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BIOLOGICAL INVESTIGATION ON DIFFERENT EXTRACTS OF COMMON WETLAND PLANT; *TYPHA LATIFOLIA.*

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ABSTRACT

Typha latifolia is also known as "pater", belonging to family Typhaceae. In the present study antibacterial activity of successive extracts of different parts of the plant like rhizome, shoot, leaves and pollen have been evaluated against both gram positive and gram-negative bacterial strains by disc diffusion method. The methanolic and aqueous extracts of all the plant parts showed effectiveness against all microorganisms as compared to standard penicillin while petroleum ether, chloroform and ethyl acetate extracts of some parts were found to be resistant for few organisms. All the extracts showed significant values of minimum inhibitory concentration (MIC).

Key words*: Wetland, Zone of inhibition, Typhaceae, MIC.*

INTRODUCTION:

India has a good wealth of medicinal plants throughout its geographical region. Aquatic plants have been used as a source of nutrition in folk medicine since eons. Three species of Typha namely *T.latifolia*, *T. angustifolia* and *T. elephantina*[1] are of common occurrence in India. In folk medicine Typha has been used as refrigerant, aphrodisiac and for treating dysuria, burning of skin and disorders of bile. It acts in the same way as medicated cotton wool in local dressing in allaying wounds and ulcer[2]. The pollen of Typha possesses diuretic, emmenagogue and haemostatic[3] properties. The dried pollen has been used internally in the treatment of kidney stones[4], internal hemorrhage of almost any kind[5], painful menstruation, abnormal uterine bleeding, post –partum pains[6], sedative[7], ecchymosis, epistaxis, erysipelas[8], fever, gonorrhea[9] etc.

Different plant parts of Typha can be boiled and eaten like potatoes or macerated and then boiled to yield sweet syrup. [10] Rich in protein, its powder is used to make biscuits. Young flowering raw stem of the plant when cooked or made into a soup[11], tastes like sweet corn and is a protein rich additive to flour used in making bread, porridge etc[12]. Although the whole plant is used in folk medicine and as an economical source of nutrition in various parts of the world but its antibacterial property remains unevaluated. In present study an effort has been taken to explore anti bacterial property of different parts of *Typha latifolia.*

SUBJECTS AND METHODS:

Extraction:
Typha latifolia was collected from Dehradun, Uttarakhand, in the month of October. Its identity was confirmed from the Krishi Vikash Kendra, Sultanpur, U.P. The collected plant parts like rhizome, shoot, leaves and pollen were washed and dried in shade and crushed to coarse powder. Extraction of powdered plant materials (500g) were carried

out using different solvents i.e. petroleum ether, chloroform, ethyl acetate, methanol and water in a soxhlet apparatus. All the successive extract were collected, filtered and concentrated in vacuum under reduced pressure and dried and stored in desiccators.

Qualitative chemical test in the different extracts of *Typha latifolia were* carried out and the tests have shown the presence of alkaloids, sterols, sugars, flavonoids and tannins were present in different extract of rhizome, leaf, shoot and pollen. Absence of resins, anthracene glycoside, cardiac glycosides, volatile oil and gums in all the selected extracts was also found[13]. The plant is well established for the treatment of various common diseases in traditional medicines.

Test Organisms

The antibacterial activity of the extracts were tested in vitro using *Staphlococcus aureus, Escherchia coli, Pseudomonas aeruginosa* and *Basillus* collected from department of microbiology, ANDCP, Babhnan, Gonda, U.P. The growth Medias used were nutrient agar and nutrient broth.

Preparation of inoculums[14]

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of microorganism from the stock cultures to test tubes of nutrient broth, and incubated for 24 hrs at 37°C. The cultures were diluted with fresh nutrient broth.

Preparation of Media

The medium was prepared by dissolving the different ingredients in water and autoclaved at 121^0C for 15 minutes. This was used for preliminary antibacterial studies.

Antibacterial activity[15-16]

In vitro antibacterial activity was screened by disc diffusion method using nutrient agar (NA) made from Himedia (Mumbai). The different extracts were loaded on different 3mm sterile disc till saturation. The discs were allowed to diffuse solvents for 5 minutes. The loaded disc was placed on the surface of medium containing microorganisms and the plates were kept for incubation at 37°C for 24 hrs in an incubator. At the end of incubation, zone of inhibition formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate by using standard drugs (10 mcg/disc Penicillin).

Minimum Inhibitory Concentration by Serial Dilution technique[17]

Testing was done in seeded broth containing 106 to 107 colonies forming units per ml (Cfu/ml). The crude extracts were taken at different concentrations ranging from 1000, 500, 250, 125, 62.5, 31.25 µg/ml to determine MIC by using seeded broth as diluent. Similarly, standard penicillin preparations were formulated at same concentrations as used in plant extracts**.** DMSO was used as solvent system for the extracts and standard drug in the experiment. The study involved a series of six assay tubes for the test compounds against each strain. In the first assay tube, 1.8 ml of seeds broth was transferred and 0.2 ml of test solution was added and mixed thoroughly to obtain a concentration of 1000 µg/ml for the extracts. To the remaining five assay tubes, 1 ml of seeded broth was transferred and then from the first assay tube, 1 ml content was pipetted out into the second assay tube and this was mixed thoroughly. This type of dilution was repeated up to 6th assay tube serially. The same procedure was followed for standard drugs. All these experimental procedures were carried out under absolute aseptic conditions. The experiments were done in

triplicate. The assay tubes were then incubated at 37 ± 1°C and resultant turbidities were measured using turbidity meter and MIC was calculated. Solvent controls were also observed for inhibitory action**.** DMSO did not show any inhibition.

For crude extract and fractions, an MIC below 100 µg/mL was considered as an excellent effect, 100 to 500 µg/mL as moderate, 500 to 1000 µg/mL as weak and over 1000 µg/mL as inactive.

RESULTS

The antibacterial activity of different parts of *Typha latifolia* indicated that the chloroform, methanolic and aqueous extracts of rhizome of the plant has significant antibacterial activity against all the test microorganisms. Petroleum ether and ethyl acetate extracts were not found to be significantly effective against *P. aureginosa* and *Basillus*. methanolic and aqueous extracts of shoot of the plant exhibited significant antibacterial activity against all bacteria while the petroleum ether and ethyl acetate extracts against *P. aureginosa* and *Basillus*, Pet ether against *E coli*, Chloroform extract against S.aureus were ineffective. Petroleum ether, chloroform, methanolic and aqueous extracts of leaves showed significant antibacterial activity against all bacteria whereas the ethyl acetate extract was ineffective against *P. aureginosa* and *Basillus*. Petroleum ether, methanolic and aqueous extracts of pollen had significant antibacterial activity against all bacteria while the chloroform and ethyl acetate extracts were found ineffective against *P. aureginosa* and *Basillus* respectively. The zones of inhibition in diameter (mm) recorded for all the extracts of rhizome, shoot, leaves and pollen has been presented in table and figure 1-4 respectively. Minimum inhibitory concentrations were measured in µg/ml for all the extracts. Readings were presented in table and figure 5. methanolic and aqueous extract of all the plant parts showed variations in MIC i.e. 1000-31.25 µg/ml, for different test microorganisms. Petroleum ether and ethyl acetate extracts showed MIC >1000 µg/ml against *P. aureginosa* and *Basillus* ;shoot extracts in petroleum ether and ethyl acetate against P. aureginosa and Basillus, pet ether against *E coli*, chloroform extract against S.aureus were found inactive (MIC >1000 µg/ml). Extracts of leaves in pet ether, chloroform, alcohol and water showed moderate to weak activity against all bacteria whereas ethyl acetate extract against *P. aureginosa* and *Basillus* was inactive. Petroleum ether, methanolic and aqueous extracts of pollen has excellent activity against all bacteria. Chloroform and ethyl acetate extracts were inactive against *P. aureginosa* and *Basillus*. MIC for standard penicillin was 10 µg/ml.

DISCUSSION:

Typha is a versatile herb, its rhizome is used as vegetable [18] and other parts of the plant are used to prepare different recipes. It has been used to treat wounds and urinary disorders[19]. Its antibacterial property is untouched and hence this property has been taken up for detailed assessment in the current study. The zone of inhibition of different extracts and standard have been recorded in table and figure 1. All the extracts were effective against S.aureus. Significant antimicrobial activity was observed for aqueous, ethyl acetate and petroleum ether extracts. The aqueous extract was also found to be significantly effective against bacillus; whereas the other extracts were found to be resistant. Minimum inhibitory concentration was measured in µg/ml for all the extracts. Reading was presented in table and figure 2. All the extracts showed variations in the MIC 1000-31.25 µg/ml.

Present findings support the applicability of *Typha latifolia* in traditional systems for its claimed uses like urinary infectious diseases. Microorganisms are developing rapid resistance towards many commonly used antibiotics.

Hence it is the need of the hour to seek out novel antibiotics. Plants continue to be a rich source of therapeutic agents and the present study reveals the potential value of *Typha latifolia* in this regard.

Table 1. **Zone of inhibition of different extracts of *Typha latifolia* in mm.**

S.N.	Zone of inhibition of different extracts in mm							
	Plant Part	**Microorganisms**	**Pet.Ex**	**Chlo.Ex**	**Eth.Ac**	**Methanol.E**	**Aq.E**	**STD Penicillin**
1	**Rhizome**	***E.c***	3	2	5	8	4	10
2		***P.a***	0	6	0	4	9	
3		***S.a***	6	4	8	5	7	
4		***B.***	0	3	0	2	6	
5	**Shoot**	***E.c***	0	3	2	6	7	
6		***P.a***	0	1	0	5	3	
7		***S.a***	1	0	8	3	8	
8		***B.***	0	4	0	6	4	
9	**Leaf**	***E.c***	6	9	3	5	4	
10		***P.a***	4	8	0	4	1	
11		***S.a***	2	2	6	7	4	
12		***B.***	6	9	0	2	7	
13	**Pollen**	***E.c***	6	8	0	7	2	
14		***P.a***	9	6	9	4	6	
15		***S.a***	3	4	6	9	4	
16		***B.***	4	0	8	7	9	

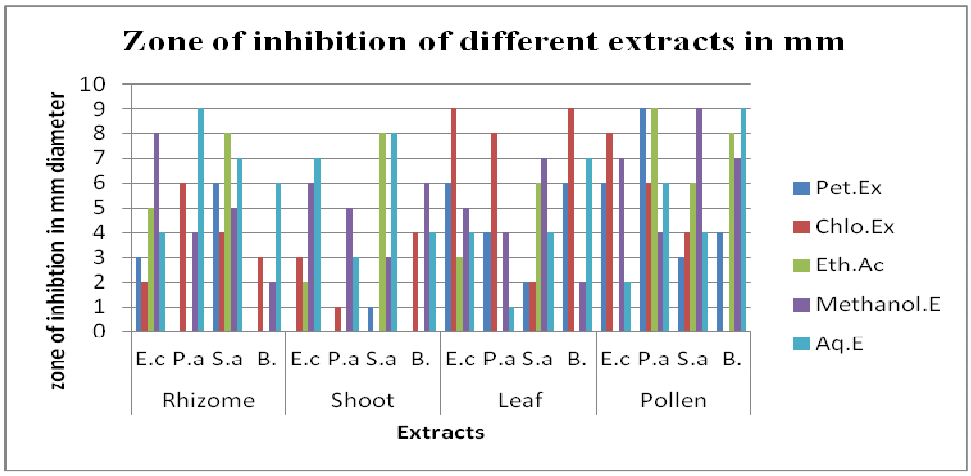

Figure 1. Zone of inhibition of different extracts of *Typha latifolia* in mm.

Table *2*. Minimum Inhibitory Concentration of different extracts *of Typha latifolia in µg/ml.*

Minimum Inhibitory Concentration of different extracts in µg/ml								
S.N.	**Minimum Inhibitory Concentration of different extracts in µg/ml**							
	Plant Part	**Microorganisms**	**Pet.Ex**	**Chlo.Ex**	**Eth.Ac**	**methanol.E**	**Aq.E**	**STD Penicillin**
1	**Rhizome**	***E.c***	500	1000	500	31.25	500	10
2		***P.a***	**nd**	125	**nd**	500	31.25	
3		***S.a***	500	500	125	500	250	
4		***B.***	**nd**	1000	**nd**	1000	500	
5	**Shoot**	***E.c***	**nd**	500	1000	500	250	
6		***P.a***	**nd**	1000	**nd**	250	500	
7		***S.a***	1000	**nd**	125	500	31.25	
8		***B.***	**nd**	500	**nd**	500	125	
9	**Leaf**	***E.c***	500	31.25	1000	250	125	
10		***P.a***	500	125	**nd**	125	1000	
11		***S.a***	1000	1000	500	250	250	
12		***B.***	500	31.25	**nd**	1000	250	
13	**Pollen**	***E.c***	250	125	**nd**	250	1000	
14		***P.a***	31.25	250	31.25	500	125	
15		***S.a***	500	500	250	31.25	500	
16		***B.***	500	**nd**	125	250	31.25	

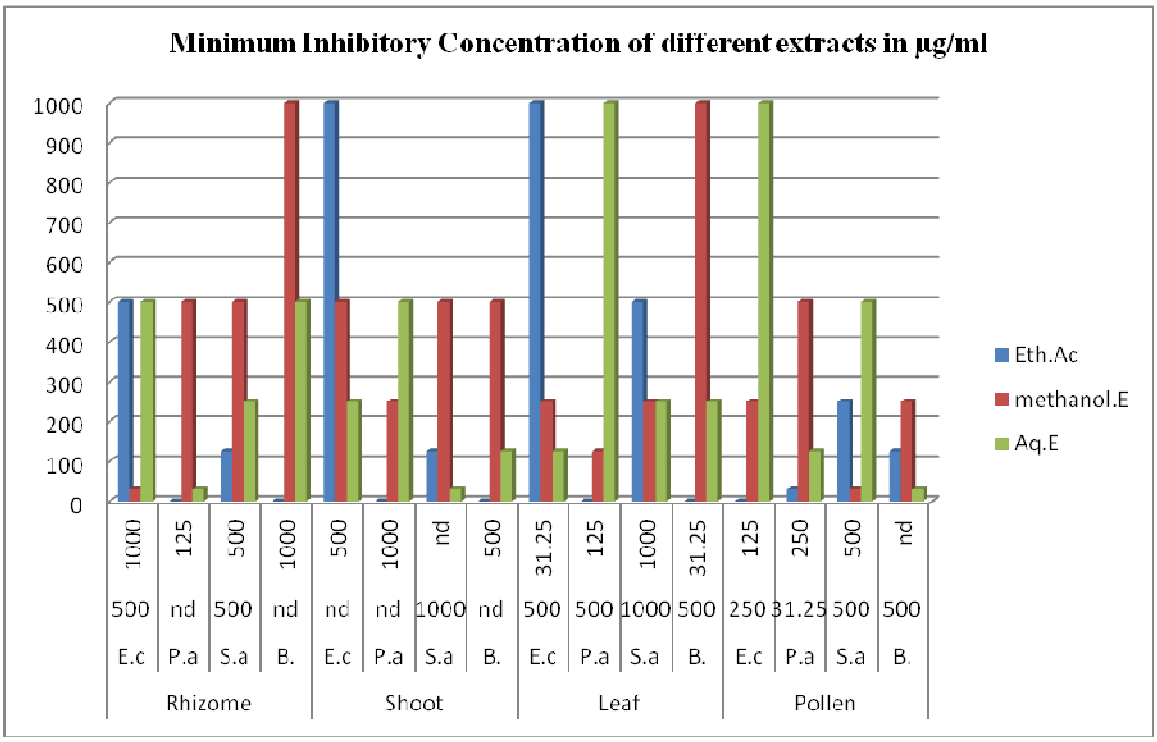

Figure 2. Minimum Inhibitory Concentration of different extracts of *Typha latifolia* in µg/ml.

Where E.c= E.coli P.a= P.aureginosa S.a= S.aureus B.= Bacillus**,** *nd : not determined as the MIC was >1000 µg/ml.*

Pet.Ex= Petroleum ether extract, Chlo.E= Chloroform extract, Eth.Ac= Ethyl acetate, Alco.E= Alcoholic extract, Aq.E=Aqueous Extract, STD=Standard (Penicillin)

DISCUSSION:

Typha is a versatile herb, its rhizome is used as vegetable [18] and other parts of the plant are used to prepare different recipes. It has been used to treat wounds and urinary disorders[19]. Its antibacterial property is untouched and hence this property has been taken up for detailed assessment in the current study. The zone of inhibition of different extracts and standard have been recorded in table and figure 1. All the extracts were effective against S.aureus. Significant antimicrobial activity was observed for aqueous, ethyl acetate and petroleum ether extracts. The aqueous extract was also found to be significantly effective against bacillus; whereas the other extracts were found to be resistant. Minimum inhibitory concentration was measured in µg/ml for all the extracts. Reading was presented in table and figure 2. All the extracts showed variations in the MIC 1000-31.25 µg/ml.

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