International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF *TYPHONIUM TRILOBATUM* PLANT

Sourav Kanti Roy[1], Pratyush Kumar Mishra[1], Subhangkar Nandy*[2] and Viren kumar Patel[3]

[1]Department of Pharmacology, School of Pharmaceutical Sciences, SOA University, Bhubaneswar, Orissa, India
[2]Department of Pharmacology, Vedica College of Pharmacy, RKDF Group, Bhopal, MP, India
[3]Department of Pharmaceutical chemistry, Vedica College of Pharmacy, RKDF Group, Bhopal, MP, India

***Corresponding author e-mail:** subhangkarnandy@gmail.com

ABSTRACT
Typhonium trilobatum is a genus in the Araceae family endemic to tropical Asia, the South Pacific, and Australia. Most of the plant parts of *Typhonium trilobatum* are used in traditional systems of medicine in India. According to Ayurveda, the rhizome is used with effect for treating vomiting, cough, asthma, excessive expectoration, pyogenic sore throat, headache, gastric ulcer, abcess and snake bite. The present work objectives focus on scientific exploration of anti microbial activity of whole plant of *Typhonium trilobatum*. The test all extracts exhibited prominent antimicrobial activity against all tested organism, where chloroform extract shown the maximum zone of inhibition. The order of antimicrobial activity of all extract are, chloroform > Methanolic >Ethyl acetate against bacteria i.e. *Escherichia Coli, P. aeruginosa, Staphylococcus aureus and S. Epidermidis* and fungi i.e. *Candida albicans* and *Aspergillus niger* .

Keywords: *Typhonium trilobatum*, Antimicrobial activity, Human pathogens, MIC, chloroform, methanol and Ethyl acetate extract.

INTRODUCTION

Many plants are used as folk medicines to infectious diseases such as urinary tract infections, diarrhea, cutaneous abscesses, bronchitis and parasitic diseases [1-4]. Due to the indiscriminate use of antibacterial drugs, the microorganisms have developed resistance to many commercial antibiotics. Therefore, investigation of the chemical compounds within medicinal plants has become desirable [5]. Even though certain plants have been demonstrated for their effects on pathogenic bacteria [6], a number of them have not yet been investigated for their antimicrobial activities. Hence, it is essential to establish the scientific basis for their therapeutic actions as these may serve as the source for the development of effective drugs. Antibacterials which destroy are bactericides or germicides; those which merely suppress growth are bacteriostatic agents of the thousands of antimicrobial agents, only a small number are safe chemotherapeutic agents, effective in controlling infectious diseases in plants, animals, and humans. A much larger number are used in almost every phase of human activity: in agriculture, food preservation, and water, skin, and disinfection. Some antimicrobial agents are Sulphonamide, Chloramphinicol, Erythromycin, Polymaxin, Griseofulvin, Chlorin, Glycolic acid, etc. [7-8]

Typhonium trilobatum is a genus in the Araceae family endemic to tropical Asia, the South Pacific, and Australia. It consists of approximately 50 species that are typically found growing in wooded areas [9]**.** It has been valued in Ayurveda and Unani systems of medicine for possessing variety of therapeutic properties. Most of the plant parts of *Typhonium*

trilobatum are used in traditional systems of medicine in India. According to Ayurveda, the rhizome is used with effect for treating vomiting, cough, asthma, excessive expectoration; pyogenic sore throat, headache, gastric ulcer, abcess and snake bite [10].

Therefore such plants should be investigated to understand their properties, safety and efficacy and for a search of new potent antimicrobial compounds and fractions. The present study was undertaken to investigate the potential of medicinal plant, *Typhonium trilobatum* as antimicrobial agent of their different extract against some pathogenic bacteria and some pathogenic fungi. These lead us to evaluate the antibacterial, MIC and antifungal activities of the methanolic extract and its different fractions of *Typhonium trilobatum* against four pathogenic bacteria and two fungi.

MATERIALS AND METHODS

Plant Collection: The whole plant was collected from rural belt of Westbengal, medinipore district, during the month of July and August 2010 in the early morning. The plant was identified, confirmed and authenticated by Prof. Sushil Ku. Mallick taxonomist in the Department of Botany, S.V.M Autonomous College, Bhubaneswar. After authentication the plant was collected in bulk and washed under running tap water to remove adhering dirt and soil particles.

The plants were dried under shade at room temperature, after washing. The dried materials were made into coarse powder by grinding in mechanical grinder and passed in sieve no 40 and used for further study.

Preparation of Extracts: The coarse powder of the plant was taken in soxhlet apparatus and extracted with methanol. The extraction with each solvent is done for 72 hours and the temperature was maintained in between 37-40^0c to prevent the loss of thermosensitive constituent of the plant.

The methanolic extract is mixed with water(1:1) and used for fractionation with chloroform and ethylacetate by the use of separating funnel. The liquid extracts and fractionation were concentrated separately under vacuum and resulting dried extracts were preserved in a desiccator until further use.

In vitro Antibacterial activity:

Bacterial Strains: The various organisms used in the present study include *Escherichia Coli, P. aeruginosa, Staphylococcus aureus and S. Epidermidis* were collected from National collection of industrial microorganisms (NCIM), National Chemical Laboratory, Pune, Maharastra, India. These organisms were maintained on nutrient agar slopes and the organisms were confirmed by biochemical test.

Preparation of standard bacterial suspension: The average number of viable *Escherichia coli* (ATCC 25922), *Staphylococcus aureus*(NCTC 25953),*Pseudomonas aeruginosa*(ATCC 27853*)* and *S.Epidermidis* organisms per ml of the stock suspensions was determined by means of the surface viable counting technique. About (108-109) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Preparation of Test Solutions: The solution of the test compound was prepared by dissolving different extracts of plant *Typhonium trilobatumin* Dimethyl sulphoxide(DMSO) separately in a beaker and stored in refrigerator. The solution was removed from the refrigerator one hour prior to its use and allowed to warm upto room temperature. The solution of test compound were prepared of concentration of 50mg/ml and 100mg/ml. similarly the standard drug solution of ampicillin of a concentration of 100mg was used for finding zone of inhibition. Solvent control of DMSO was also maintained paralley throughout the experiment.

Preparations of Media for Bacterial strain: Nutrient Agar was used as a media preparation. For 1000 ml Nutrient Agar preparation, Peptone-10gm, Sodium Chloride-5.036, Beef Extract-5.036, and Agar-15.225 was weighed and dissolved in 1000 ml of distilled water and adjusted to pH 7.3-7.4 which was sterilized by autoclaving at 121^0C for 15 minutes at 15 psi pressure and was used for sensitivity tests.

Testing for antibacterial activity: The cup-plate agar diffusion method was adopted to assess the antibacterial activity of the prepared extracts, 0.6ml of standardized bacterial stock suspensions (108-109) colony-forming units per ml was thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile

Petri dishes .The agar was left to set and in each of these plates 2 cups, 6mm in diameter, were cut using a sterile cork borer No.4 and the agar discs were removed. Alternate cups were filled with 300µl of each extracts using microtiter-pipette and allowed to diffuse at room temperature for two hours .The plates were then incubated in the upright position at 37^0C for 18 hours. Two replicates were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation the diameters of the results and growth inhibition zones were measured, averaged and the mean values were tabulated [11-15].

In vitro Antifungal studies

Fungal Strain: The fungal strain is used *Candida albicans* and *Aspergillus niger* procured from C.B.T laboratory were used for study. The temperature was maintained at 28^0c.the same concentration of test compound was prepared as done previously. Solvent control was DMSO and fluconazole was positive drug control for these antifungal studies.

Preparation of Media: Sabourand Dextrose Agar (Hi-media) media (SDA) was used for cultivation of fungi and particularly pathogenic fungi associated with skin infections. Formula gm/litre Peptone 10g, dextrose 40g and agar 15g; pH5.6 ± 0.2. 65 gm of SDA was dissolved in 1000ml of distilled water. The medium was sterilized by autoclaving at 121oC for 15 minutes at 15psi pressure.

Preparation of standard fungal suspensions: The fungal cultures *Aspergillus niger* (ATCC 9763), *candida albicans*(ATCC 7596),were maintained on saboraud dextrose agar, incubated at 25^0C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in (100ml) of sterile normal saline and the suspension was stored in refrigerator till used.

Testing for anti-fungal activity: The same method as for bacteria was adopted. Instead of nutrient agar, yeast and mould extract agar was used. The inoculated medium was incubated at 25^0C for two days for the Candida albicans and three days for Aspergillus niger [16-17].

The estimation of MIC by the dilution method in the tube: Dilution method was used to measure MIC colony made from 24 hour culture of bacterium inoculated to Mooler Hinton Berath culture medium. This suspension was inoculated at 37ºC for about 4 to 6 hours in order to get the bacteria to the dynamic level and compared to Macfarland 0.5 standard at last. As results the suspension contain 10 bacteria in each ml. Microbial suspension was diluted to the proportion of 1/100 in order to reach 106 bacteria in each ml. To measure the MIC, 1ml of Mooler Hinton Berath culture was poured in 10 tubes and mixed right after adding 1ml of the extract to the first tube . One ml of first tube was added to the second and 1 ml of the second to the third tube respectively. Thus the dilution is obtained. Then 1ml of the microbial suspension was added to each tube to make concentrations. The tubes were incubated at 37ºC and MIC was appointed by the growth or non-growth of the bacterium in the tubes.

***Statistical Analysis*:** Results are expressed as the mean value ± standard error of mean (S.E.M.). Within group comparisons were performed by the analysis of variance using ANOVA test. Significant difference between control and experimental groups was assessed by student's t test. A probability level of less than 5 %($P < 0.05$) was considered significant.

RESULTS AND DISCUSSION

The antimicrobial activity of Methanolic extract and its chloroform and ethyl acetate fractions of aerial parts of the plant *Typhonium trilobatum* Linn. was studied against two gram positive (*S. aureus ,s. epidermidis*), two gram negative (*E. coli, P. aeruginosa*) bacteria and two fungus (*C. albicans, A. niger*) by zone of inhibition and Minimum inhibitory concentration method using two con. levels (50mg and100mg/ml).

The zone of inhibition study result is depicted in Table 1. The result indicates that, methanolic extract, chloroform fraction and ethyl acetate fraction showed zone of inhibition of 15-20, 10-15, 13-24, 13-20, 13-17 and 11-14 mm against *S.aureus, S.epidermidis, E.coli, P.aeruginosa, C.albicans and A.niger*. However the standard drug ciprofloxacin and griseofulvin registered zone of inhibition of 10-28 and 20-22 mm against *S.aureus, S.epidermidis, E.coli, P.aeruginosa* as bacteria and *C.albicans and A.niger* as fungi. The solvent control group is kept parallel throughout the experiment and found that there is no zone of inhibition observed against the tested bacteria and fungi. Chloroform fraction showed zone of inhibition of 17.66, 11.33, 13.02 14.33, 13.02 and $0mm^2$ at 50mg/ml concentration

while at 100mg/ml it became 20.33, 15.66, 24.01, 20.02,15.66 and 14 mm^2 against *S.aureus, S.epidermidis, E.coli, P.aeruginosa, C.albicans and A.niger*. . Methanolic extract showed zone of inhibition of 15.02, 10.33, 15.02,13.33, 13.02 and 0 mm^2 at 50mg/ml concentration while at 100mg/ml it become 16.66, 13.02, 16.66,14.66, 17.66, 11 $mm^{2,}$, similarly ethyl acetate fraction showed against the same tested organism at two concentrations of 50mg/ml as 15.02,11.33,14.03,14.66, 14.66, 0 mm^2 and at 100mg/ml as16.66,13.66, 15.02,16.66, 16.66 mm^2 respective to the above order of micro-organisms. The statistical analysis of the measured zone of inhibition indicates that, the values (diameter) are significantly different with an extent of $p<0.001$. It has been observed that chloroform fraction is more potent with a zone of inhibition of 15-24 mm at 100 mg/ml, followed by methanolic extract 13-17 mm and ethyl acetate fraction 13-16 mm against the tested micro-organisms as per the above sequence. The test extract and fractions exhibited prominent antimicrobial activity against all tested organism, except *A.niger*, which is sensitive to methanolic extract and chloroform fraction at 100mg/ml con. only. The phyto constituent (s) present in the chloroform fraction like Saponin, other phenolic compound may be responsible for the said activity. The order of antimicrobial activity of extract and fractions are, Chloroform fraction> Methanolic extract>ethylacetate fraction against *S.aureus, S.epidermidis, P.aeruginosa. E.coli, C albicans and A.niger*[18-19]. **(Figure 1 and 2)(Table 1).** The minimum inhibitory concentration (MIC) of Methanolic extract, chloroform fraction and ethyl acetate fraction was carried out by two-fold serial dilution technique against the microorganisms, *S.aureus, S.epidermidis ,E.coli ,P.aeruginosa, C.albicans, A.niger*. The MIC of methanolic extract against the above order of microorganism are found as 50, 100, 50,100,100,50mg/ml. While Chloroform fraction showed 100,50,25,100,50,100mg/ml,and Ethyl acetate fraction registered 100,100,50,100,100,100 mg/ml. respectively. Methanolic extract is active against S.aureus, and E. coli at 50 and 50mg/ml. while chloroform fraction is more active against *S.epidermidis* (50 mg/ml), *E. coli* (25 mg/ml), *C. albicans* (50 mg/ml) similarly ethyl acetate fraction showed active against E. coli (50 mg/ml). As a whole the test extract and fractions are more active against E. coli followed by others with MIC value of 50-100 mg/ml in all test drugs against all tested bacteria and fungi except E. coli in which the MIC of chloroform fraction showed 25 mg/ml. But the solvent control (DMSO) registered zero (MIC) against all tested organism. From the study it indicates that the extract and fraction posses antimicrobial activity against all tested microorganism. Chloroform fraction is more potent because it shows MIC at 25mg/ml on E. coli, where as the remaining test drugs showed 50-100 mg/ml. The phytoconstituent present in the chloroform fraction may be responsible for the said activity. The phyto constituent (s) present in the chloroform fraction like Saponin, some phenolic compound may be responsible for the said activity [20]. **(Table 2)** (Cowan MM, 1999 and OkwuDE et al, 2006)

ACKNOWLEDGEMENTS

The authors are thankful to Chairman, Principal (School of Pharmaceutical Sciences, SOA University, Bhubaneswar, Orrisa).

Figure 1: Graph showing the Zone of Inhibition of methanolic, chloroform and ethyl acetate (100mg/ml) extract of *Typhoniuum trilobatum* plant on six different microorganisms.

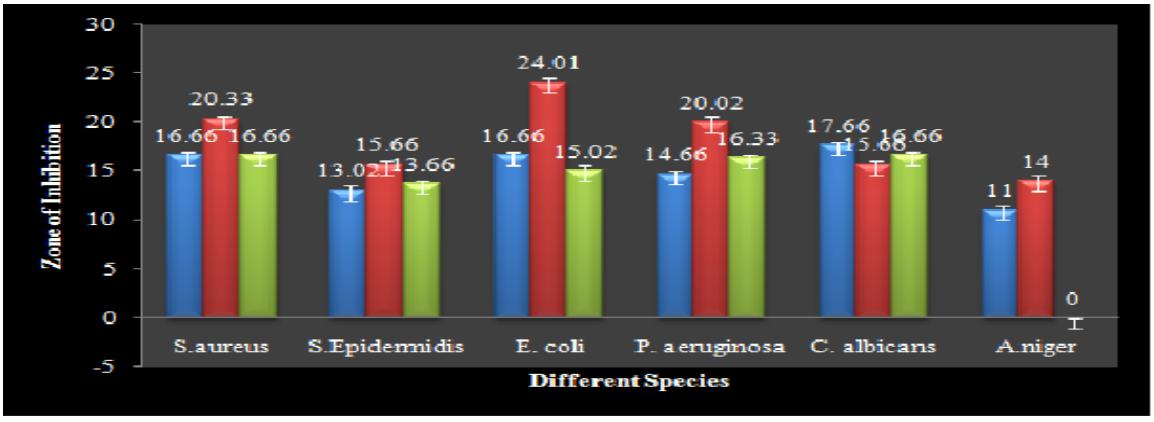

Figure 2: Graph showing the Zone of Inhibition of Griseofulvin (20µg/ml) and Ciprofloxacin (100µg/ml) as standard antimicrobial agents on six different microorganisms

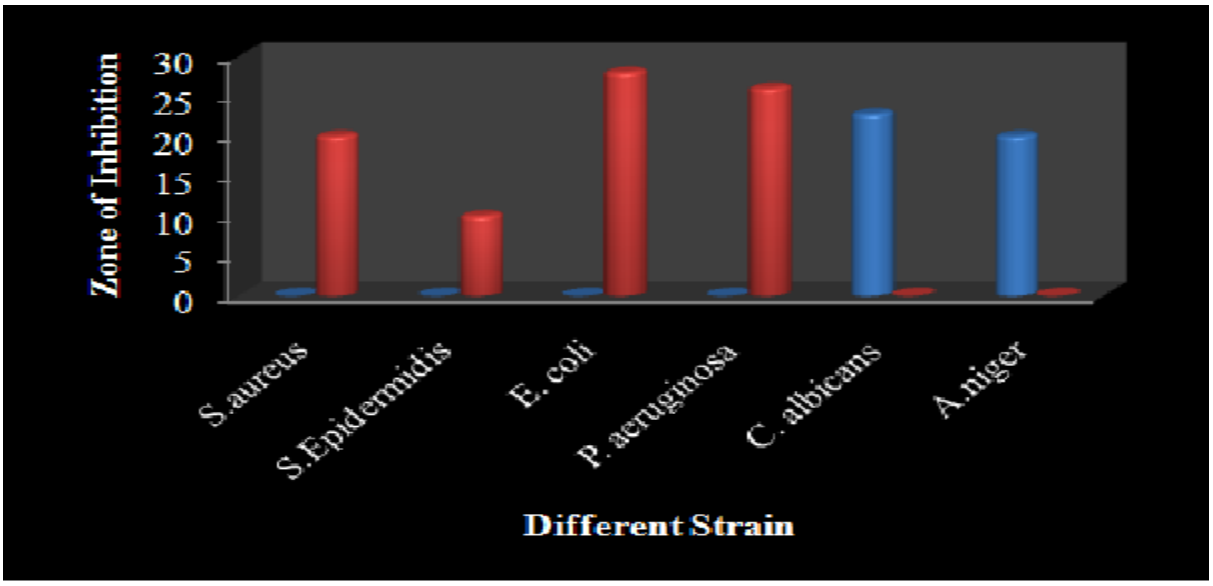

Table-1: Effect of the methanolicextract and its chloroform and ethyl acetate fraction of *Typhoniuum trilobatum* on zone of inhibition against selected bacteria and fungi

Sample	Concentration	Zone of inhibition in(mm)					
		S.aureus	*S.Epidermidis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A.niger*
DMSO	300(µg/ml)	-	-	-	-	-	-
Griseofulvin	20(µg/ml)	NA	NA	NA	NA	22.8±1.1^{c}	20±1.1^{c}
Ciprofloxacin	100 (µg/ml)	20.00±0.00	10.00±0.57	28±0.00	26±0.00	NA	NA
Methanolic extract	50 (mg/ml)	15.02±0.57^{c}	10.33±0.33^{c}	15.02±0.57^{c}	13.33±0.33^{c}	13.02±0.57^{c}	-
	100 (mg/ml)	16.66±0.33^{c}	13.02±0.57^{c}	16.66±0.33^{c}	14.66±0.33^{c}	17.66±0.33^{c}	11±0.57^{c}
Chloroform fraction	50 (mg/ml)	17.66±0.33^{c}	11.33±0.33^{c}	13.02±0.57^{c}	14.33±0.33^{c}	13.02±0.57^{c}	-
	100 (mg/ml)	20.33±0.33^{c}	15.66±0.33^{c}	24.01±0.57^{c}	20.02±0.57^{c}	15.66±0.33^{c}	14±0.57^{c}
Ethyl acetate fraction	50 (mg/ml)	15.02±0.57^{c}	11.33±0.33^{c}	14.03±0.57^{c}	14.66±0.33^{c}	14.66±0.33^{c}	-
	100 (mg/ml)	16.66±0.33^{c}	13.66±0.33^{c}	15.02±0.57^{c}	16.33±0.33^{c}	16.66±0.33^{c}	-

Values are mean ± S.E.M. of 3 replications. t-value represents significance at $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$ respectively, when compared with solvent control group.
S.aureus- Staphylococcus aureus, S.epidermidis -Staphylococcus epidermidis, E.coli- Escherichia coli, P.aeruginosa-Pseudomonas aeruginosa,C.albicans-Candida albicans. (–)- no measurable zone. Aqueous extract, methanol extract and Chloroform extracts respectively each having concentration 300 µg/disc.

Table- 2: Effect of the methanolic extract and its chloroform and ethyl acetate fraction of *Typhoniuum trilobatum* on MIC against selected bacteria and fungi

Sample	Minimum Inhibitory Concentration (mg/ml)					
	S.aureus	***S.epidermidis***	***E.coli***	***P.aeruginosa***	***C.albicans***	***A.niger***
DMSO	-	-	-	-	-	-
Methanolic extract	50	100	50	100	100	100
Chloroform fraction	100	50	25	100	50	100
Ethyl acetate fraction	100	100	50	100	100	100

S.aureus: Staphylococcus aureus, S.epidermidis: Staphylococcus epidermidis, E.coli: Escherichia coli, P.aeruginosa: Pseudomonas aeruginosa, C.albicans: Candida albicans, A.niger: Aspergillus niger.

REFERENCE

1. Yasunaka K, et al. J Ethnopharmacol, 2005; 97: 293-99.
2. Bhuiyan MNI, Chowdhury JU, Begum J. Bangladesh J Pharmacol, 2009; 4: 9-12.
3. Zakaria ZA, Mohamad AS, Chear CT, Wong YY, Israf DA, Sulaiman MR. Med Princ Pract, 2010; 19: 287-94.
4. Yob NJ, et al.. Evid Based Complement Alternat Med, 2011.
5. Ahmad I, Mehmood Z, Mohammad F. J Ethonopharmacol, 1998; 62: 183-93.
6. Voravuthikunchai SP, Popaya W, Supavita T. J Ethnopharmacol, 2004; 33: 60-5.
7. Verpoorte R, Contin A Memelink J. Phytochem. Rev, 2002; 1: 13-25.
8. Kumar A, Nehar S, Rawat M. 2007; 9: 24-8.
9. Mohammed Rahmatullah, et al. Ame Eurasian J Sustainable Agriculture, 2010; 4: 55-64.
10. Das P, Palai SK, Samantaray S. Plant Growth Regulation, 1999; **27:** 193–97.
11. Kokoska L, Polesny Z, Rada V, Nepovim A, Vanek T. J Ethnopharmacol, 2002; 82: 51-3.
12. Lehmann PF. Vet immunol Immunopatholo, 1985; 10: 33-69.
13. Gerard J. Tortora, Berdell R. Funke, Christine L. Microbiology An introduction, Pearson education; 8th edition: 591-592.
14. Pelczar MJ, Reid JD, Jr. ECS Chan. Microbiology, Tata Mcgraw Hill, New Delhi; 5th edition, 1974; 473-491.
15. Frobisher M, Hinsdill RD, Crabtree KT, Goodheart CR. Fundamentals of Microbiology. Philadelphia, PA, W.B. Saunders Company, Chapter 23 Antibiotics, 9th Ed, 1974; 319- 330.
16. Sunil Christudas, Kulathivel TM, Agastian P. Asian Pacific J Tropical Biomed, 2012; 2: 159-61.
17. Golam Kader, Farjana Nikkon1, Mohammad Abdur Rashid, Tanzima Yeasmin. Asian Pacific J Tropical Biomed, 2011; 2: 409-12.
18. Periyasamy N, Srinivasan M, Balakrishnan S. Asian Pacific J Tropical Biomed, 2012; 36-40.
19. Umesh Kumar, Kunal Kumar, Hindumathy CK. Int J Microbiol Res, 2012; 4: 182-5.
20. Cowan MM. Clinical Microbiol Rev, 1999; 12: 564-82.