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IN *Vitro* ANTIBACTERIAL ACTIVITY OF ASTERACEOUS PLANTS AGAINST TEST PATHOGEN *Shigella* AND *Klebsiella* sp.

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ABSTRACT

Hydroethanolic extract of different locally available plants of Asteraceous plants were evaluated for their antibacterial activity against the test pathogen *Shigella* and *Klebsiella* sp. The whole plant extract of *Tridax procumbens* Linn. exhibited maximum antibacterial activity forming an inhibition zone 25.0 mm.& 16.0mm.diameter against test pathogen *Shigella* and *Klebsiella* sp. respectively. Other sample showed less or no inhibition zone. The result indicate that the whole plant of *Tridax procumbens* Linn. can be exploited as good sources of innocuous bactericide.

KEYWORDS : Antibacterial, Asteraceous Plants, *Tridax procumbens*, *Shigella* sp. *Klebsiella* sp.

Plants develop a variety of chemicals inside their defensive mechanism to resists the natural antigens the face. There are several synthetic bactericides available in the market but several of them have been proved carcinogenic, teratogenic or they cause a variety of side effects in plants or in human beings. In contrast, the fungicides and bactericides of plant origin are easily biodegradable and they do not cause undesired side effect in living beings. A lot of work has been done in recent years for the investigation of antibacterial and antifungal activity in plants (Srivastava D. & Yadav H.L ,2008; Britto S.J. et al., 2001). Since long the use of plants is in practice for the control of bacterial diseases. In comparison to the synthetic compounds, the pesticidal compounds of plants origin are more effective (Dikshit et al., 1979) and have little or no side effects in human being (Kumar et al., 1995). A number of Asteraceous plants have been found by various workers which contain antifungal compounds (Naquvi et al.,1991; Garud et al, 2004). These facts inspired us to investigate antibacterial activity in Asteraceous plants of this locality.

In the present investigation 50% hydroethanolic extract of some locally available of Asteraceous plants parts have been evaluated for their antibacterial activity against the both test pathogens *Shigella* sp. and *Klebsiella* sp.. Only the whole plant extract of Tridax procumbens Linn. was found to be most active forming the largest inhibition zone in our experimentation. It was fractionated in different organic solvents by differential solubility methods only the petroleum ether fraction was found to contain antibacterial constituents in it and the minimum inhibitory concentration was worked out.

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MATERIALS AND METHODS

Different available parts of 7 Asteraceous plants were locally collected and identified with the help of Ph.D. thesis on Azamgarh (Chandra V. 1984). 50% hydroethanolic extract of plant part was made by crushing 5.0 g plant material in 25.0 ml of 50% ethanol (v/v) and the mixture was left whole night for the maximum extraction of compound in solution. 2.0 ml of the filtered extract was soaked in disk (diameter 10 mm) of Whatman paper no.1, dry with the help of hair drier and sterilized forceps. The disk was designated as treatment disk, side by side control disk were also prepared by impregnating 2.0ml of 50% (v/v) ethanol only. Results were obtained by comparing the activity in treatment and control disks by measuring the diameter of the inhibition zone.

The test pathogen *Shigella* sp. was collected from feacal matter of patients suffering from dysentery & *Klebsiella* sp. from sputum of patients. However, the pathogens were identified on the basis of colony characters and biochemical tests.

The nutrient agar medium was prepared and sterilized medium was poured in presterilised petriplates (3”diameter) to the extend that it may cover the base of plate. The treatment and control disks were assayed for the antibacterial activity against the both test pathogen by inhibition zone technique. First of all peptone water was prepared and it was inoculated with both test pathogen separately prepare the seed of test pathogen. Now already prepared agar plates were seeded with test pathogen *Shigella* sp. and side by side *Klebsiella* sp. The treatment

disk and control plates were incubated at 37±1ºC for 48 hours.

The compounds in the impregnated treatment disks gradually diffused into the medium where the compound(s) present in the disks had antibacterial activity they inhibited the test pathogen of the seeded agar plates and an inhibition zone around the disk was formed. On the other hand no zone was formed around the inactive treatment disks. Similarly, inhibition zone was not formed around the control disks. The diameter of inhibition zone in each plate was measured in perpendicular directions and mean value was recorded in (Table-1).

The hydroethanolic extract of active plant parts was fractionated by 'Differential Solubility Method'. It is the general principle that different organic solvents have different polarity i.e. efficiency of dissolve specific compounds into them. On the basis of this principle the hydroethanolic extract of active plant parts was treated with different organic solvents one by one. The non-polar solvent used first and then other solvents were used in increasing order of polarity i.e. in the order of petroleum ether, benzene, carbon tetrachloride, chloroform, acetone and finally methanol to fractionate the compounds in different fractions. All the fraction were tested for antibacterial activity against the both test pathogens by the usual inhibition zone technique and the result were tabulated in (Table-2).

To prepared different concentration of the constituents of the active fraction, the solvent of the active fraction are evaporated at the room temperature. The remained was weighted on a chemical balance and again dissolved in a known volume of the solvent (10% methanol) to get a known concentration of the active fraction. To find out the MID for one loopful of inoculum of the test pathogen, Whatman No.1 paper disks (5.0mm diameter) were taken (as per performance standards for antimicrobial disk susceptibility tests; Fourth Edition, National Committee for Clinical Laboratory Standards, Vol. 13 No. 24, 1993). These disks were weighed on chemical balance and to the weight of these disks 2mg., 3mg., 5mg., 7mg., 9mg.,12mg.,14mg and 15 mg. of the constituents of solvent ether fraction were impregnated separately. These impregnated disks carrying measured amounts of active fraction constituents (i.e. 2, 3, 5, 7, 9, 12, 14, and 15mg.) were aseptically placed equidistantly on a presterilised and 'seeded' nutrient agar plate, on the surface of the medium. The disks were gently pressed on the medium surface with the help of a sterilized forceps so that the disks may come in complete contact of the medium. Allowed the plate to stand at room temperature for 30 minutes (pre diffusion time) and then it was incubated for 72 hours at 37±1ºC . Observations were made 12 hourly for MID and inhibition zone recorded in (Table-3).

RESULTS AND DISCUSSION

Different available Asteraceous plants and parts were screened for their antibacterial activity. The results have been recorded in table-1, where names of the plants are arranged alphabetically.

Out of different parts of plant screened the hydroethanolic extract of the Tridax procumbens Linn. exhibited most antibacterial activity against both test pathogens. It is useful to screen all the available plant parts to obtain the knowledge about the distribution of bactericidal factor in a plant. The use of 50% ethanol has been made in the present work which ensures extraction of maximum compounds (ethanol and water have maximum polarity) as well as facilitates further purification of active (Dhar et al., 1968). Aqueous extracts or expressed juices may to their efficiency due to degradation of active constituents by continued enzymatic activity.

Several workers first isolated different compounds from the plants and then their antimicrobial activity were tested in vitro (Cottiglia et al., 2004; Rahaman. M. M. & Gray A.I. 2002). Another aspect is that some workers first isolated the antimicrobial activity of plants and if they were found antimicrobial activity then fractionation was done and each fraction was assayed against the test pathogen to find out the active fraction (Sukul and Chaudhuri; 2001). In present investigation the hydroethanolic extract of Tridax procumbens Linn. was found to contain antibacterial activity and it was fractionated by differential solubility method , after fractionation of hydroethanolic extract in different organic solvents, each fraction was assayed against the both pathogens. Only the petroleum ether

fraction was found to possess antibacterial activity.

Several compounds of different plants parts have been reported by different workers to shows antimicrobial activities at minimum inhibitory concentration (MICs) in the range 25-127 micro g/ml (Rahman and Gray, 2002; Austin et al., 2003). In the present study emphasis has been given to find out the minimum inhibitory dose (MID) by the inhibition zone technique. In this technique the concentration of compounds decreases gradually as it diffuses out from the sensitivity disk to the periphery. Though the disks impregnated with 2,3,5,7,9,12,14 and 15mg contents of active fraction formed inhibition zones around them, all the zones except 14mg disk-zone in seeded plate of test pathogen *Shigella* sp. and 15mg disk-zone in seeded plat of test pathogen *Klebsiella* sp. were gradually invaded by the test pathogen after 24 hrs. However zone around 14mg and 15mg disks persisted and remained unaffected by the test pathogen *Shigella* sp. and *Klebsiella* sp. respectively throughout the experimentation period (72 hrs.). It was concluded that the 7,9 and 12mg disks were bacteriostatic upto 24 hrs. only but 14mg disk proved bactericidal against *Shigella* sp. during 72 hrs. and 7,9&14mg disks were bacteriostatic upto 24 hrs. only but 15mg disk proved bacteriostatic during the experimention period (72hrs.) against *Klebsiella* sp. (Table-3), one loopful inoculum was taken to seed 3 diameter nutrient agar plate. Here, it is required to mention that after seeding the nutrient agar plate the number of bacteria would increase on the medium surface and it is difficult to count their exact numbers, although Skinner (1955) has mentioned that the efficacy of antibiotics depends upon the number of bacteria they have to act on. However, to fix the exact dose requires

Table 1: The Diameter of Inhibition Zone in Each Plate Was Measured in Perpendicular Directions and Mean Value Was Recorded

Sl. No.	Name of Plants	Plants Part	Diameter of Inhibition Zone (mm)	
			Shigella sp.	*Klebsiella sp.*
1.	*Ageratum conyzoides* Linn.	Whole Plant	00.0	14.0
2.	*Blumea lacera* D.C.	Leaf	15.0	15.0
3.	*Launaea nudicaulis* Hook.f	Leaf	16.0	00.0
4.	*Partheniumhysterophorus* Linn	Leaf	12.0	15.0
5.	*Tagetes erecta* Linn.	Inflorescence	00.0	12.0
6.	*Tridax procumbens* Linn	Whole Plant	25.0	16.0
7.	*Xanthium strumarium* Linn	Leaf	00.0	00.0

Table 2 : Antibacterial Activity in Different Fractions of Whole Plant Extraction of *Tridax procumbens* Linn

Sl.No.	Different fraction of the active plant parts	Inhibition Zone(mm.)	
		Shigella sp .	***Klebsiella sp.***
1.	Petroleum ether	30.0	26.0
2.	Benzene	00.0	00.0
3.	Carbon tetrachloride	00.0	00.0
4.	Chloroform	00.0	00.0
5.	Acetone	00.0	00.0
6.	Methanol	00.0	00.0

Table 3 : 12 Hourly Observation of Inhibition Zones Around Treatment Disks of Different Strengths in *Klebsiella spp.* and *Shigella spp* seeded Plates

S.No.	Hours	Observation
1.	12 hrs.	Zones were indistinct
2.	24 hrs.	Zones started to appear in 7, 9, 12, 14 & 15 mg. No zone were formed around 2, 3, & 5mg. disks.
3.	36 hrs.	The zones around 7, 9, 12, 14 & 15 become distinct and clearly visible. 2, 3, and 5 mg. disks did not affect the test pathogens.
4.	48 hrs.	Invasion of bacteria started in the zone around 7, 9, and12 mg. disks. Zone around 14 and 15 mg. disks were unaffe cted and 2, 3, and 5 mg. disks still remained incapable to form any zone.
5.	60 hrs.	Zone around 15 mg. disk remained unaffected. The zone around 7, 9, 12 & 14 mg. disks were invaded by the test pathogen *Klebsiella spp.* but zone around 14 mg. disk remained unaffected in the test pathogen *Shigella spp.* seeded plate.
6.	72 hrs.	Zone of 15 mg. disk still remained unaffected. The zone around 7, 9, 12 & 14 mg. disks were vigorously invaded by the test pathogen but the zone of 14 mg. disk still remained unaffected in the test pathogen *Shigella spp.* seeded plate. No zone around 2, 3, and 5 mg disk.

further investigations. In the present study first time reported active fraction of Tridax procumbens Linn. having strong bactericidal activity and broad antibacterial spectrum at MID.

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