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SCREENING OF TWO ARID ZONE PLANT EXTRACTS AGAINST SOME BACTERIA AND FUNGUS

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The present study describes the isolation and identification of active antimicrobial principle from crude extract of flavonoids in different plant parts (root, stem and flower) of *Heliotropium curassavicum* L. (Boraginaceae) and *Spergula arvensis* L. (Caryophyllaceae) *in vivo* against Gram-positive bacteria, Gram-negative bacteria and fungal pathogen.

Keywords : Active antimicrobial principle : Crude extract; *Heliotropium curassavicum; Spergula arvensis.*

Introduction

Staphylococcus aureus (Gram+ve coccus) is responsible for food poisoning, osteomylitis and septicaemia, *Bacillus cerus* (Gram+ve bacillus) causes foodborne illnesses, severe nausea, vomiting and diarrhoea, *Salmonella typhi* (Gram-ve bacillus) causes typhoid fever, paratyphoid fever, and the foodborne illness salmonellosis, *Escherichia coli* (Gram-ve bacillus) causes diarrhoea and urinary tract infections and *Candida albicans* (fungal pathogen) causes skin diseases.

Medicinal values of *Heliotropium curassavicum* and *Spergula arvensis* for diarrhoea, urinary infection, treated wounds and ulcers and use as ingredients in the pharmaceutical and cosmetic industries have been reported. All above diseases are directly related to *S. aureus, B. cerus, S. typhi, E. coli* and *C. albicans.* Hence, these microorganisms were used for testing the antimicrobial activity of extracted flavonoids (antimicrobial principle).

Material and Methods

For present investigation fresh, healthy and fully matured plant parts root, stem and flower of *H. curassavicum* and *S. arvensis* were collected from different areas of Bikaner. These plant parts were dried, powdered and weighed for further experiment.

The plant parts selected for the present studies were screened for their antimicrobial activity against some test microorganisms *Staphylococcus aureus* (NCIM 2454), *Bacillus cerus* (NCIM 2156), *Salmonella typhi* (NCIM 2501), *Escherichia coli* (2685) and *Candida albicans* (NCIM 3684), repsectively, by paper disc method.

Extraction and preparation of test samples (Isolation of Active principles) - Dried, weighed and powdered samples (root, stem and flower) of *H. curassavicum* and *S. arvensis* were Soxhlet extracted with 80% hot ethanol[1] on a water bath for 24 hrs. This crude extract, dried and dissolved in minimal amount of distilled water, was used as test sample. Each of extracts was concentrated and reextraced with petroleum ether (40°C-60°C, Fraction I), ethyl ether (Fraction II) and ethyl acetate (Fraction III) in succession. Each of the steps was repeatd three times to ensure complete extraction in each case. Fraction I was rejected due to its being rich in fatty substancs where as fraction II was analysed for the free flavonoids and fraction III was analysed for bound flavonoids in each of the samples.

Fraction III of each of the test sample was hydrolysed by refluxing with 7% H_2SO_4 (10 ml/gm residue) for 2 hrs. The mixture was filtered and the filterate extracted with ethyl acetate in separating funnel. The ethyl acetate layer was washed with distilled water to neutrality, dried *in vacuo* and was analysed for bound flavonoids.

Identification of Active Principles - The isoates were identified by Co-TLC (silica gel 'G' coated plates) along with standard reference compounds *viz.* Luteolin, Kaempferol, Quercetin, Isorhamnetin, Scopoletin and Nigretine etc.

The plates were developed in an organic sovent mixture of n-butanol : acetic acid : H_2O (4 : 1 : 5, upper layer). The developed plates were seen under UV light, placed in a chamber saturted with ammonia and were sprayed with ethanolic ferric chloride. Each of the isolates was purified by preparative TLC. Isolates were eluted with ethyl acetate and crystallized from chloroform. The purified isolates were subjected to mp, mmp, UV and IR spectral studies for identification and confirmation. These isolates were quantitatively estimated and used as test

Table 1. Antimicrobial activity of ethanolic extract of various plant parts of *H. curassavicum* and *S. arvensis.*

Organism Bacterial/Fungus	Ethanolic Extract						Antibiotic
	Heliotropium curassavicum			*Spergula arvensis*			
	Root	Stem	Flower	Root	Stem	Flower	
Staphylococcus aureus	0.52 (++)	0.4 (+)	0.4 (+)	0.37 (+)	0.48 (+)	0.34 (+)	Streptomycin
Bacillus cerus	0.37 (+)	0.31 (+)	0.41 (+)	-	-	-	Streptomycin
Salmonella typhi	0.66 (++)	0.93 (++)	1.26 (+++)	-	-	-	Ampicillin
E. coli	-	-	-	-	-	-	Ampicillin
Candida albicans	-	-	-	-	-	-	Fluconazole

materials.

Quantitative estimation of isolates- These isolated compounds were quantitatively estimated with the help of Spectronic-20 colorimeter (Bausch and Lomb) and used as test material.

Antimicrobial testing of isolates- All the test organisms were clinical isolates, obtained from different patients diagnosed for having bacterial and fungal infections, were procured from the Department of Microbiology, M. N. Institute, Bikaner. The microorganism used for screening were *Staphylococcus aureus* (Gram positive), *Bacillus cerus* (Gram positive), *Salmonella typhi* (Gram negative) and *Escherichia coli* (Gram negative) and a fungal pathogen *Candida albicans* (fluconazole).

The bacterial cultures of *S. aureus, B cerus, S. typhi* and *E. coli* were maintained on nutrient Agar medium (Peprone 5 gm/liter, Yeast extract 3gm/litre, NaCl 5gm/ litre, Agar 1.8% D/W 1000ml, pH 7.2-7.4), whereas, *C. albicans* on Sabouraud liquid medium (Peptone 10.0 gm/ litre, Detrose 40.0 gm/litre, Agar 1.5-1.8, pH±5.6). These microorganisms were allowed to grow at 35-37°C. The inoculum used for screening studies was prepared by adjusting the concentration of microorganism in the medium using Spectronic-20 colorimeter (Bausch and Lomb) set at 630 nm. 40% transmittance was used in case of *S. aureus, B. cerus, S. typhi* and *E. coli* and 65% transmittance in case of *C. albicans.*

The reference Antibiotic discs - The antibiotics known to be effective against each of the test microorganisms in their established doses were used as reference for comparison of the antimicrobial activity of the test samples. These were Streptomycin (30mcg units) for *S. aureus* and *B. cerus,* Ampicillin (10 mcg units) for *S. typhi* and *E. coli,* fluconazole for *C. albicans.*

Testing for Antimicrobial activity- Petri plates were rinsed with sterile distilled water, dried, wrapped in tin foils and sterilized in an oven at 100°C for 18hrs. Each of the sterilized Petri plates was then preceded with 10 ml of growth medium[2-4], 4.0 ml of the inoculums in case of bacteria *(S. aureus, B. cerus, S. typhi* and *E. coli*) and 6.5 ml of the inoculums in case of *C. albicans.* Each of the mixture was thoroughly shaken to ensure uniform distribution of the inoculums. Experiment was carried out in five replicates.

Paper discs measuring 6mm diameter which absorb about 0.1 ml of the test samples (Isolates) were employed for the screening pupose. Thus, each of the tests Petri plate contained paper discs of the reference antibiotic(s) of desired dose and paper discs of isolated compounds. Petri plates containing the paper discs (6mm) diameter (dipped in ethyl ether and 50% ethanol) were run as controls.

All the test and conrol Petri plates were kept at 5°C for 40-45 minutes so as to allow the diffusion of the substances and then were incubated at 35-37°C for 18 hrs.

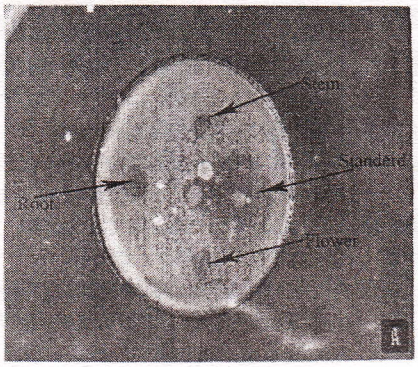
The inhibition zones formed by the test samples were measured and compared with those of the standard reference antibiotic discs (s) as given below :-

$$\text{Antimicrobial activity} = \frac{\text{Inhibition zone formed by the test samples}}{\text{Inhibition zone formed by the standard reference discs}}$$

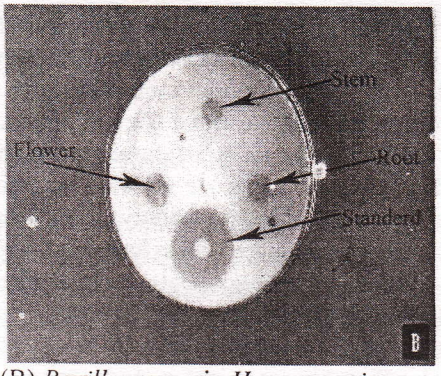
Results and Discussion

Kaempferol and quercetin flavonoids were isolated from root, stem and flower of *Heliotropium curassavicum* and *Spergula arvensis.*

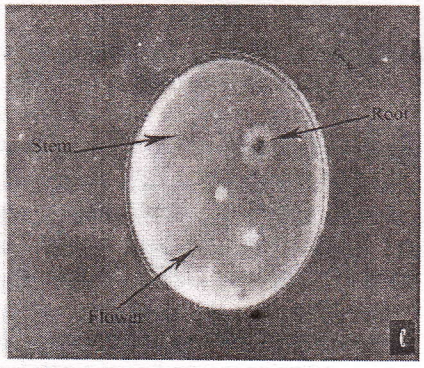
H. curassavicum and *S. pergula arensis* both plant species selected for the present investigation showed antibacterial activity against the *Staphylococcus aureus. H. curassavicum* showed more activity than *S. arvensis* against above bacterial strain. The antibacterial activity observed was maximum in the root of *H. curassavicum* (0.52mm) as compared to the root of *S. arvensis* (0.37mm) against bacterial strain *Staphylococcus aureus,* however, in the stem and flower of *H. curassavicum* it was detected



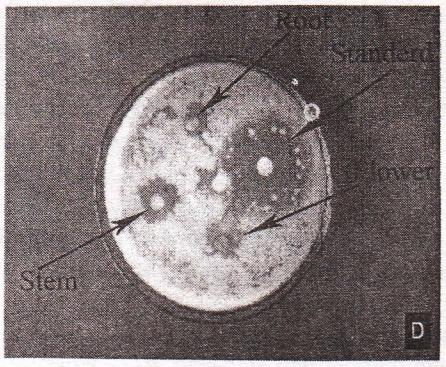
(A) *Staphylococcus aureus* in *H. curassavicum*



(B) *Bacillus cerus* in *H. curassavicum*



(C) *Salmonella typhi* in *H. curassavicum*



(D) *Staphylococcus aureus* in *S. arvensis.*

Fig.1. Showing inhibition zone due to antimicrobial activity of isolated substances against bacterial strain.

to be same (0.4mm). In the stem of *S. arvensis* (0.48mm) activty was observed and in the flower of same plant it showed minimum activity (0.34mm) against the same bacterial strain (Table 1 and Fig.1).

The antibacterial activity against *Bacillus cerus, Salmonella typhi* was observed due to extracts of all plant parts (root, stem and flower) of *H. curassavicum* whereas, those of *S. arvensis* did not show any activity against these bacterial strain (Table 1 and Fig.1).

The activity against *Bacillus cerus* observed in the root, stem of *H. curassavicum* was almost similiar (0.37mm, 0.31mm, respectively) whereas, in the flower of same plant little higher activity was detected 0.41mm. (Table 1 and Fig.1).

The antibacterial activity against *salmonella typhi* showed by the root, stem and flower of *H. curassavicum* (0.66mm, 0.93mm, 1.26mm, respectively), was observed to be highest in the flower of *H. curassavicum* (1.26mm) (Table 1 and Fig. 1).

H. curassavicum and *S. arvensis* did not show any activity against bacterial strain *Escherichia coli* and fungal pathogen *Candida albicans.*

Antimicrobial activity studied in vivo- Antimicrobial activity of the plants *in vivo* and *in vitro* has extensively been studied. Skinner[5] and Nickell[6] have reviewed antimicrobial principles and their distribution in plants. Nickell[6] has surveyed 174 plants belonging to 157 families of vascular plants for antimicrobial activity.

Many workers have observed antimicrobial activity in different parts of various plants. Kabadi and Hammarlund[7] observed antimicrobial activities in leaves of *Arbutus menziesii,* Ross *et al.*[8] in seeds of *Peganum harmala,* Grover[9]. in shoot and fruits of *Seetzenia orientalis.* Khanna and Staba[3] and Khanna *et al.*[4] reported that tissue cultures of plants also have antimicrobial activities. Bhojak[10] in *Calligonum polygonoides,* Badia[11] in *Peganum harmala,* Bains[12] in *Withania somnifera,* Reddy[13] in *Cassia angustifolia,* Bedawat[14] in *Balanites aegyptiaca* and Agarwal[15] in *Maytenus emarginata,* Yadav[16] in *Cistanche tubulosa* and *Orobanche aegyptiaca* have observed antimicrobial activity against microorganisms.

Kujumgieva *et al.*[17] investigated Propolis (bee glue) samples from different geographic origins for their antibacterial (*S. aureus* and *E. coli)* and antifungal (*C. albicans)* activities. Rauha *et al.*[18] observed the antimirobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds.

Nkere and Lroegbu[19] studied antimicrobial activity of the root, seed and stembark extracts of *Picralima nitida*. Ghosh *et al.*[20] studied antimicrobial activity of various fractions of ethanol extract of *Bacopa monnieri* Linn. Thus, it can be concluded, by all above reports, that the flavonoids present in plants are working as active principles to show the antimicrobial activity against many Gram+ve and Gram-ve bacteria as well as fungal pathogen. Although the intensity of antimicrobial activity depends and varies with amount of flavonoids present in plant parts or unorganized tissue. Flavonoids are found to be effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to make complex with extracellular and soluble proteins and with bacterial cell wall; more lipophilic flavonoids may also disrupt microbial membrane. The antimicrobial activity exhibited by *H. curassavicum* and *S. arvensis* may be attributed to flavonoids present in these plants, which either due to their individual or combined action, exhibit antimicrobial activity. Hence, the present findings provide a scientific base for some of the medicinal claims of *H. curassavicum* and *S. arvensis*.

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