylococcus aureus*-induced ablation wound in rats [4]. Vinyl hexylether, a compound isolated from roots of *Tragia involucrata* demonstrated a wide spectrum nature of antibacterial action [5]. Antiinflammatory action of aqueous extract of *Tragia involucrata* was proved in hind paw oedema, induced with carrageenan and cotton pelleted granuloma designs in albino rats. [6]. Hydrocarbon esters found in *Tragia involucrata* exhibited antimicrobial activity [7]. The response of methanolic extract of *Tragia involucrata* was tested in different laboratory animal models and affirmed that the extracts hooked prominent analgesic and anti-inflammatory activity [8]. Tribal and non-tribal indwellers of Andhra Pradesh used *Tragia involucrata* for managing asthma [9]. Roots of *Tragia involucrata* used for controlling skin diseases by Kani tribals in Kouthalai region of Tirunelveli hills, Tamil Nadu state of India [10]. Roots of *Tragia involucrata* have larvicidal and oviposition deterrence activity [11]. Present study seeks to evaluate antibacterial potential of the plant in different gradient extracts. The decoctions are prepared in varied solvents with rising polarity with respect to pathogenic microorganisms that cause varied skin infection.

MATERIALS AND METHODS

Formation of plant extract

Fresh specimens procured in the December from Kottayam District in Kerala State, India. A voucher specimen (TT 1140) was stored at the herbarium, present in St. Thomas College Palai. The shade-dried roots of the plant body (100 g) was milled and employed for formulating extracts. Soxhlet extracts made in successive manner in petroleum ether, acetone, ethanol and water [12].

Microorganisms employed

Organisms procured from the culture mass of the Institute of Microbial Technology (IMTECH) Chandigarh. Microorganisms comprise *Escherichia coli* (MTCC-443), *Staphylococcus aureus* subsp *aureus* (MTCC-96), *Serratia marcescens* (MTCC-97), *Pseudomonas aeruginosa* (MTCC-741), and *Klebsiella pneumoniae* subsp *pneumoniae* (MTCC-109). The bacteria mentioned above are involved in different skin

associated diseases [13]. The microorganisms were sub cultured at regular intervals on nutrient medium, incubated at 37°C for about 24 hours; it was kept at 4°C in the refrigerator, as the stock form culture.

In vitro antibacterial test

The disc diffusion method as explained by Bauer et al. [14] was employed for determining the growth suppression of bacteria by various plant extracts. Sterilized Mueller Hinton Agar (pH 7.4 ± 2) media was emptied into aseptic petri dish. It was allowed to solidify and bacterial suspension (1 ml broth of around 10⁵ CFU) was swabbed with the help of a sterile swab prepared with cotton under aseptic environment. Sterile discs were developed using Whatman type No. 4 Filter Paper. Discs with 5-mm diameter were utilised for the experiment. Solvent used for preparing the extract was used in one of the discs and is denoted as the control disc. Materials used for testing were liquefied in the exact solvent to get a stock solution with a concentration around 100 mg/ml. 10 µL of the extract was poured in each and every disc to get a concentration around 1 mg/disc. All the discs were employed after drying it in an incubator set at 50°C to eliminate whatever remnant of solvent. Then the discs were put onto the superficial surface on the medium. Finally, the plates were incubated overnight at suitable incubation condition. Now, microbial growth was measured by taking the inhibition zone diameter. All the experiments were performed around three repeats and average inhibitory zone diameter was recorded with its standard deviation.

Minimum inhibitory concentration (MIC)

The MIC of the extracts was tested by placing various amounts (128–0.125 mg/ml) of the extract into various sets of test tubes containing the culture medium [15]. 50 µl of the bacterial broth culture mixed into every test tube. Then, the microbial cultures with the plant extracts were nurtured at 37° C for 24 hours. Test tubes with the growth medium and each of the test organisms, nurtured under the exact conditions were employed as positive controls. The minimum inhibitory concentration was articulated as the lowest concentration of the extracts that could not permit visible growth of bacteria as it was compared with the control culture tubes.

Minimum bactericidal concentration (MBC)

Test tubes in the MIC assays, which could not display any visible development after a specified time of incubation, were again cultured on a freshly developed bacterial nutrient setting [16]. The MIC was taken as the minimum titre of the extract which could not develop any colony on the bacterial nutrient medium after twenty-four hours of kept in proper incubator temperature.

Preliminary testing of phytochemicals

The crude samples employed for phytochemical testing, especially for the detection of phenolics, alkaloids, Triterpenoids, flavonoids employing the method of Harborne [17].

RESULTS AND DISCUSSION

Antibacterial potential and phytochemical testing of *Tragia involucrata* were tested with petroleum ether, acetone, ethanol and water as distilling solvents, and utilises in the nature of rising polarity. The acetone decoction of *Tragia involucrata* showed maximum activity as it was compared to others. The results are disclosed in the Table 1 and 2. The plant showed antibacterial activity towards *Serratia marcescens*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* at the same time, acetone decoction was not working against *Staphylococcus aureus*. Similar result obtained by Gobalakrishnana et al. [18] for acetone extract of *Tragia involucrata*. Maximum activity was found in the case of *Pseudomonas aeruginosa*. Roots of *Tragia involucrata* prepared in petroleum ether extract of did not exhibit antibacterial action towards all worked out bacterial species. Water extracts demonstrated little antibacterial activity towards the tested organisms. Ethanol extract acted in the case of bacteria, *Pseudomonas aeruginosa*.

Table 1: Antibacterial activity of *Tragia involucrata*

<i>Tragia involucrata</i> prepared in solvents	Inhibition zone (mm) Value = Mean ± SD				
	K.p (G-) MTCC-109	S.a (G+) MTCC-96	P.a (G-) MTCC-741	E.c (G-) MTCC-443	S.m (G-) MTCC-97
Petroleum ether (60°C to 80°C)	-	-	-	-	-
Acetone	8.73± 0.19	-	9.2 ± 0.24	8.97± 0.23	9.03 ± 0.32
Ethanol	-	-	7.23± 0.21	-	-
Water	-	-	-	-	-

*(-) = No antibacterial action. Value = Mean \pm SD; Disc diameter 6 mm; K.p, *Klebsiella pneumoniae* subsp *pneumoniae*; P.a, *Pseudomonas aeruginosa*; S.a, *Staphylococcus aureus* subsp *aureus*; S.m, *Serratia marcescens* and E.c, *Escherichia coli*, negative control (solvent blank) G+ Gram positive; G- Gram negative

Table 2. Phytochemicals detected in different extracts of *Tragia involucrata*

Extract used	Phytochemicals found (values: + present; - absent)			
	Flavonoids	Alkaloids	Triterpenoids	Phenolics
Petroleum Ether	-	+	-	-
Acetone	+	-	-	+
Ethanol	+	+	-	+
Water	-	-	-	+

Pseudomonas aeruginosa was the most sensitive organism towards acetone extract and hence acetone extract was employed for further antibacterial tests, MIC and MBC. MIC value of 50mg/ml and MBC value of 100mg/ml were found in the case of *Pseudomonas aeruginosa*. Ayyanar [10] reported that roots of *Tragia involucrata* used for controlling skin diseases by Kani tribals found Kouthalai region of Tirunelveli hills, Tamil Nadu state, India. The present work approved the antibacterial property of the root and it is working towards tested pathogens. These tested pathogens have role causing various skin diseases. The phytochemical experimental work of *Tragia involucrata* revealed that phenolics and flavonoids were common in active acetone extract. Petroleum ether extract possessed the presence of alkaloids. Ethanol extract also showed the presence of alkaloids and flavonoids when tested. The occurrence flavonoids and phenolics in acetone extract of roots of the plant could be one of the reasons responsible for its antibacterial action of roots. The present result could support the ethnobotanical capacity of the plant in regulating skin diseases as earlier reported earlier by Ayyanar [10], and Savithamma et al. [9]. Another observation indicated that, if we proceeded down the extraction from non-polar to polar solvents, a gradual reduction in activity was noticed.

Conclusion

Roots of *Tragia involucrata* demonstrated for the antibacterial action and phytochemical constituents in different solvent extracts of root of the plant in ascending polarity towards disease causing bacterial species found in skin diseases associate with human beings. The results showed that the roots showed antibacterial activity mainly in acetone extract. Petroleum ether and water extracts exhibit little antibacterial activity towards any of the evaluated bacterial species. As the extraction continued from non-polar to polar solvents, gradual reduction in activity was noticed. Phytochemical analysis indicated that phenolics and flavonoids were predominant in acetone extracts.

Conflict of interests

The authors have not declared any conflict of interests.

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