

Physicochemical characterisation and antifungal, CNS depressant activity of certain Ethnomedicinal plants of Tripura state, India

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ABSTRACT

Methanolic extract of edible parts of certain ethno medicinal plants (10 Nos.), growing in state Tripura, India, which are used by tribal people, were physicochemically characterized and screened for antifungal activity against *Candida albicans* and also for CNS depressant property in mice. It was found that extracts were able to exhibit antifungal activity except O3 extracts. Among them, extract of *I. aquatica* showed zone of inhibition 1.6 cm, which was nearer to the effect of standard. Extracts of *S. torvum* and *L. spinosa* showed CNS depressant property in 100mg/kg body weight comparable to the effect of standard in 5mg/kg body weight.

Key words: Edible parts, Tribal, Physicochemical, *Candida albicans* and CNS depressant.

INTRODUCTION

Since the time immemorial man made use of plants in the treatment of diseases. The pharmacopoeias of many countries of the World even today include a large number of drugs of plant origin. The history of medicinal plants dates back to Rigveda, perhaps the oldest repository of human knowledge, which was written in about 4500-1600 B.C. Then there is the Ayurveda (about 2500 B.C.), which gives us details accounts of many herbal drugs. Our North East India including Tripura is very rich in plant and herbs because of plenty of rainfall and availability of deep forest. Above all we are rich with the knowledge of Tribal communities including 19 Schedule Tribes of Tripura¹. Over 9500 wild plant species used by tribals for meeting the varied requirements have been recorded so far. Out of 7500 wild plant species used by tribals for medicinal purpose, about 950 are found to be new claims and

worthy of scientific scrutiny. Out of 3900 or more wild species used as edible (as subsidiary food/vegetables) by tribals, about 800 are new information and at least 250 of them worthy of attention of developed as alternative source of food that the world would need in near future.²

In this compilation, we are reporting the physicochemical properties (colour, pH, density, specific gravity and R_f values of the spots obtained in TLC) and antifungal activity (against *Candida albicans*) of the methanolic extract of the edible parts of few plants used by tribal people of Tripura (India), were carried out by paper disc agar diffusion method^{3,4} to observe zone of inhibition in cm and reported in Table -1 and compared with a standard drug Amphotericin-B. The MIC⁵ in $\mu\text{g/ml}$ were also carried out and reported in Table -1. Further, the CNS depressant property⁶ of all extracts were observed and depicted in Table -1.

EXPERIMENTAL

The edible parts of few plants used by tribal people of Tripura were collected from the local market of Agartala, Tripura, India and was authenticated by the expert of Tribal Research Institute, Agartala, Tripura.

Edible parts were cleared from extraneous matters and were shade dried with occasional shifting of material to avoid any growth of fungi. Completely dried edible parts were powdered and passed through sieve 40. Extraction was done by using Soxhlet apparatus in methanol (5gm in 500ml). Liquid extract was collected, filtered, air dried followed by keeping at desiccator, for further works.

The chemicals used for all purposes were of analytical grade.

The colour of the extracts were recorded on visual observation through naked eye. The pH of the extracts were observed in a pH meter (Toshniwal Instrument Mfg. Ltd., CAT No. CL 52) at 25°C. The density in gm/ml and specific gravity⁷ of all extracts were also recorded. The TLC^{8,9} was also performed for the extracts by taking Butanol: Acetic acid: Water = 4:1:3 as mobile phase and silica gel-G as stationary phase. The spot identification was done under iodine vapour. The R_f value of all the spot were calculated accordingly.

Antifungal Investigation by Paper disc Agar Diffusion Method

Antifungal studies were carried out by paper disc agar diffusion method for the extracts of edible parts of few plants used by tribal people of Tripura against *Candida albicans*. The zone of inhibition in cm were recorded and compared with the standard drug Amphotericin-B of 100 µg/ml w/v concentration. From the stock, the subculture was prepared by dissolving the methanolic extract of edible parts in dimethyl formamide (DMF) to obtain 100 µg/ml w/v solution. In petri dishes the Sabouraud Dextrose Agar (SDA) media were spreaded along with the organism (Pour plate technique) followed by placing the paper discs (6 mm dia) soaked with test and standard solutions aseptically and then allowed for 48 hours incubation

at 20°C to note down the zone of inhibition. The SDA media composition was as dextrose-40 gm, peptone-10 gm, agar-25 gm and distilled water up to volume-1000 ml. Each petri dish was containing three discs, one of standard, another of control and 3rd one of test solutions.

MIC (Minimal Inhibitory Concentration)

By adopting serial dilution technique and using SDA broth, the MIC in µg/ml of all extracts against *Candida albicans* were determined. Total 15 Nos. of test tube were taken for each extracts. 2 ml broth, 2 ml test culture and 2 ml extract solution of different concentration in DMF were taken separately in all test tubes and then all the test tubes were incubated for 48 hours at 20°C. The optical density (O.D.) of all content were determined spectrophotometrically at 600 nm wave length by using the mixture of broth and test culture in equivalent ratio (not incubated) for base line correction. As per requirement for bracketing of concentration, later more numbers of test tubes were required for more different concentrations of individual extracts. The concentration was recorded as minimal inhibitory concentration, in which the O.D. was recorded just less than the O.D. of control (2ml broth + 2ml test culture + 2ml DMF).

CNS Depressant property: Study on the locomotor activity of mice

Healthy and adult albino Swiss mice weighing between 20-25 gm in groups of six each were taken for present investigation. One group was marked as standard, where the animals were treated intraperitoneally with Chlorpromazine HCl in the dose of 5 mg/kg (body weight) dissolved in normal saline water. Another group was considered as control, where the animals were treated with only normal saline water. Other groups were studied by the intraperitoneal injection of test compounds dissolved/suspended in normal saline water in the dose of 100 mg/kg (body weight). Before treating with drugs individually each mouse of each group was kept in photoactometer (INCO Photoactometer) for 5 minutes. The basal activity score of all the animals were recorded. After the intraperitoneal injection the animals were kept aside for 15 minutes and then the animals were re-tested individually in Photoactometer for 5 minutes. The average percent decrease of movement (scores) i.e. decrease in

Table -1: Physicochemical characterisation, antifungal activity and CNS depressant property of Methanolic extracts of edible parts of plants used by tribes of Tripura (India)

Name of the Plants(Family)/ Standard Compounds	Edible parts	Colour (pH)	Density in gm/ml (Specific gravity)	Spots in TLC (R _f values)	ZOI (cm) (MIC in µg/ml)	CNS depressant: Average % of decrease of movement ± SEM
<i>Monochoria hastata</i> Linn. (Pontederiaceae)	Stems and leaves	Yellowish green (6.9)	0.9174 (0.9201)	3 (0.62, 0.83, 0.74)	0.8 (1000)	43.01 ± 1.05
<i>Diplazium polypodioides</i> Bl (Athenaceae)	Tender coiled leaves	Yellow (6.1)	0.8927 (0.8953)	3 (0.66, 0.72, 0.87)	Not active (>1000)	44.39 ± 1.14
<i>Alocasia odora</i> Roxb. (Araceae)	Stems	Grey (4.3)	0.9507 (0.9598)	4 (0.27, 0.4, 0.54, 0.6)	0.9 (1000)	49.27 ± 1.74
<i>Musa paradisiaca</i> Linn (Musaceae)	Soft immature bud	Yellowish green - brown (5.2)	0.9681 (0.9709)	3 (0.33, 0.34, 0.36)	1.0 (900)	52.28 ± 0.82
<i>Ipomoea aquatica</i> Linn (Convolvulaceae)	Twigs and leaves	Yellowish green (4.8)	0.8756 (0.8782)	3 (0.36, 0.59, 0.83)	1.6 (250)	40.83 ± 3.58
<i>Dioscorea hamiltonin</i> Hook (Dioscoreaceae)	Tubers	Grayish yellow (4.4)	0.8492 (0.8517)	3 (0.38, 0.58, 0.89)	1.4 (550)	46.26 ± 2.1
<i>Solanum torvum</i> Swartz (Solanaceae)	Fruits	Brownish yellow (9.8)	0.9286 (0.9313)	3 (0.14, 0.52, 0.76)	1.1 (700)	54.99 ± 0.76
<i>Solanum indicum</i> Linn (Solanaceae)	Tender fruits	Greenish & brownish yellow (8.9)	0.8897 (0.8923)	4 (0.16, 0.28, 0.76, 0.89)	Not active (>1000)	44.55 ± 2.68
<i>Canavalia gladiata</i> Jacq (Papilionaceae)	Fruits	Yellow (8.8)	0.9535 (0.9563)	4 (0.13, 0.43, 0.71, 0.92)	1.5 (300)	51.33 ± 1.67
<i>Lasia spinosa</i> Linn (Araceae)	Tender stems and leaves	Greenish deep yellow (9.7)	0.9336 (0.9363)	3 (0.17, 0.61, 0.77)	Not active (>1000)	55.28 ± 0.82
Amphotericin-B (for antifungal activity)		-		-	1.8 (52)	-
Chlorpromazine HCl (for CNS depressant) [Control]		-		-	-	57.88 ± 2.31 [No significant difference]

motor activity was calculated. Reduction in the motor activity indicates CNS depressant property of the drug.

RESULTS AND DISCUSSION

The pH of extracts were found in the acidic range except four i.e. in case of *S. torvum*, *S. indicum*, *C. gladiata* and *L. spinosa*. In maximum cases (extracts) spots were found 03 and 04 spots were found in *A. odora*, *S. indicum* and *C. gladiata* in the study of TLC, where the mobile phase was Butanol : Acetic acid : Water = 4:1:3 and silica gel-G was stationary phase. These spots were due to the presence of organic compounds, identified in iodine vapour.

Among the plants subjected for antifungal screening, total 07 plants were found to show antifungal activity against *Candida albicans*, though

no extract exhibited at par activity to the standard drug Amphotericin-B (zone of inhibition- 1.8cm and MIC in $\mu\text{g/ml}$ -52). Comparable activity were found for the extracts of the plants *I. aquatica* and *C. gladiata*. Significant activity was found for the plants *M. paradisiaca*, *D. hamiltonin* and *S. torvum*. No zone of inhibition was recorded for the plants *D. polypodioides*, *S. indicum* and *L. spinosa*. The MIC value found for all extracts were not at par to the value of standard.

All the extracts were showing the CNS depressant property in the dose of 100 mg/kg body weight, but not at par to the standard drug Chlorpromazine HCl in the dose of 5 mg/kg body weight. Considering the intensity of the activity, it has been observed that the extracts of *S. torvum* and *L. spinosa* showed CNS depressant property comparable to the standard. Significant activity was recorded for *M. paradisiaca* and *C. gladiata*.

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