ANTAGONISTIC ACTIVITY OF CITRULLUSCOLOCYNTHIS

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Abstract - The antimicrobial activity of methanolic, ethanolic and water extract of the plant C. colocyn this was investigated by disc-diffusion method on Mueller-Hinton broth. It was performed using a 24 h old bacterial culture at 37°C reseeded on Nutrient Broth. The cultures were adjusted to 5.6 x 106 CFU/ml with sterile water. One milliliter of the suspension was added over the plates containing Mueller-Hinton broth to get a uniform microbial growth on both control and test plates. The extract of C. colocyn this were dissolved in methanol, ethanol and water (30 mg/ml) and sterilized. Under aseptic conditions, empty sterilized discs (Whatman no. 5, 6 mm diameter) were impregnated with methanol extract (300 µg/ml), and placed on the agar surface. The plates were left for 30 min at room temperature to allow the diffusion of the oil and extract, and then they were incubated at 37°C. After the incubation period (24 h), the zone inhibitions were measured and presented in millimeter.

1 INTRODUCTION

In view of increasing resistance to existing antimicrobial agents, herbal drugs are being looked as very importance source for discovery of new agents for treating various ailments related to bacterial infections. Traditional medicine is an important source of potentially useful compounds for the development of phototherapeutics agents. Antimicrobials of plant origin have enormous therapeutic potential in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Lwu et al 1999). The antimicrobial research is geared towards the discovery and development of novel antibacterial agents. The spread of multi drug resistant strains of Microorganisms necessitate the discovery of new classes of antimicrobial compounds that inhibit these drug resistance mechanisms. Natural products continue to play a major role as active substances, model molecules for the discovery and validation of drug targets. Medicinal plants continue to be an important therapeutic aid for alleviating ailments of human kind and there is an ever-increasing demand for more and more drugs from plant sources (Nair et al., 2008).

A number of plants from different families of angiosperms have been reported to show antimicrobial activity (Palombo & Semple 2001). In angiosperms Citrulluscolocyn this (Linn.) commonly known as colocynth is member of the melon family of Cucurbitaceae and it produces bitter flavoured fruits about the size of cantaloupe and seeds rich in oiland protein. It is a long lived perennial and grows wild in sandy shore under xerophytic conditions. Young fruits are fleshy, mottled with dark green and usually turn yellow when ripe. The fruit of Citrulluscolocyn this had been used medicinally since ancient times. Traditionally fruit of C. colocyn this is used for the treatment of diabetes, microbial diseases, ulcer, inflammation, and jaundice andurinary diseases in Asian and African countries (Nmila et al., 2000). The present study aimed at evaluating the in vitro antimicrobial activity of the different parts of plant like leaf, fruit, and stem in ethanol and chloroformextracts against gastrointestinal problem causing Enterobacteriace aebacteria in northern Rajasthan, India.

2 MATERIALS & METHODS

Collection of plant materials - Citrulluscolocynthisplant collected from agricultural field of northern part of Rajasthan state, encompassing the Cucurbitaceaefamily was utilized and transferred in department for further searching to microbiological activity. In research laboratory all plant materials were thoroughly washed using water, dried and carefully separated into leaves, stems and roots with suitable equipment. The dried plant materials were each macerated and ground into a fine powder, using clean, dry electric blender.

Preparation of extract; the powder materials were extracted with two organic solvents methanol (50%), ethanol (50%) and one aqueous (water) extraction respectively. 30g of the

accurately weighed each parts of plant powder was put in the Soxhlet thimble made by Whatman filter paper No. 1 and 300ml of the each solvents (distilled water, ethanol & methanol) in a round bottomed flask in soxhlet flask. After this samples were allowed for extraction at 20-30^{0}C. Following this the extracts were concentrated under pressure using rotary vacuum evaporator. The concentrated extract was weighed and labeled appropriately. All residues were kept in tightly stoppered bottle until used for the anti-microbial tests.

Bacterial strains and antibiotics-The antimicrobial activity was assayed utilizing two groups of well-known microorganisms. One group of MTCC gram negative, pathogenic strains:Escherichia coli 1692; Vibrio cholerae 3906; Salmonella typhi 0733; Pseudomonas aeruginosa 4676;other the gram positive Bacillus cereus 1272; and Staphylococcus aureus 7443. The microorganisms were maintained in nutrient agar at 4°C until the assays were carried out. Different (Himedia) antibacterial antibiotics (Streptomycin, Ampicillin) were used in work.

2.1 Antimicrobial susceptibility test

The antimicrobial screening of the bio extract were carried out by determining the zone of inhibition using disc diffusion method.[8] The sterilized Mueller-Hinton agar (MHA) plates were prepared and labeled appropriately with the name of the bacterial strains and the weed plant extracts. Using sterile forceps, sterile 6mm discs (cut from Whatman No. 1 filter paper with a paper punch device and sterilized before use) were picked and submerged in each of the graded concentrations of weed plant extracts namely; leaf, stem and root of Citrulluscolocynthis. Overnight bacterial culture 1X10^{-6} CFU/mL viable count inoculums were used and applying the bio extract impregnated discs (6mm). Commercially prepared antibiotic (Himedia) disc were used as a positive control and discs soaked in distilled water as a negative control in each agar plate. The plates were allowed to stand for 30minutes and then incubated at 37°C for 24 hours. Antimicrobial activity of each extract against the test organisms were indicated by a growth-free zone around the respective discs and the diameters of the zones of inhibition to the nearest millimeter with a ruler were obtained by measuring the distance from one end of the inhibition zone, across the disc to the other end.

3 RESULTS& DISCUSSION

Plants are a potential source of therapeutic activities due to the presence of bioactive components. Many reports are available on the antibacterial, antifungal, antiviral, antihelmic, antimolluscal and anti-inflammatory properties of plants (Samy RP &Ignacimuthu S 2000; Palombo&Semple 2001; Kumaraswamy Y et al 2002).

In ethanol extract maximum inhibition zone (22 mm) was observed in root extract against Staphylococcus aureuswhile minimum (10 mm) was observed in stem extract against Salmonella typhi. Vibrio choleraeshowed no inhibition in this extract. The non-activity of the ethanol extract against bacterial strain investigated in study is in agreement with previous works which show that aqueous extracts of plant generally showed little or no antibacterial activities (Koduru et al 2006; Aliero et al 2006; Ashafa et al 2008; Aiyegoro et al 2008).

In fungal isolates maximum inhibition zone (10 mm) was observed against A. fumigatus in leaf extract while minimum zone (7.3 mm) was showed in the stem extract of A. niger. In leaf extract maximum inhibition zone (18 mm) reported against Escherichia coli while minimum against (11.2 mm) Bacillus cereus. In stem extract maximum inhibition zone was 20 mm against Staphylococcus aureus. Minimum (10 mm) inhibition zone was observed against Salmonella typhi. In root extract maximum zone of inhibition of this plant was (22 mm) occurred against Staphylococcus aureuswhile minimum zone (12.5 mm) against Pseudomonas aeruginosa.

In the respect of water extract maximum and minimum inhibition zone (15.5 mm & 10.5 mm) was observed in stem extract against Bacillus cereus&Pseudomonas aeruginosa respectively. Vibrio cholerae also showed no inhibition in this extract. In leaf extract maximum inhibition zone reported against Staphylococcus aureus(14 mm) while minimum against Staphylococcus aureus, Salmonella typhiwas observed. In stem extract maximum inhibition zone was 15.5 mm against Bacillus cereus while minimum zone (10.5 mm)

against Pseudomonas aeruginos a was observed. In root extract maximum zone of inhibition was (13.6 mm) occurred againstEscherichia coli.Minimum (11.1 mm) was observed against Pseudomonas aeruginosa. Maximum and minimum (11.6 mm & 10mm) zone of inhibition was occurred against leaf extract of A. fumigates and root extract of A. niger.

In the respect of methanol extract maximum inhibition zone (22.9 mm) was observed in root extract against Escherichia coli while minimum (6.5 mm) was observed in leaf extract against Staphylococcus aureus. In leaf, stem and root extract maximum (19.5 mm, 17.8 mm & 22.9 mm) inhibition zone was reported against Escherichia coli while minimum zone (6.5 mm, 7.3 mm, 8.4 mm) was observed against Staphylococcus aureus. In fungal strains A. fumigates showed maximum inhibition (11.5 mm) against leaf extract while minimum inhibition zone (8.6 mm) was reported in stem extract against A. niger. So from these data in can be concluded that this plant extract is very effective against Eshcerichia coli. Parekh and Chanda (2007) reported that methanolic extracts of Lagenaria vulgaris, Momordicacharantia and Mukiamaderaspatana showed inhibitory effect against K. pneumoniae, while no zone observed against E. coli. Similarly, fruits, leaves, stems and roots of Citrulluscolocynthis showed no response against E. coli and P. aeruginosa (Memonet al2003).

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Table 1.- Showing antimicrobial activity of C. colocyn this using ethanol extract against bacterial and fungal Strains

Microorganisms	50% ethanol	Leaf (mm)	Stem (mm)	Root (mm)	*Standard Drug (1 or 2)	**Standard Drug (3 or 4)
Gram- Negative Bacteria						
E. coli	0±0	18±.88	16.6±.90	20±.57	18.3±.95	21±.52
V. cholerae	0±0	------	-------	------	17.6±.56	13.3±.57
S. typhi	0±0	12±1.22	10±.66	15±1.15	21±1.55	29±.90
P. aerunginosa	0±0	13.6±0.78	12.5±0.24	12.5±0.54	-------	------
Gram- Positive Bacteria						
B. cereus	0±0	11.2±.33	10.3±.95	14.5±.66	16.6±.38	25.6±1.20
S. aureus	0±0	17±.33	20±.91	22±1.45	21.3±.92	33.3±.66
Fungi						
A.fumigatus	0±0	10±1.33	7.8±.85	8.6±1.15	13±.88	12.2±1.45
A. niger	0±0	8.4±.91	7.3±.66	8.2±89	12±1.15	11.3±1.20

Result as per shown in Mean±S.E; -------- No inhibition
*Standard Drug – 1- Streptomycin (10mcg) or 2-Flucanozole,** 3-ampicillin (10mcg) or 4-Amphotericin B

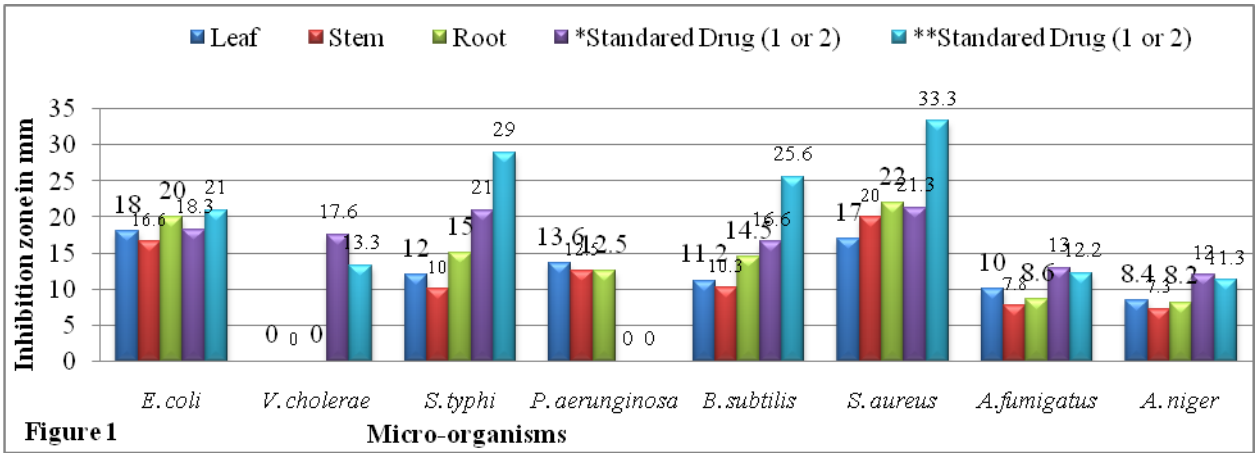

Figure 1- Showing antimicrobial activity of C. colocynthis using Ethanol extract against bacterial and fungal Strains

Table 2- Showing antimicrobial activity of C. colocynthis using ethanol extract against bacterial and fungal Strains

Microorganisms	50% ethanol/ methanol/water	Water extract			Methanol extract		
		Leaf (mm)	Stem (mm)	Root (mm)	Leaf (mm)	Stem (mm)	Root (mm)
Gram- Negative Bacteria							
E. coli	0±0	12.8±0.39	11.5±0.47	13.6±0.58	19.5±0.25	17.8±0.67	22.9±0.89
V. cholerae	0±0	------	------	------	9±1.24	8.5±0.33	11.2±0.94
S. typhi	0±0	11.5±0.27	13.1±0.83	12.6±0.74	14±1.45	13.2±0.58	15.5±0.79
P. aerunginosa	0±0	12.1±0.16	10.5±0.64	11.1±0.28	13.9±0.64	13±1.10	16±0.48
Gram- Positive Bacteria							
B. cereus	0±0	14±0.34	15.5±0.24	13.3±0.98	13.6±0.28	14.5±0.94	15.7±0.73
S. aureus	0±0	11.5±0.88	12.6±0.61	12.7±0.79	6.5±1.15	7.3±0.86	8.4±0.68
Fungi							
A.fumigatus	0±0	11.6±1.05	10.9±1.23	11.2±0.98	11.5±0.29	9.6±0.39	9.9±0.92
A. niger	0±0	11.2±0.45	10.2±0.52	10.0±0.62	9.5±0.76	8.6±0.18	9.5±1.11

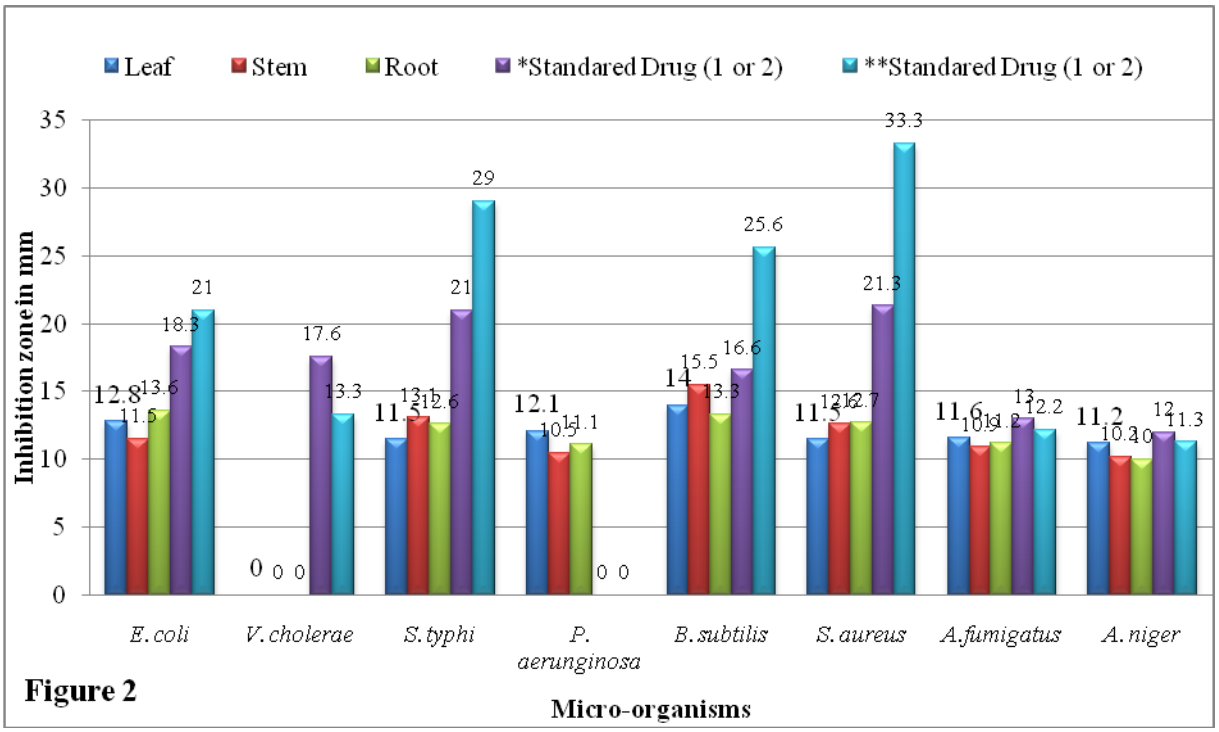

Figure 2- Showing antimicrobial activity of C. colocynthis using Water extract against bacterial and fungal Strains

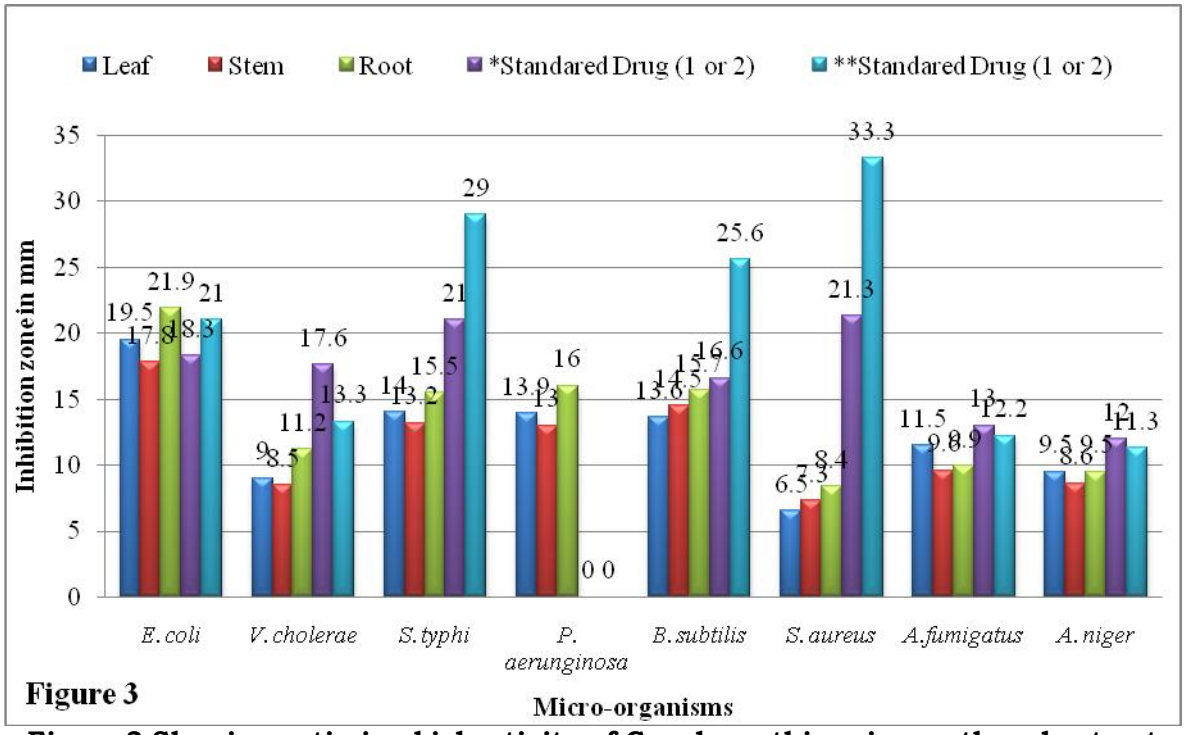

Figure 3-Showing antimicrobial activity of C. colocynthis using methanol extract against bacterial and fungal Strains