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IN-VITRO SCREENING OF ANTIBACTERIAL ACTIVITY OF SEEDS OF *CROTALARIA VERRUCOSA L.* AND *DURANTA ERECTA* L.

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ABSTRACT

Antimicrobial activities of seeds of *Crotalaria verrucosa L.* (*Fabaceae*) and *Duranta erecta* L. (*Verbenaceae*) were tested in the present study. The crude extracts were prepared by hot continuous and successive extraction using soxhlet apparatus. Extractions were done in the order of increasing polarity of the solvents in the sequence of hexane, petroleum ether, ethyl acetate, chloroform, acetone, ethanol, methanol and water. Anti bacterial activities were investigated by "Disc Diffusion Method" against the Human pathogenic bacteria: viz., *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.* Water and methanolic extracts of *C. verrucosa* showed maximum inhibition effects against *Bacillus subtilis* and *Pseudomonas aeruginosa.*

KEY WORDS: Anti Bacterial Activity, *Bacillus subtilis, Escherichia coli. Pseudomonas aeruginosa, Staphylococcus aureus.*

1. INTRODUCTION

Since time immemorial, plants have been a valuable source of natural products for maintaining human health going together with intensive studies for natural therapies. Now days, the use of phytochemicals for pharmaceutical purpose has gradually increased in

many countries to cope increasing number of infectious agents, becoming resistant to commercial antimicrobial drugs.[1] In recent years, multiple resistances in human pathogenic microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious disorders.[2] The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of new anti- infective agents.[3-6] Medicinal plants represent a rich source of antimicrobial agents and source of new drugs which are extremely useful for synthetic modification of optimal of biological activity.[7-9] Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper than modern medicines.[10]

Crotalaria verrucosa L. (Fabaceae) commonly called, blue rattlesnake; globally distributed in the Pan tropics, within India, in the tropical regions, from Himalayas to Ceylon.[11] In almost all districts, is weed of roadsides, waste places, gardens and fields. Much Branched herbaceous, usually annual plant with blue, sometimes white, flowers.[12] This ethno botanical herb is known to have medicinal properties. Juice of leaves is used in scabies and impetigo both internally and externally, also considered efficacious in diminishing salivation.[13] The leaf decoction is given orally to cure jaundice.[14-15] The aqueous ethanolic extract of aerial parts of *C.verrucosa*. Has shown very significant hepato-protection against paracetamol induced hepatotoxicity study models in wistar rats.[16] Aqueous and ethanolic extracts of aerial parts of *C. verrucosa* were found to be affective against fertility and estrogenic implantation in *Albino* rats.[17]

Durunta erecta L., a member of *Verbenaceae* family, commonly known as Golden dewdrop, is widely used as an ornamental plant in tropical and subtropical gardens throughout the world. The leaves are light green, elliptic to ovate, opposite. The flowers are light-blue or lavender, produced in tight clusters located on terminal and axillary stems. The fruit is a small globose yellow to orange berry. The leaves and berries of the plant are toxic, and are confirmed to have killed children, dogs and cats [18]. *D. erecta* is used medicinally for a wide variety of ailments. The fruit and leaves have been used as vermifuge, diuretic, in malaria and in intestinal worms. Saponins in the fruits and foliage cause gastro enteric irritation, drowsiness, fever, nausea, vomiting, and convulsions. Dermatitis sometimes occurs from handling the plants.[19-20] Ethyl acetate and aqueous extracts of leaves showed significant antimalarial activity when administered to mice.[21] In small quantities, fruits are used to treat

intestinal worms.[22] From the genus *Duranta* several iridoid glycosides as durantosides and lamiide, flavanoids and c-alkylated flavonoids and some alkaloids were isolated.[22]

2. METHODOLOGY

Seeds of *Crotalaria verrucosa,* (Voucher No.0146) and *Durunta erecta* (Voucher No 087) were collected from Osmania University campus, in wild condition from mature pods in the month of November, 2014. And the plants were authenticated by Prof. P. Ramachandra Reddy, Professor, (Plant Anatomy and Taxonomy Laboratory) Department of Botany, Osmania university, Hyderabad, Telangana.

3. PROCESSING OF PLANT MATERIAL

Fresh seeds were washed thoroughly under running tap water, sterilized by distilled water and dried in hot air oven at 45^{o} C.[23] Until concurrent dry weights were obtained using electronic balance (Type BL-22OH, NO.D427600501). The seeds were ground into fine powder by using mechanical pulverizer. The powdered material was meshed through 0.3mm mesh (Jayanth scientific IND. Mumbai.) and stored in airtight sterile container.

4. AQUEOUS EXTRACTION

20 g of the fruit and seed powder was taken in a flask and heated with 200ml of distilled water for five hours at 80^{o} C by agitating gently at regular intervals. The contents were then filtered through Whatman's No.1filter paper (W and R balson Ltd, England) and the filtrate was used for microbial activity procedures.

5. HOT CONTINUOUS EXTRACTION

As the target compounds may be non-polar to polar and thermally labile, the suitability of the methods of extraction was considered. Various methods, such as sonication, heating under reflux, soxhlet extraction and others are commonly used.[24-27] For the plant samples extraction. An earlier study on phytochemical extraction suggests that soxhlet extraction process provided standard results. Successive extractions were carried out using soxhlet apparatus. 20gr of powdered materials of *C. verrucosa* and *D. erecta* seeds were packed in porous cellulose "thimble" made from filter paper, was placed in extraction chamber. The seeds powders were extracted successively with n-hexane at 70^{o}C, petroleum ether at 60^{o}C, ethyl- acetate at 77^{o}C and chloroform at 61^{o}C, acetone at 56^{o}C, ethanol at 78^{o}C, methanol at 65^{o}C, and water at 80^{o}C. Extraction temperatures were adjusted to boiling points of solvent to allow a faster rate of cycling of fresh solvent. Six hours of duration was allocated to each

solvent for hot continuous and successive extraction. The extracts were cooled and filtered through Whatman No.1 filter paper. Extractions were done in the order of increasing polarity of the solvents, from hexane to water. The extracts were concentrated by rotary evaporator and were stored at 4^{o}C.

6. IN-VITRO ANTIMICROBIAL SCREENING

Antibacterial tests were carried out by disc diffusion method.[27] Against different human pathogenic bacteria viz., *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli*. The pathogenic bacteria were obtained from General Microbiology, Chemotherapy laboratory, Department of Microbiology, Osmania University Hyderabad, Telangana, India. Using 100 µL of the suspension, containing 10^{8} colony forming units (CFU) mL^{-1} of bacteria spread on potato dextrose agar (PDA) medium. The discs (6 mm in diameter) impregnated with seed extractions of *C. verrucosa* and *D.erecta* were placed on the inoculated agar.

Anti Bacterial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay was repeated 3 times.

7. RESULTS AND DISCUSSION

The ability of the plant extracts to kill or inhibit the growth of pathogenic microorganisms viz.,*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi* were evaluated by in vitro conditions and the results are presented in table 1& 2. The four different fractions obtained were screened for their antibacterial activity by determining the zone of inhibition (ZOI) with the help of scale against tested organisms by agar cup diffusion method given by. The diameter of zone of inhibition (ZOI) measures for the estimation of potency of the antimicrobial substances.

Extracts obtained from the seeds of *Crotalaria verrucosa L.* and *Durunta erecta* using a series of solvents by successive extraction method were tested against human pathogenic Bacteria; viz., *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli*. The solvents used for hot continuous extraction were hexane, petroleum ether, ethyl acetate, chloroform, acetone, ethanol, methanol and water.

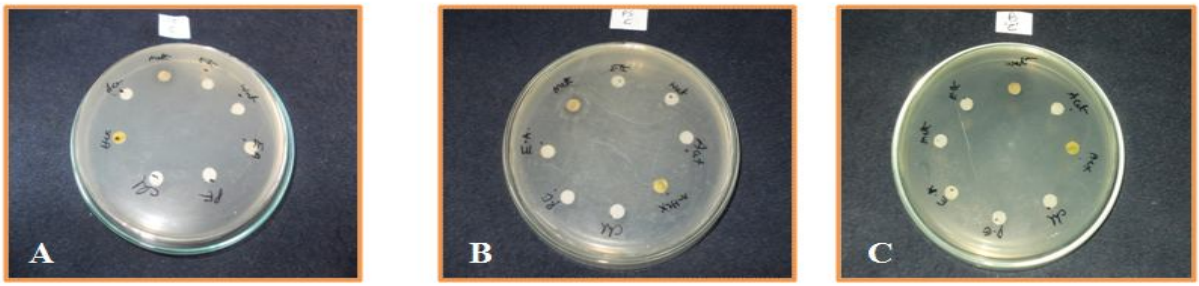
Water extracts of *Crotalaria verrucosa* showed maximum inhibition (6mm) among various extracts tested followed by, acetone and ethanol extract with inhibition of 2mm and methanol

extract showed minimum inhibition (1mm) against B. *subtilis*. Only methanol seed extract of *C. verrucosa* showed antibacterial activity (3mm) towards *Pseudomonas aeruginosa,* and the same showed maximum inhibition (55mm) against *S. aureus.* Ethanolic and acetone extracts showed low inhibitory effect (1mm) against *Staphylococcus aureus.* However, petroleum ether, n-hexane, ethyle acetate and chloroform extract showed no antibacterial activity against the tested pathogenic bacteria (*B. subtilis* .*P. aeruginosa*, *S. aureus* and E. *coli*). The extracts of methanol, ethanol, acetone, and water did not show any inhibition effect against the growth of *E. coli* (Table 1).

Methanolic seed extract of *D. erecta*. Showed no inhibition against *B. subtilis.* However petroleum ether, n-hexane, ethyl acetate, chloroform, ethanol, acetone and water extracts showed no antibacterial activity towards *P. aeruginosa, S. aureus* and *E. coli.* (Table 2). On the contrary, the findings of revealed that the seeds extracts of *Duranta repens* are biologically inactive against *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* and *Staphylococcus aureus.*[28]

Plant based products have been effectively proven as source of antimicrobial compounds. Many reports are available on the antibacterial efficacy of leaf, root and bark extracts. In the present study, seeds of *C. verrucosa and D. erecta* were screened. Results obtained revealed that the seed extracts of *C. verrucosa and D. erecta are* potentially antibacterial against *B. subtilis.* Only the methanolic extract of *C. verrucosa* was effective against *P.aeruginosa, S.aureus* growth the methanolic, ethanolic and acetone extracts of *C. verrucosa* shown inhibitory effect.

Antimicrobial screening revealed that Duranta repens seeds extract were biologically inactive against *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa and Staphylococcus aureus.* Literature review revealed that the seeds of Duranta repens have antimicrobial activity as our investigation differs from the previous studies. It may be due to the seasonal variation, different geographical location of the plant *D. repens* and different experimental conditions.

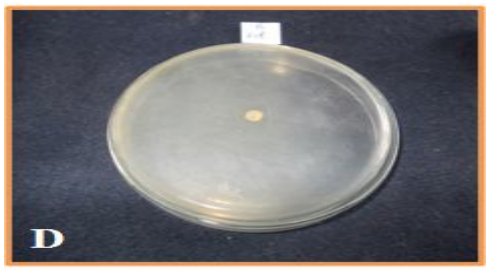


A *Crotalaria verrucosa* against *Pseudo Monous*

B *Crotalaria verrucosa* against *Staphylococcus*

C *Crotalaria verrucosa* against *Bacillus subtilis*

Figure1: Anti Bacterial Activity of *Crotalaria verrucosa Against* A- *Pseudomonous,* B- *Staphylococus Aureus,* C- *B.Stutillis.*



D *DurantaErecta* Against *Bacillus subtilis*

Figure 2: Anti Bacterial Activity of *Duranta* seeds against D- *B.stutillis.*

Table 1. Antibacterial activity of Seed extract of *Crotalaria verrucosa*

	Inhibition zone (mm)			
Seed extract of *Crotalaria verrucosa*	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Escherichia coli***
Petroleum ether	-	-	-	-
n-Hexane	-	-	-	-
Ethyl acetate	-	-	-	-
Chloroform	-	-	-	-
Methanol	1	3	6	-
Ethanol	2	-	1	-
Acetone	2	-	1	-
Water	6	-	-	-

−No activity.

Table 2. Antibacterial activity of Seed extract of *Duranta erecta*

	Inhibition zone (mm)			
Seed extract of *Duranta erecta*	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Escherichia coli***
Petroleum ether	-	-	-	-
n-hexane	-	-	-	-
Ethyl acetate	-	-	-	-
Chloroform	-	-	-	-
Methanol	3	-	-	-
Ethanol	-	-	-	-
Acetone	-	-	-	-
Water	-	-	-	-

−No activity

9. CONCLUSION

The results of present investigation clearly indicate that the antibacterial activity varies with the species of the plants and plant material used. Thus, the study ascertains the value of plants used in pharmacy, which could be of considerable interest to the development of new drugs.

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