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Antimicrobial Potential of Tropical Plant *Trichodesma indicum* and *Trichodesma sedgwickianum*

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ABSTRACT

Traditionally important plant *Trichodesma sedgwickianum* and *Trichodesma indicum* has therapeutic utility in folk medicine having anti-inflammatory, wound healing and anticancer properties; this led us to carry out the a research based study on antimicrobial properties and identification of phytoconstituents. The antimicrobial activity has been checked against five gram positive and gram negative bacteria and three fungi. The zone of inhibition of extracts ranged from 12 to 29 mm. The MIC was found in the range of 5 to 0.625 mg mL^{-1}. Amongst the extracts, ethanol extract of both species was more active against gram positive bacteria, *S. aureus* and *B. subtilis* whereas aqueous extract had having strong inhibitory effect against gram negative bacteria like *E. coli* and other organism. Phytochemically they contain steroidal, b-sitosterol and phenolics, catechin and gallic acid (HPLC). Plants have proved to be significant natural resources as effective antimicrobial and chemotherapeutic agents and offer a broad spectrum of activity with greater emphasis on preventive action.

Key words: *Trichodesma sedgwickianum*, *Trichodesma indicum*, antimicrobial, catechin, gallic acid, gram negative, positive bacteria

INTRODUCTION

The emergence of multi-drug resistant bacterial strains throughout the globe limits the effectiveness of current drugs and significantly limits treatment, leading to prolonged infections (Hancock, 2005). The increasing resistance of bacteria to the antibiotics has been the emergence of antibiotic-resistant pathogens and resulting in a serious threat to global public health over prescription of the drugs and as resistance to the antibiotics spreads, the development of new antimicrobial agents has to be expedited if the problem is to be contained. Thus, the resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria there is a need to develop new antibiotics to delay or prevent the arrival of a post-antibiotic era (Malik and Singh, 2010; Prasad *et al*., 2008). The idea of mankind to use medicinal plant and its product as a potential healing agent is long before the discovery of microbes (Arora and Kaur, 1999). These early attempts very useful in minimizing the toxic effects of various chemotherapeutic agents because the primary benefits of using plant derived medicines are that they are relatively safer than synthetic (Sofowora, 1982; Oladunmoye *et al*., 2007).

Trichodesma sedgwickianum (TRS) and *Trichodesma indicum* (TRI) are important medicinal plants of Boraginaceae family. It is cultivated mainly for their pyrrazolidine alkaloids; these species possess known antibacterial, strong wound healing agent, anti-inflammatory, cough reflux depressant and antidiabetic properties. Phytochemically they contain monocrotolin, suspinine as pyrrazolidine alkaloids and steroidal compounds hexcosane, amylin and lupeol (Kirtikar and Basu, 1935). Due to its strong ethnopharmacological claim on antibacterial property, the present study sought to obtain data on antibacterial potential of *Trichodesma* species. In present study, we examine the plant as a potential antimicrobial crude drugs as well as a source for natural compounds that act as new anti-infection agents.

MATERIALS AND METHODS

Microorganisms: Microbial species, *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10535), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 13315), *Candida albicans* (ATCC 10231), *Aspergillus flavons* (ATCC 15517) and *Aspergillus niger* (ATCC 16404) from the stock cultures of microorganisms (Department of Microbiology, Amravati University) were used. Sabouraud 2% (w/v)-glucose agar, Muller-Hinton agar and Muller-Hinton broth were supplied by Merck (Germany) and RPMI 1640 broth by the Institute of Immunology.

Herbal material: Aerial parts of both the species of *Trichodesma* were collected in the month of August-September, 2009 from Amravati District, Maharashtra and it is authenticated by Prof. Dr. Bhowagaokar, VIHS, Amravati, Maharashtra (India). A voucher specimen (AMT-36) has been preserved for future reference.

Extraction and phytochemical analysis: The aerial parts of plants was successively extracted with petroleum ether, chloroform, ethanol and water which yielded non-polar Petroleum Ether extract (PE), less polar Successive Chloroform, (SCH) and highly polar Successive Ethanol, (SEE) and Successive Aqueous, (SAE) extracts. The presense of different types of secondary metabolites including flavones, flavonoids, phenolics, quinones, saponins and triterpenoids were confirmed using conventional phytochemical tests (Khandelwal, 2010).

HPLC analysis of extracts: High Performance Liquid Chromatography (HPLC) is an important analytical tool for separating and quantifying components in complex liquid mixtures. By choosing the appropriate equipment (i.e., column and detector), this method is applicable to samples with components ranging from small organic and inorganic molecules and ions to polymers and proteins with high molecular weights (Skoog *et al*., 1998).

The presense of various constituents in the various extracts of TRS and TRI was confirmed by HPLC using standard marker (Sigma-Aldrich Chemie, Steinheim, Germany). Shimadzu HPLC system with LC-10AT, UV detector (Spectra System UV1000), a Luna C18 reverse-phase column (250×4.6 mm, i.d. particle size 5 μm) was used. The mobile phase, flow rate and detection wavelength for different constituent has been summarized in Table 1. Data was acquired and analyzed using Chromquest version 3.0 software.

Table 1: HPLC method for different phytoconstituents

Plants	Extracts	Marker phytochemical	Mobile phase	Flow rate (mL min^{-1})	Detection wavelength (nm)
TRS	SCH	B-sitosterol	Acetonitrile: Water (90:10)	0.9	220
	SEE	Gallic acid	Methanol: Water (70:30)	0.7	280
		Catechin	Acetonitrile: Water (80:20)	0.3	280
TRI	SCH	B-sitosterol	Acetonitrile: Water (90:10)	0.9	220
	SEE	Gallic acid	Methanol: Water (70:30)	0.7	280
		Catechin	Acetonitrile: Water (80:20)	0.3	280

TRS: *Trichodesma sedgwickianum*, TRI: *Trichodesma indicum*, SCH; Successive chloroform extract, SEE: Successive ethanol extract

Antibacterial susceptibility testing: A disk diffusion method, according to NCCLS (1997a), was employed for the determination of the antibacterial activity of the extracts. Inoculums were prepared with fresh cultures of microbial strains, cultured on Sabouraud 2% (w/v) glucose agar for 18 h (48 h for fungi) at 37°C with saline. All agar plates were prepared in 90 mm petri dish with 22 mL of agar, giving a final depth of 4 mm. One-hundred microlitres of a suspension of the tested microorganisms (108 cells mL^{-1}) were spread on the solid media plates. Sterile filter paper disks were impregnated with 50 µL (10 mg mL^{-1}) of the extracts of TRS as well as TRI and placed on inoculated plates. These plates, after standing at 4°C for 2 h, were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for the fungi. Standard disks of Tetracycline (10 mg mL^{-1}) were used individually as positive controls. The diameters of the inhibition zones were measured in millimeters (to the nearest 0.1 mm) using Antibiotic Zone Reader. Each test was performed in quintuplicate and repeated three times.

Antifungal assay: Anti-fungal study was carried out through the same procedure as for the antibacterial study except that Sabouraud Dextrose Agar Media (SDA MEDIUM) was used. Ketaconozol was used as a standard.

Minimal inhibitory concentration: Minimal Inhibitory Concentration (MIC) was determined by the twofold micro dilution method in Muller-Hinton broth for bacterial strains and RPMI 1640 for yeast and mould according to the Clinical and Laboratory Standards Institute (formerly NCCLS) M-27A recommendations (NCCLS, 1997b).

RESULTS AND DISCUSSION

The phytochemical screening revealed the presense of secondary metabolites including flavonoids, alkaloids, steroid and phenolic compound as confirmed by phytochemical tests and analysis (Table 2). Secondary metabolites are well known for their inhibitory action against pathogenic microorganisms (Pereira *et al*., 2006; Rauha *et al*., 2000; Duru and Onyedineke, 2010). Phytochemical analysis has been performed by HPLC. HPLC analysis of different extracts of plants revealed that the presense of B-sitosterol in the SCH of TRS and TRI while catechin and gallic acid was found in the SEE of TRS and TRI when compared with the standards. The retention time of B-sitosterol was found to be 11.10 while gallic acid and catechin was obtained at 3.30 and 2.60 (Table 3).

The antimicrobial potency of extract can be estimated by measuring zone of inhibition, zone diameters and MIC values. The zone of inhibition of all extracts ranged from 12 to 29 mm of both *Trichodesma* species. The ethanolic extract of both species was more active against gram positive bacteria, *S. aureus* and *B. subtilis* having zone of inhibition range from 24.12-26.30 mm. While

Table 2: Phytochemical study of *Trichodesma* species

Tests	Species	PE	SCH	SEE	SAE
Carbohydrates	TRS	-	-	-	+
	TRI	-	-	-	+
Cardiac Glycosides	TRS				
	TRI	-	+	+	-
Saponin Glycosides	TRS	-	-	-	-
	TRI	-	-	-	-
Steroids	TRS	+	+	-	-
	TRI	+	+	-	-
Fatty acids	TRS	+	-	-	-
	TRI	+	-	-	-
Alkaloids	TRS	-	+	-	-
	TRI	-	+	-	-
Flavonoids	TRS	-	-	+	-
	TRI	-	-	+	-
Tannins	TRS	-	-	-	+
	TRI	-	-	-	+

+: Present, -: Absent, TRS: *Trichodesma sedgwickianum*, TRI: *Trichodesma indicum*, PE: Petroleum ether extract, SCH: Successive chloform extract, SEE: Successive ethanol extract, SAE: Successive aqueous extract

Table 3: Retention time of phytoconstituents in HPLC

Plant	Extract	Compounds	Retention time (min)
TRS	SCH	B-sitosterol	11.10
SEE	Gallic acid	3.3	
		Catechin	2.60
TRI	SCH	B-sitosterol	11.10
SEE	Gallic acid	3.3	
		Catechin	2.60

TRS: *Trichodesma sedgwickianum*, TRI: *Trichodesma indicum*, SCH: Successive chloform extract, SEE: Successive ethanol extract

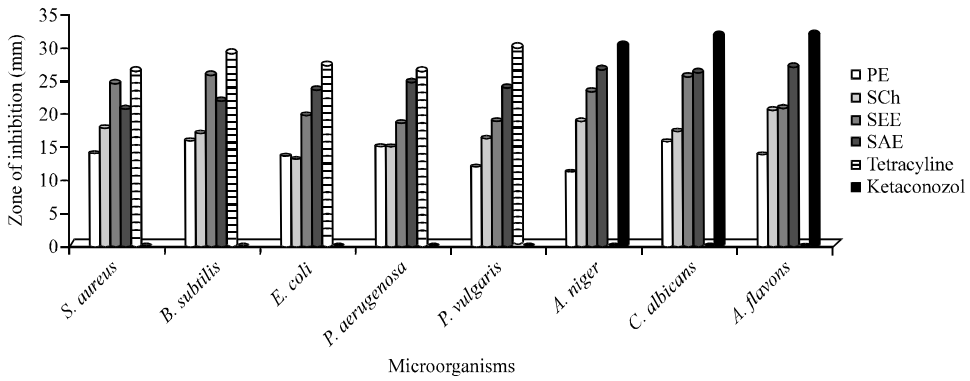

Fig. 1: Zone of inhibition of TRS extracts against microorganisms

the Aqueous extract, SAE showing strong inhibition against *E. coli*., *P. aeruginosa*, *P. vulgaris* and fungi, *A. flavons*, *A. Niger* and *C. albicans*. Their inhibition zone ranges from 23-28 mm (Fig. 1, 2). The MIC was found in the range of 5 to 0.625 mg mL^{-1} (Table 4). The petroleum ether and chloroform extract of both species also had inhibitory effect on tested organism but less as compared to ethanol and aqueous extracts.

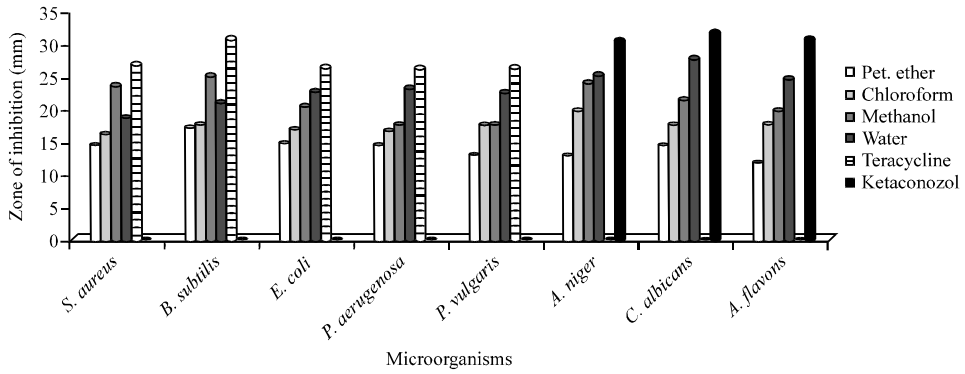

Fig. 2: Zone of inhibition of TRI extracts against microorganisms

Table 4: MIC values of *Trichodesma* species

		Gram positive		Gram negative			Fungi		
Extract	Conc. (μg mL^{-1})	*S. aureus*	*B. subtilis*	*E. Coli*	*P. aeruginosa*	*P. vulgaris*	*A. niger*	*C. albicans*	*A. flavon*
SAE (TRS)	10.000	-	-	-	-	-	-	-	-
	5.000	-	-	-	-	-	-	-	-
	2.500	-	-	-	-	-	-	-	+
	1.250	++	++	+	+	+	+	+	+
	0.625	+++	+++	+	+	+	+	+	+
SEE (TRS)	10.000	-	-	-	-	-	-	-	-
	5.000	-	-	-	-	-	-	-	-
	2.500	-	-	+	+	-	+	-	-
	1.250	+	+	+	++	++	++	+	+
	0.625	+	+	++	+++	+++	++	++	++
SAE (TRI)	10.000	-	-	-	-	-	-	-	-
	5.000	-	-	-	-	-	-	-	-
	2.500	++	+	-	+	+	-	-	-
	1.250	+++	++	+	++	+	+	+	+
	0.625	+++	+++	++	++	++	++	++	++
SEE (TRI)	10.000	-	-	-	-	-	-	-	-
	5.000	-	-	-	-	-	-	-	-
	2.500	-	-	+	+	+	+	-	+
	1.250	+	+	++	++	++	++	+	+
	0.625	++	++	+++	+++	+++	++	++	++
TC	10.000	-	-	-	-	-	*	*	*
	5.000	-	-	-	-	-	*	*	*
	2.500	-	-	-	-	-	*	*	*
	1.250	-	-	-	+	-	*	*	*
	0.625	+	+	+	++	+	*	*	*
KC	10.000	*	*	*	*	*	-	-	-
	5.000	*	*	*	*	*	-	-	-
	2.500	*	*	*	*	*	-	-	-
	1.250	*	*	*	*	*	-	+	-
	0.625	*	*	*	*	*	+	++	+

TRS: *Trichodesma sedgwickianum*, TRI: *Trichodesma indicum*, SCH: Successive chloform extract, SEE: Successive ethanol extract, TC: Tetracycline, KC: Ketaconozol, -: No growth, +: Less growth, ++: Moderate growth, +++: Heavy growth, *Not performed

The HPLC analysis of extracts revealed the presense of catechin and gallic acid. Both the phytoconstituents are well known antimicrobial agent (Taylor *et al*., 2005; Chanwitheesuk *et al*., 2007). The presense of these constituent along with other compounds will be the responsible for above observed activity.

CONCLUSION

The overall results of this study indicated that the antimicrobial activity of the different extract of both species of plant *Trichodesma* could be due to the presense of secondary metabolites, mainly phenolics. The biological activity of this plant against bacteria and fungi as well the presense of phenolic compounds, catechin and gallic acid is reported first time in this study. The present study provides important baseline information for the use of *Trichodesma* as well as its constituents for the treatment of infections associated with the studied microorganisms.

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