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Antibacterial Activity of *Scoparia dulcis* Extract on Uropathogenic *Escherichia coli* and *Staphylococcus aureus.*

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ABSTRACT

Time immemorial, medicinal plants have been the source of various therapeutic agents. *Scoparia dulcis* is a well-known medicinal plant in India known for its immense potential in the treatment of the diabetes. Though the plant is used to treat only diabetes, it is also known to possess antibacterial properties. The antibacterial property of *Scoparia dulcis* against *Escherichia coli* and *Staphylococcus aureus* is of interest because these bacteria are the most common pathogens involved in urinary tract infections (UTI). *In vitro* antibacterial activity of the four extracts (petroleum ether, ethanol, chloroform, and acetone) was compared by means of agar well diffusion method. Further analysis was carried out with the ethanol extract exhibiting the highest antibacterial activity. Minimum inhibitory concentration was then determined by micro-dilution based method and found to be 510µg/ml for *E.coli* and 437µg/ml for *S.aureus*. The rate of killing of the uropathogens was also found by performing the modified method of time kill assay. Furthermore, morphological changes on the cell induced by the extract were analyzed using Scanning Electron Microscopy. *Scoparia dulcis* is a potent inhibitor of both *E.coli* and *S.aureus* exhibiting bactericidal and bacteriostatic action respectively. The characterization of the bioactive components will help in determining the efficiency of the plant extract in being used as a commercial drug against urinary tract infections in future.

Keywords: *Scoparia dulcis, Escherichia coli, Staphylococcus aureus,* uropathogens, ethanolic extract, time kill assay, agar well diffusion method.

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INTRODUCTION

The search for natural plant compounds with antimicrobial activity has been ever increasing due to continuous emergence of antibiotic resistance in pathogenic bacteria [1]. *Scoparia dulcis* L. is a widely distributed tropical and subtropical plant commonly known as sweet broom. Traditionally the plant has been used for the treatment of diabetes in India .The fresh or dried plant has also been used as remedies for stomach troubles, hypertension, inflammation, bronchitis, hemorrhoids, hepatosis and also as an analgesic and antipyretic. Extensive research has been focused on the secondary metabolites of the plant for its bioactivity such as antimicrobial, cytotoxic, analgesic and hypoglycemic [2-5]. The bioactivity of the various extract of this plant has been attributed to the presence of the various novel phytochemical compounds including scoparic acid A, scoparic acid B, scopadulcic acid A and B, scopadulciol and scopadulin[6,7]. Several authors have reported the antimicrobial activity of the *Scoparia dulcis* extracts against a wide range of bacteria and fungi [8,9,10]. Reports on the activity of the plant on UTI (urinary tract infection) causing agents are very few. In the present study we report the antibacterial activity of the ethanol extract of the *Scoparia dulcis* leaves on UTI causing *E.coli* and *S.aureus.*

E.coli and *S.aureus* are opportunistic pathogens being the most common causative agents of UTI. In most UTI cases, patients are usually indulged in indiscriminate usage of antibiotics [11, 12]. The extensive unsystematic usage and improper prescription of antibiotics involved in the treatment of UTIs are significant causative factors to the appearance and increase of bacterial resistance to the commonly used antimicrobial drugs [13].

MATERIALS AND METHODS

Fresh and healthy plant parts of *Scoparia dulcis* were collected from Marthandam region of Kanyakumari district, Tamilnadu. The plant was taxonomically identified by Dr.V.Chelladurai , Former Research Officer, CCRAS (Central Council of Research in Ayurveda and Siddha, Govt. of India).

Preparation of leaf extract

The leaves were surface sterilized by successive washings with distilled water and sodium hypochlorite.The sterilized leaves were shade dried at 27 ± 2 ^{o}C for a few days. After drying the leaves were grinded coarsely to powder was then stored for future use in a sealed container [14]. The leaf extract was prepared by the method of Devendra *et al.,* 2011[15].

The dried powder was soaked in four fifferent solvents like petroleum ether, chloroform, ethanol and acetone at a concentration of 2 ml/g of powder for 2 days and later filtered using Whatman No.1 filter paper. The filtrate was dried to evaporate the solvent. A rotary vacuum evaporator was used for drying the filtrate while the powder was allowed to dry separately. A dark green paste was obtained after drying and this paste was resuspended in DMSO for further analysis.

Antibacterial screening

The urinary tract infection causing pathogens *Escherichia coli* and *Staphylococcus aureus* were obtained from the clinical isolates obtained from Madurai Medical College. The mother culture was sub-cultured on nutrient agar slants and these subcultures were used for further investigations. Prior to experimentation the preserved culture was re-suspended in nutrient broth medium and incubated for 24 hrs at 37˚C.

Agar well diffusion technique

Sterile petriplates were taken and 15-20 ml of nutrient agar medium was poured into the plates. Once the agar has solidified, about 50-100µl of bacterial broth cultures were poured and swabbed using sterile cotton swabs. After swabbing, agar wells were made on the plates by puncturing the agar using sterile micro-tips. Wells of about 5mm diameter were made. In each well about 20µl of the extract was injected. The positive control used was an antibiotic disc (chloramphenicol, 25 µg, Himedia, Mumbai, India). The incubation

was done at 37˚C for overnight. The results were observed after incubation. Clear zones of inhibition were observed around the wells that contained antibacterial agents.

Minimum Inhibitory Concentration (MIC)

Dilution susceptibility testing is used to determine the minimum concentration of a drug or antibacterial agent required to inhibit or kill the microorganism.

The procedure was based on broth micro-dilution method technique as described by the National Committee for Clinical Laboratories standards [16]. The uropathogenic strains were suspended in sterile physiological Tris buffer (of pH 7.4, 0.05 M). This was then homogenized and adjusted to an optical density of 0.05 at 530 nm (1 X 10^6 CFU/ml -McFarland standard). 100µl of these bacterial suspensions (both *E.coli* and *S.aureus)* were inoculated into the wells of 96 well plate. One positive control using chloramphenicol (antibiotic) and one negative control (culture without any sample) was also inoculated into the wells. The extract was added at different volumes such as 5, 10, 20, 30, 40, 50, 60, 70, 80 and 90µl into the wells. The well plate was incubated at 37˚C for 24hrs. After 24hrs, the readings were taken using an ELISA reader at 620nm. A graph was plotted to determine the MIC for both the uropathogens.

SEM Analysis

The extract treated cells were subjected to SEM analysis using JEOL (JSM-6380 LA) scanning electron microscope by following a modified method of biofilm inhibition assay [17]. Both treated and non treated cultures of *E.coli* and *S.aureus* strains were added to the wells. The treated cultures contained the minimum inhibitory concentration of *S.dulcis* extract (specifically for both the strains) in the broth. The plate was incubated at 37˚C for 72hrs. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 minutes.

Time kill assay

The time kill assay is performed to determine the rate of killing of the uropathogens by the *Scoparia dulcis* extract. The test was carried out by following modified method as described by Olufunmiso *et al.,* [18].

RESULTS AND DISCUSSION

Various polar and non polar solvents have been used for the extraction of bioactive components from the different parts of the plant. Our present study, the ethanolic extract was used for the antibacterial assay based on the observations of the agar diffusion assay (Table 1) and also on previous reports on the inhibition of virulence factors of the uropathogens by the ethanolic extract [19].

Table 1 Zones of inhibition observed for the different extracts against the two strains (mm)

Extract	*E.coli*	*S.aureus*
Petroleum ether	6±0.2	4±0.3
Ethanol	12±0.32	14±0.21
Chloroform	8±0.2	11±0.5
Acetone	11±0.4	12±0.3

It was observed that in the present study the ethanolic extract exhibited better activity against both strains in comparison with other solvent extracts. The next potent inhibitor of both the strains was the acetone extract. Followed by acetone, was the chloroform extract with a medium level activity and the petroleum ether extract was the least effective extract for both the strains *S.aureus* and *E.coli.* Generally studies indicated the bioactivity of the plant to be extracted effectively in polar solvents such as ethanol [10, 15]. The leaf extract was also found to exhibit antibacterial activity against S.aureus and *E.coli* which correlate to the prior studies [20]. The MIC of the extract that inhibited the growth of the uropathogens, was determined from the graph drawn using the OD values taken at 620nm.

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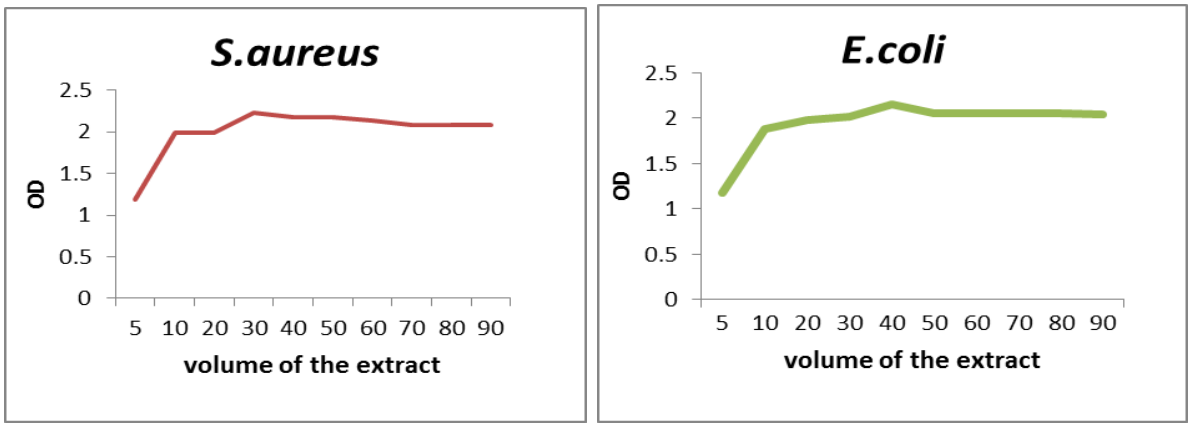

Figure 1: Determination MIC by microdilution method

From the graph, it was found that *S.aureus* was inhibited at a volume of 40 µl and *E.coli* at 50 µl. Upon calculation, the MIC of the ethanolic extract of the *Scoparia dulcis* leaves was found to be 510µg/ml for *E.coli* and 437µg/ml for *S.aureus.* Abu Hasanat *et al.,* [21] found the MIC of the ethanolic extract of the whole plant to be 256µg/ml against *Salmonella typhi* and *Staphyloccocus aureus* while attributing bioactivity to the synergistic effect of the bioactive metabolites of the whole plant.

The possible mode of action of the crude ethanol extract on the morphology of the treated cells was determined by scanning electron microscopy. The microscopic analyses clearly showed differences in morphology between untreated and treated cells**.** The SEM micrograph for untreated *S.aureus* showed well defined shape with normal and smooth surface of clustered cells (Characteristic of *S.aureus* cells). Comparatively extract treated cells showed disaggregation of the cell cluster (Figure 2). The SEM micrograph for untreated *E.coli* cells showed well defined, smooth surfaced normal cells. On the other hand, extract treated cells had changes in the membrane morphology (Figure 3).

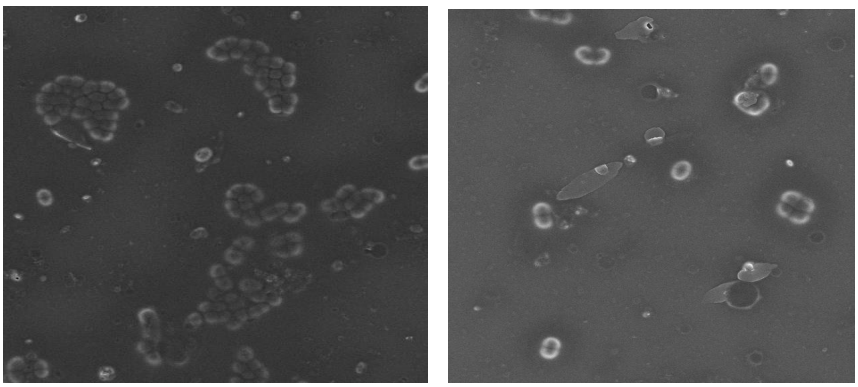

Figure 2: SEM micrograph of untreated and extract treated *S.aureus* cells

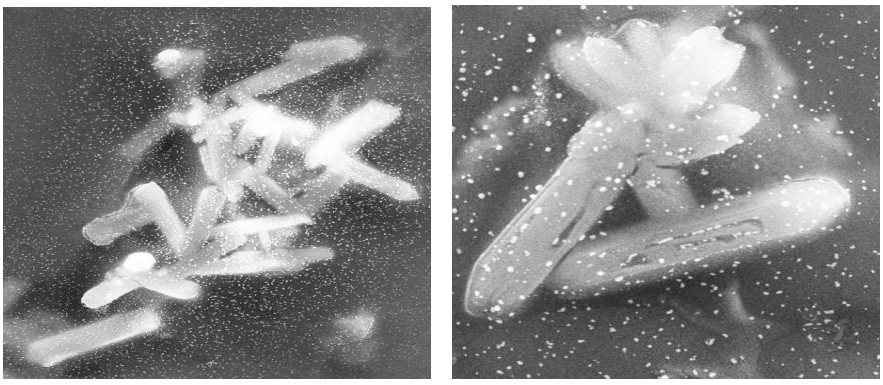

Figure 3: SEM micrograph of untreated and extract treated *E.coli* cells

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A modified method of time kill assay was used to determine the effect of the crude ethanolic extract on the growth pattern of *E.coli* and *S.aureus*. The bacterial strains were incubated along with the extract at the MIC and the growth monitored every hour. Soroj kumar reported that the rate of kill of test bacterial cells varied with concentrations of extract, duration of exposure and the bacterial strains tested [22]. Our present study, the extract was used at the MIC specifically indicating the inhibition of the growth pattern of both the strains. It was observed that the growth of *E.coli* was inhibited from the 6th hour of incubation. On the other hand the growth of *S.aureus* was inhibited from the 4th hour of inhibition (Figure 4and Figure 5). These observations indicated the bactericidal action of the crude extract. Bactericidal action was acknowledged by the significant reduction in the cell counts between 4th hour and 8th hour of incubation according to Olufunmiso *et al*., [18, 23]. It was observed from the graph that after the 8th hour, the uropathogenic colonies were completely wiped out. The negative control reveals the increasing order of growth of the bacteria and the positive control shows a straight line that indicated the completed inhibition of bacterial colonies from the time of incubation.

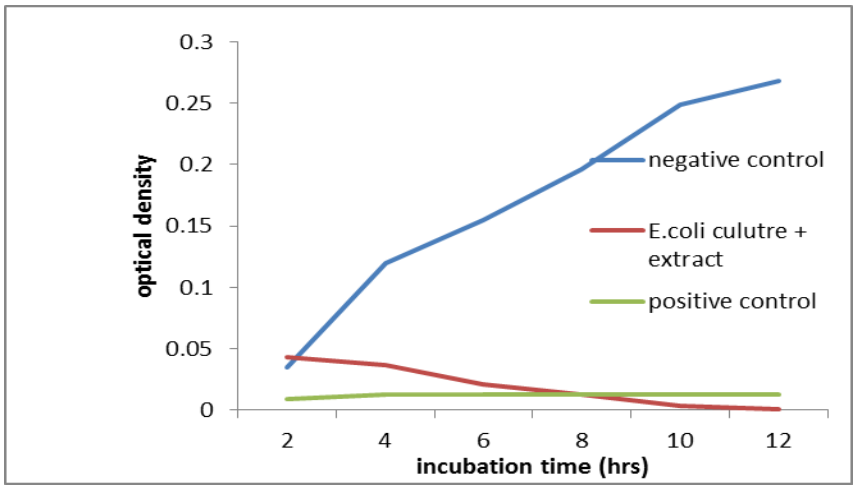

Figure 4: Effect of the crude ethanolic extract on the growth pattern of *E.coli*

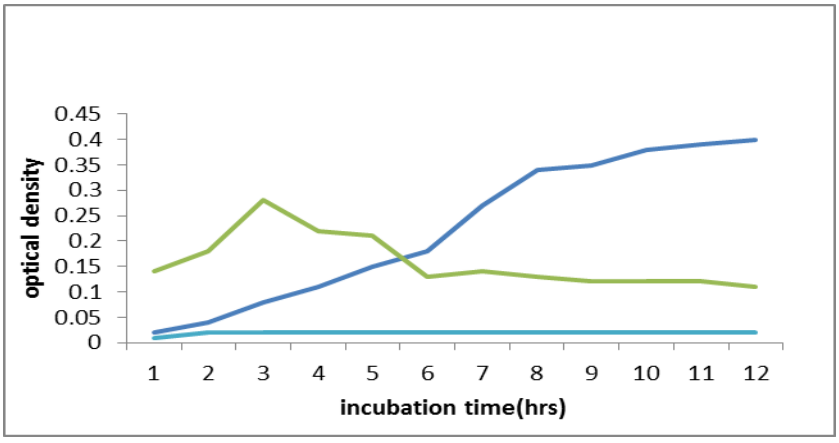

Figure 5: Effect of the crude ethanolic extract on the growth pattern of *S. aureus*

According to Ojo et al, a constant logarithmic rate of kill has been assumed during a time-kill. A 90% kill at 6h is equivalent to a 99% kill at 24h [24].

CONCLUSION

On the basis of the results of our study, we conclude that the crude ethanolic extract of the leaves of *Scoparia dulcis* possess antibacterial activity against uropapathogenic *E.coli* and *S.aureus*. *E.coli* colonies were completely inhibited at 4th hour of incubation along with the extract indicating a strong bactericidal action of

the extract which is further confirmed with the morphological changes observed by SEM. While the *S.aureus* colonies were inhibited from, 6th hour of incubation along with the extract indicating a rather bacteriostatic activity of the extract. Further studies on the purification and characterization of the bioactive components of the extract are essential for development as a drug.

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