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ASSESSMENT OF FOOD, ETHNOBOTANICAL AND ANTIBACTERIAL ACTIVITY OF *TRICHOSANTHES CUCUMIRINA* L.

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ABSTRACT: *Trichosanthes cucumirina* L., locally known as “Chachindra” among the tribal and rural mass of Similipal Biosphere Reserve (SBR) forest. Plant parts are used as ethnomedicine among inhabitants of SBR. They use the fruits, leaves and roots against various diseases and traditional practices are continuing since long. Leaves are used against microbial infections, roots against diabetic and fruits against killing of stomach worm. Young fruits are used as vegetables as well as medicine. Keeping this traditional practice in view, assessment of food values and antibacterial properties were done. Fruits are rich with carbohydrate (26.24 %), lipid (2.20 %), protein (1.50 %), fiber (1.96 %) and good amount of moisture. Plant parts showed diverse bioactive compounds such as tannin, saponin, and flavonoid present in most of extracts of leaves, fruits and roots of *T. cucumirina* L. The antibacterial activity of plant extracts showed excellent against MTCC 1252, MTCC 1457, MTCC 3906, MTCC 1926, MTCC *497. Presents study highlights the nutritional, ethnobotanical and antibacterial activity of the plant and emphasis upon the conservation of this bioresource in the state in general and SBR in particular.

INTRODUCTION: Cucurbitaceae commonly known as “Guard family” of flowering plants in Plantae¹. It includes about 825 species in 118 genera currently recognized worldwide². In India, it represents 100 species belong to 36 genera³ including 22 species in 12 genera⁴ reported for Odisha. The family is known as; the species are having wide uses, come in groups of economically important food plants, domesticated wild species, therapeutic medicinally important horticultural plants and common medicinal weeds.

Many researchers have paid attention towards the Cucurbitaceae family, because the fruits, leaves, root and seeds are traditionally consumed as food and therapeutic medicine throughout the world. The major genera of the family are *Trichosanthes*, *Lagenaria*, *Luffa*, *Benincasa*, *Momordica*, *Cucumis*, *Citrullus*, *Cucurbita*, *Bryonopsis*, *Corallocarpus* etc^{1, 4, 5}. Among them, *T. cucumirina* L. (**Fig. 1**) belongs to the genera *Trichosanthes* are very common in India in general and Similipal Biosphere Reserve (SBR), Odisha in particular. It is a climber, monoecious and annual herb with 2-3 branched tendrils. Stem is slender and pentangled. Leaves are alternate, simple. Lamina is furrowed into 5-7 lobes. Petiole is 2-10 cm long and ex-stipulate. Flowers are unisexual (**Fig. 1**), actinomorphic, pentamorous and white in

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colour. Fruits are spindle-shaped and rostrate having greenish-white strips in raw and turn red while ripen⁴⁻⁶. It is distributed in Bangladesh, Sri Lanka, Myanmar, Malaysia, Australia and throughout in India⁷. It is also rich in Odisha and locally known as “Ban Chhachindra”. In Odisha, indigenous people traditionally use a wide range of plants including cucurbits as food and medicine. These plants constitute great reservoir of a wide variety of compounds which exhibit some medicinal and nutritive properties; thus are used as spices, food or medicinal purposes. Many of these indigenous plants contain bioactive compounds that show physiological activities against bacteria and other microorganisms and are precursors for the synthesis of useful drugs. Literature survey

revealed that *T. cucumirina* L. posses the food and medicinal values. The parts of the plant have been used as vegetables and in various diseases among the rural and tribal communities of SBR. The communities are commonly using the plant parts in diabetic, gastroprotective and muscle inflammation. Plant parts are also used as antioxidant, in lipid lowering, in treatments of wounds including boils, sores, skin eruption such as eczema and dermatitis⁸⁻¹¹. Keeping the rich distribution of the species in SBR and other parts of state as well as food and pharmacological values of *T. cucumirina* L., an attempt has been made to evaluate its distribution in SBR, its ethnobotanical values, food and antibacterial activities against selected Gram-positive and Gram-negative bacterial strain.



FIG 1: PLANT PARTS OF *T. CUCUMIRINA* (FLOWER, FRUITS AND LEAVES WITH FLOWER)

Study area: SBR is situated in the Mayurbhanj district of Odisha. Biosphere derives its name from the abundance of semul or red silk cotton that blooms vividly. Government of India declared SBR

as a Biosphere Reserve in 1994. UNESCO (United Nations Educational, Scientific and Cultural Organization) added this National Park to its list of Biosphere reserve in May 2009.

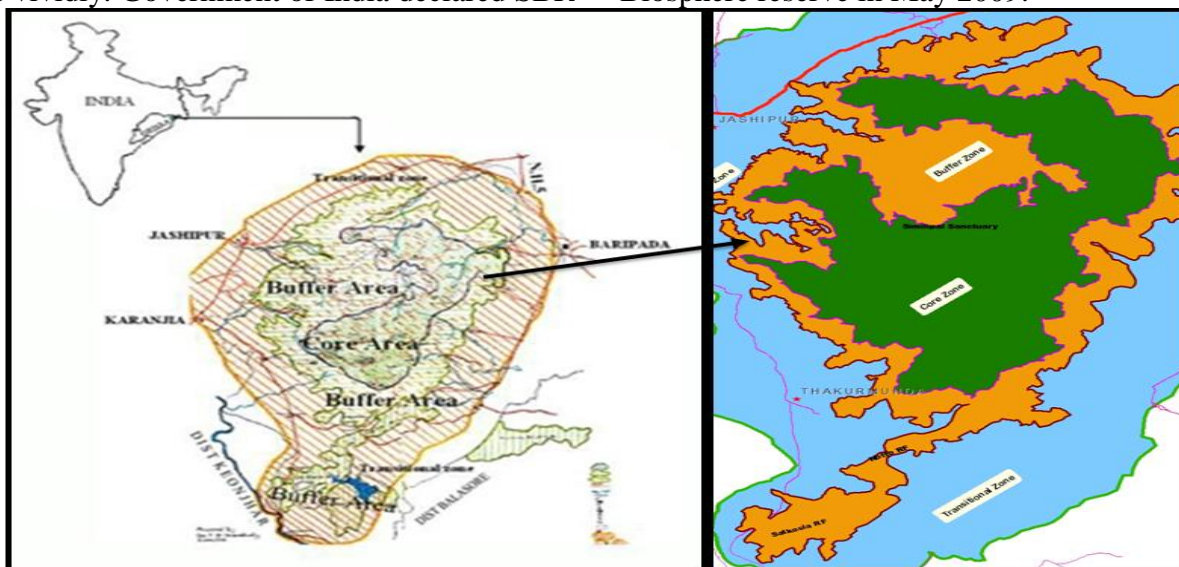


FIG 2: GEOGRAPHICAL LOCATION OF STUDY AREA (SBR AND ITS ADJOINING AREAS)

There are 10,000 people living in 65 villages inside the SBR (Fig. 2). That is why SBR is yet to be declared a fully fledged park, despite its having the status of one of the fifteen biosphere of India. The park has total area of 2,750 km². The average elevation is 559.31 meters. At least 12 mountain rivers cut across the plain area. The prominent among them are Budhabalanga, Khairi and Deo¹²⁻¹⁶. It has a mixed type of vegetation known as Orissa semi-evergreen forests with tropical moist broadleaf forest and tropical moist deciduous forest with dry deciduous hill forest along with high level sal forests. There are different types of tribal communities are found in and around of SBR. Some of the tribes namely Kol, Kharia, Mankdias and Saharas are still primitive in the state of living. They depend solely on their surrounding plants for their requirement of food and medicine¹¹.

METHODOLOGY:

The present study is based on the field survey for collecting ethnobotanical data and lab work for investigation of bioactive compounds and antimicrobial activity.

Ethnobotanical data of collection: A field work was conducted for the collection of ethnobotanical data from rural and tribal communities of SBR (Fig. 3) and its adjoining areas (Jashipur, Karanja, Bisoi Ghati, Hatibadi, Kendumundi, Manda, Angarpada and Padampur) during 2010-2013. The methodological framework was followed as per

standard technique of ethno-biological approaches and Christian and Brigitte, (2004)¹⁷. The information on plant parts used as traditional medicine against different pathogens and disorders were collected through cross questioners with different rural and tribal communities of SBR and its adjoining areas. The pharmacological and medicinal properties of *T. cucumirina* L. were confirmed by cross check with informants. Plant species was identified by second author and confirmed with the Flora's Book^{4, 5}.

Collection of plant materials: Plant parts of *T. cucumirina* L. were collected from the Manda, Kendumundi Hatibadi and Padampur. Collected plant materials were thoroughly washed under tap water followed by distilled water twice and left for air dry. The dried plant parts were powdered by the mechanical grinder and kept in air tight container for the further work.

Preparation of plant extracts: As per polarity index four solvents (n-butanol, acetone, methanol and aqueous) were selected for extraction. Extraction was done using Soxhlet apparatus followed by Tiwari *et al.*, (2011)¹⁸. The powder of plant parts were poured in the thimble at the ratio of 20 g per 250 ml of solvents. Collected residue was then dried at room temperature. The dried samples of extracts were weighed to get yield of the extract and stored it in refrigerator for further use.



FIG. 3: ETHNOBOTANICAL DATA COLLECTION ON *T. CUCUMIRINA* L. AT SBR

Detection of bioactive compounds:

Phytochemical screenings were done using standard method of Sofowora, (1993)¹⁹ and Kumar *et al.*, (2011)²⁰.

Test for Tannin: 0.7 ml of the extract was dissolved in 50ml of distilled water and was heated for 10 minutes. After cooling few drops of 1% ferric chloride was added. Color of sample was

changed from yellow to green and dark green precipitate was observed indicated the presence of Tannin.

Test for Saponin: 5 ml of extract was dried and to it was add 1ml of Ethyl acetate. Ethyl acetate was removed and distilled water was added. The mixture was shaken vigorously and observed. The persistent foam which lasted for at least 15 minutes confirmed the presence of Saponin.

Test for Flavonoid: 0.5 gm of leaf extract was taken in a flask and dissolved in 10% NaOH. Few drops of HCl was added. Yellow color turned to colorless confirmed the presence of Flavonoid.

Test for Terpenoid: 1ml of extract was mixed with 400 µl chloroform. Then the mixture was added by drop of sulphuric acid. A reddish brown interface indicated present of terpenoid.

Test for Alkaloids: 0.5 gm of the each crude extract was stirred with 5 ml of 1% aqueous HCl and heated on water bath and then filtered. 1 ml of the filtrate in a test tube and 2-5 drops of Dragendorff's reagent was added. Occurrences of orange-red precipitated indicate the presence of alkaloid.

Test for Phenolic compounds: 0.5 gm of extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenolic compounds.

Test for Steroids: 0.2 g of each portion, 2 ml of chloroform was added, the solution was cooled well in ice followed by the addition of conc. H₂SO₄ carefully. Two layers of red and green were developed which indicated the presence of a steroidal ring.

Antimicrobial activity: The extracts of leaves and root of *T. cucumirina* L. were screened for antibacterial activity against 2 Gram Positive bacteria *Streptococcus pyogenes* (MTCC 1926), *Streptococcus mutans* (MTCC *497) and 3 Gram Negative bacteria *Salmonella enteric typhi* (MTCC 1252), *Vibrio cholera* (MTCC 3906) and *Shigella flexneri* (MTCC 1457). Antimicrobial activity was

done using Agar Well Diffusion assay adopted from Allen *et al.*, (1991)²¹ with slight modification. Wells (6mm) were made using sterile borer. Stock solutions of samples were prepared in 100 % DMSO (Sigma) and twofold serial dilutions were made in amount of 100 µl per well ranged from 0.25 and 0.5 mg / ml. 100 µl of samples were added by sterile syringes into the wells in three above mentioned concentration and allowed to diffuse at room temperature for 2 h. Plates were incubated at 35 ± 2°C for 18-24 h. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings (diameter of zone of inhibition in mm) were taken and the mean ± SD values (diameter of zone of inhibition) were recorded.

Data analysis: Mean and SD (standard deviation) was performed to evaluate triplicate values of zone of inhibition (mm) of samples using Excel, Microsoft Corporation-2010, US).

RESULTS AND DISCUSSION:

T. cucumirina L. is very common in India and in SBR. The field survey revealed that the richness of *T. cucumirina* L. was rich in peripheral regions (Table 1) followed by buffer and core regions of SBR. It is indicated, it grow more near villages and light in forest. The plant is rich in Bisoi ghati, manda, Padampur, Kalika Parsad and Karanjia villages of SBR. The palatability as food was not good; authors got from two peripheral areas and selected locations that fruits used as food. The Santhal communities of Padampur, peripheral region of SBR use its young fruits as vegetables.

TABLE 1: DISTRIBUTION AND PALATABILITY OF *T. CUCUMIRINA* L. IN SELECTED CORE, BUFFER AND PERIPHERAL REGIONS OF SBR

Location	Region(s)	Richness	Palatability as food	Palatability as medicine
Bisoi	PR	++++	-	++++
Manda	PR	++++	-	++++
Padampur	PR	++++	+	++++
Jashipur	PR	++++	+	++++
Kalika Parsad	PR	++++	-	++++
Hatibadi	BR	+++	-	++++
Karanjia	PR	++++	-	++++
Kendumundi	BR	+++	-	++++
Gurguria	BR	+	-	++++
Sanuski	BR	+	-	++++
Nawana	CR	-	-	-
Pithabhata	BR	++	-	++++
Dudhani	CR	-	-	-

(PR: Peripheral region; BR: Buffer region; CR: Core region; ++++: very rich / more accepted; +++: Moderate, +: Less accepted; -: no report

In India and some other parts of the world, peoples use its fruits as vegetables. The approximate nutritional analysis revealed its food values. Fruits contain 26.24 % of Carbohydrate, 1.5 % of protein, 2.2 % of lipid and 1.96 % of fiber (Fig. 4). Ojiako and Igwe, (2008)²² reported the fruits contain, 1.85 % of protein, 0.81 % of fiber, and 0.23 % of lipid whereas, Oloyede and Abebooye, (2005)²³ reported 2.10 % of protein, 1.60 % of fiber and 0.50 % of total sugar; Adebooye et al., (2004)²⁴ reported 2.14 % of protein, 1.61 % of crude fiber and 1.82 % of total sugar; Ali et al., (2011)²⁵ reported 1.9 5 of crude fiber in *T. anguina*. Ethnobotanical survey indicated that *T. cucumirina* L. is very popular as a medicinal climber among the tribal communities of SBR and its adjoining areas. The leaves are used against wounds, swelling and different types of skin infections (Table 3). Fruits are used to balance sugar level among the Santhal communities of Handipua village (Fig. 3) Roots are very useful against diabetic and to kill lice. The medicinal values with collection sites and information on Informants are listed in Table. All informants were

more than 30 years. It showed the lack of traditional knowledge among the new generation. The tribal Santhal have more knowledge among the tribal who lived in the adjoining areas of SBR such as Jashipur and Bisoi etc. The qualitative analysis of bioactive compounds present in leaves and root indicated its sound pharmacological values. Tannin, saponin, phenolic compounds and flavonoids were present in leaves and root extracts (Table 2), whereas Kumar et al., (2013)⁹ reported the presence of polyphenolics and flavonoids; Murthy et al., (2012)¹⁰ reported the presence of flavones, coumarins in methanolic extract of whole plant; Sandhy et al., (2010)²⁶ reported the phenolic compounds present in alcoholic extract, flavonoids present in water extract and saponin present in alcoholic and water extract of *Tricosanthus cucumirina* L. leaves; Rahman et al., (2010)²⁷ reported present of phenolic compounds, flavonoid, terpenoids and sterols; Kage et al., (2009)²⁸ reported the presence of alkaloids, flavonoids, glycosides, lignin, sterols and tannin in ethanolic extract of whole plant.

TABLE 2: BIOACTIVE COMPOUNDS PRESENT IN LEAVES AND ROOT OF *T. CUCUMIRINA*

Plant parts	Solvents used	Detected compounds
Leaves	n-butanol	NCD
	Methanol	Phenolic Compounds, tannin, Saponin, Flavonoids
	Acetone	Phenolic compounds, Glycosides
Root	Aqueous	Tannin, Terpenoids, saponin
	n-butanol	NCD
	Methanol	Phenolic compounds, terpenoids
	Acetone	Phenolic compounds
	Aqueous	Phenolic compounds, saponin

(NCD: No compound detected)

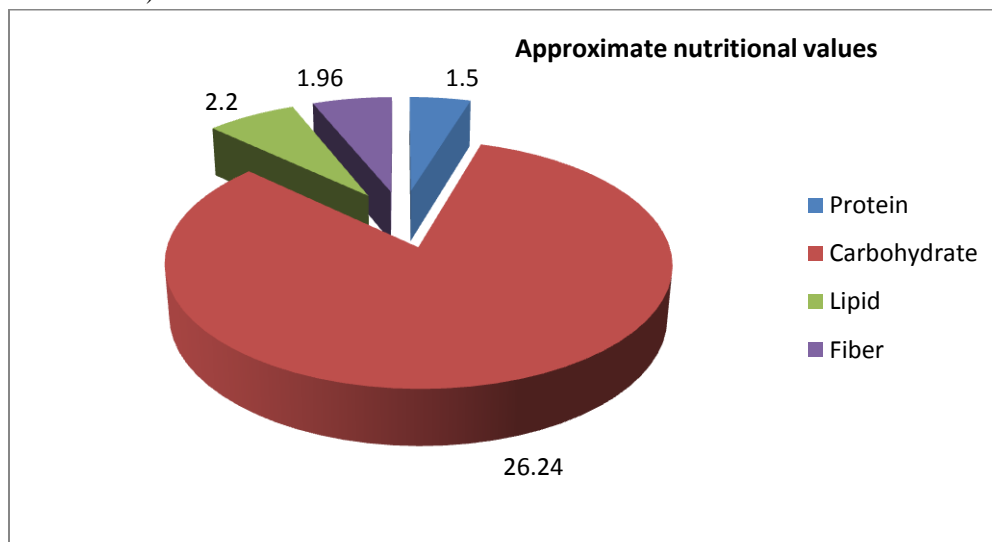


FIG. 4: APPROXIMATE NUTRITIONAL VALUES OF *T. CUCUMIRINA* FRUIT

TABLE 3: ETHNOBOTANICAL VALUES OF *TRICHOSANTHUS CUCUMIRANA* L. AMONG THE TRIBAL AND RURAL COMMUNITIES OF SBR AND ITS ADJOINING AREAS

Plant parts	Claims	Mode of uses	IF	Age of IF	Sex of IF	Races	Place of collection
Leaf	Treating wound infections	Aqueous paste of leaves is prepared and applied externally on the wounds.	Thakur das	45	M	Santhal	Jashipur
	Swelling	Thick aqueous paste is applied on swelling portion.	Sahana Bhumic	34	M	Santhal	Durdura
	Skin infections	Leaves are crushed with rhizome of <i>Costus speciosus</i> and this aqueous paste is applied on leasons thrice a day	Janki Munda	42	F	Munda	Padampur
		Leaf paste is externally applied	Adan marandi	33	M	Santhal	Handipuan
Fruits	Diabetic	A paste about 10 gram of raw young fruits is prepared in clay pot and one-third of the paste is mixed with a glass of water and taken orally to balance the sugar level in diabetes.	Ansari Soren	40	M	Santhal	-do-
			Kusal Ho	22	M	Ho	-do-
	To kill stomach worm	Young fruits are cooked and given to the children in empty stomach thrice in a week to kill the stomach worm.	Gudia Ho	35	M	Ho	Jashipur
Roots	Diabetic	About 1-2 gram of matured root is pasted with water and diluted to make a juice, taken orally in diabetes.	Parmodh Majhi	33	M	Santhal	Bisoi
			Nuangu Ho	45	M	Ho	Kiadungri
	To kill head lice	The camphor is collected from the weekly local market .Mature root is macerated with coconut oil along with camphor. The paste is applied on the hair root and left for three hours to kill the head lice.	Mangula Majhi	30	M	Santhal	Durdura
			Mentha Hembram	28	M	Kolho	Rugdi-kadodia
		Raghnandan	35	M	Bathudi	Angarpada	

(IF: Informants)

Results of antibacterial activity of four extracts of *T. cucumirina* leaves and root revealed that the methanolic, acetone and aqueous extract possess antibacterial activity against 2 Gram Positive bacteria *Streptococcus pyogenes* (MTCC 1926),

Streptococcus mutans (MTCC *497) and 3 Gram Negative bacteria *Salmonella enteric typhi* (MTCC 1252), *Vibrio cholera* (MTCC 3906) and *Shigella flexneri* (MTCC 1457) (Table 4, 5).

TABLE 4: ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS

Strain	n-butanol extract	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	0.00 ± 0.00	0.65 ± 0.007	0.67 ± 0.007	0.69 ± 0.007	0.25 mg/ml
MTCC 1457	0.00 ± 0.00	0.68 ± 0.007	0.71 ± 0.021	0.66 ± 0.014	
MTCC 3906	0.00 ± 0.00	0.71 ± 0.021	0.76 ± 0.014	0.71 ± 0.007	
MTCC 1926	0.00 ± 0.00	0.74 ± 0.035	0.63 ± 0.021	0.74 ± 0.014	
MTCC *497	0.00 ± 0.00	0.66 ± 0.021	0.63 ± 0.021	0.73 ± 0.021	
MTCC 1252	0.00 ± 0.00	0.71 ± 0.021	0.75 ± 0.028	0.81 ± 0.014	0.5 mg/ml
MTCC 1457	0.63 ± 0.014	0.76 ± 0.028	0.83 ± 0.014	0.85 ± 0.014	
MTCC 3906	0.64 ± 0.014	0.76 ± 0.014	0.91 ± 0.021	0.84 ± 0.014	
MTCC 1926	0.65 ± 0.212	0.87 ± 0.028	1.1 ± 0.212	0.80 ± 0.035	
MTCC *497	0.95 ± 0.070	0.81 ± 0.021	0.75 ± 0.014	0.80 ± 0.021	

At the concentration of 0.5 mg/ml the methanolic extracts showed excellent activity against *Streptococcus pyogenes*, hence this extracts will be use in the formulation of new drugs against

infections caused by *Streptococcus pyogenes* (Fig. 5). Kage et al., (2009)²⁸ reported the antibacterial activity of *T. cucumirina* whole plant against *Escherichia coli*, *Klebsiella pneumonia* and

Staphylococcus aureus using agar well diffusion assay and got good activity in aqueous extract against *K. pneumonia*. Using viable colony count technique, Arawwawala et al., (2011) ²⁹ reported

the inhibition activity against *Streptococcus pyogenes*, *Escherichia coli* and *Staphylococcus aureus*.

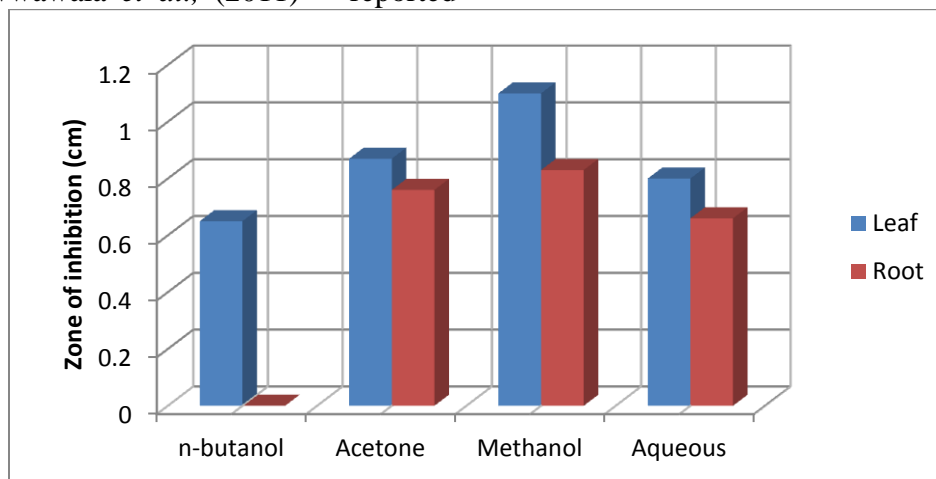


FIG. 5 : ANTIBACTERIAL ACTIVITY OF ROOT AND LEAF 11L

TABLE 5: ANTIBACTERIAL ACTIVITY OF ROOT EXTRACTS

Strain	n-butanol extract	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	0.00 ± 0.00	0.63 ± 0.021	0.65 ± 0.028	0.00 ± 0.00	0.25 mg / ml
MTCC 1457	0.00 ± 0.00	0.64 ± 0.014	0.65 ± 0.021	0.00 ± 0.00	
MTCC 3906	0.00 ± 0.00	0.61 ± 0.014	0.67 ± 0.028	0.00 ± 0.00	
MTCC 1926	0.00 ± 0.00	0.65 ± 0.028	0.73 ± 0.021	0.00 ± 0.00	
MTCC *497	0.00 ± 0.00	0.63 ± 0.021	0.71 ± 0.014	0.00 ± 0.00	
MTCC 1252	0.00 ± 0.00	0.74 ± 0.014	0.76 ± 0.014	0.61 ± 0.014	0.5 mg/ml
MTCC 1457	0.00 ± 0.00	0.66 ± 0.021	0.75 ± 0.021	0.00 ± 0.00	
MTCC 3906	0.00 ± 0.00	0.70 ± 0.014	0.77 ± 0.021	0.00 ± 0.00	
MTCC 1926	0.00 ± 0.00	0.76 ± 0.028	0.83 ± 0.021	0.66 ± 0.014	
MTCC *497	0.00 ± 0.00	0.70 ± 0.021	0.81 ± 0.014	0.64 ± 0.021	

CONCLUSION: The plant possesses sound ethnobotanical values and its parts used in various disorders and microbial infections. Extracts of leaves and root have excellent antibacterial activity against gram-positive and gram-negative bacteria. Therefore, isolation, purification and characterization of bioactive compounds from plant parts of *T. cucumirina* will be helpful to formulate new drugs to fight against microbial infections.

Present study also gives attention towards the conservation of such wild plants for proper utilization of bioresources available in SBR and its adjoining areas.

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