

Pharmacological Study of *Trichodesma Indicum*

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Abstract

Objective: To investigate the phytochemical screening and selected pharmacological activities of the methanolic extract of the plant *Trichodesma indicum*. **Methods:** The antibacterial potential of the methanolic leaf extract of the studied species was tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Streptococcus pyrogens* and *Bacillus subtilis* by agar well diffusion method. The higher zone of inhibition (15mm) was observed against the bacterium *Bacillus subtilis* (100µl) concentration of methanol leaf extract. **Results:** Preliminary phytochemical analysis of studied species shows that presence of phytochemical compounds like alkaloids, tannins, reducing sugars and flavonoids. GC-MS analysis confirms the occurrence of 20 different compounds in the methanol leaf extract of the both studied species. **Conclusions:** The obtained results provide a support for the use of this plant in traditional medicine but further pharmacokinetics studies are required.

Keywords: Methanol extract of *Trichodesma indicum*, Phytochemical Analysis, Antibacterial activity, GC-MS analysis.

1. Introduction:

Infectious disease can become a threat to public health in this world. The use of medicinal plants for the treatment of various diseases is an old practice in most countries and it still offers an enormous potential source of new anti-infective agents. Although ancient civilization recognized the antiseptic or antibacterial potential of many plant extracts, they failed to document the preservative and curative effects of plant extracts [1]. Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases [2]. The plant extracts have been developed and proposed for use as antimicrobial substances [3]. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine [4]. Thus, it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential [5, 6 and 7]. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to

side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs [8]. Antimicrobial activities of many plants have been reported by the researchers [9, 10]. Many medicinal plants that reported to have the potential for medicinal purpose were investigated for useful active compounds [11]. The present study was aimed to evaluate the antibacterial potential of methanol extract of *Trichodesma indicum* against bacterial pathogens and phytochemical analysis of *Trichodesma indicum* and to identify the compounds using GC-MS.

2. Materials and methods

2.1. Collection and Drying of plant materials

Mature leaves of *Trichodesma indicum* were collected from different places of Coimbatore, Tamil Nadu. The leaves were washed thoroughly three times with water and once with distilled water. The plant materials were air dried and powdered. The powdered samples were hermetically sealed in separate polythene bags until the time of extraction [12].

2.2. Preparation of plant extract

10 g of powdered leaves were extracted successively with 100 ml of methanol at 40-50°C in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use [13].

2.3. Test microorganisms

Five pathogenic bacteria isolated from the clinical samples, viz., *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Streptococcus pyrogens* and *Pseudomonas aeruginosa* were obtained from PSG College of arts and science, Coimbatore. The cultures were sub-cultured and maintained on nutrient agar slants and stored at 4°C.

2.4. Inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of nutrient broth and incubated at 37°C for 3-5 hours till

a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standards.

2.5.Determination of antibacterial activity (Agar well Diffusion)

Muller Hinton agar plates were inoculated with test organisms by spreading the bacterial inoculums on the surface of the media. Wells (8 mm in diameter) were punched in the agar. Methanolic extracts of the plant *Trichodesma indicum* with same concentrations of 100 mg/ml were used. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm) [15].

2.6. Phytochemical analysis

Phytochemical tests were performed to identify the active chemical constituents such as alkaloid, glycosides, terpenoids and steroids, flavonoids, reducing sugars, triterpenes, phenolic compounds and tannins by the following procedure.

2.6.1.Test for Terpenoid and Steroid

4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids[13].

2.6.2. Test for Flavonoid

4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids[13].

2.6.3. Test for Triterpenes

300 mg of extract was mixed with 5 ml of chloroform and warmed at 80°C for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well and observed for red colour formation.

2.6.4.Test for Phenolic Compounds (Ferric chloride test)

300 mg of extract was diluted in 5 ml of distilled water and filtered. To the filtrate, 5% Ferric chloride was added and observed for dark green colour formation.

2.6.5. Test for Tannins

To the 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for catecholic tannins[14].

2.6.7.Test for Saponins

2g of the powdered sample was boiled in 20 ml of distilled water in a water bath. 10ml of the filterable was mixed with 5 ml of distilled water shaken vigorously for a stable persistent broth. The following was mixed with 3 drops of Olive oil and shaken vigorously and then observed for the formation of emulsion.

2.6.8.GC-MS

The compounds were identified by using GC-MS.

3.Results

The preliminary phytochemical analysis of methanol leaf extract of the studied species is given in Table 1. The results of the phytochemical screening revealed the presence of alkaloids, steroids reducing sugars and tannins. whereas flavonoids, saponins, triterpenes and glycosides were absent. The results indicated the facts that the disparity occurrence of phytochemical compounds in the tested plant extract may be due to extracting efficacy of solvents and solubility nature of the active constituents. Analysis of GC-MS spectrum was done at the South India Textile Research Association (SITRA), Coimbatore. The spectrum of the unknown component was compared with known component stored in SITRA library. The name, molecular weight, structure of the component of the test material was ascertained. Among hundred compounds identified, twenty active ingredients with higher pharmacological properties were chosen for further analysis. The active principle, molecular weight, concentrations (%), molecular formula are presented in Table 2 and Figure 1. The prevailing compounds are Phytol (66.29%), Tetradecamethyl-cyclo-heptasiloxane (CAS) (92.11%) and Hexadecanoic acid, methyl ester (CAS) (75.40%). The antibacterial activity of the leaf extracts of the studied species was assayed *in vitro* by agar well diffusion method against the five bacterial species (Table 3). The methanol extract of *Trichodesma indicum* (100µl) showed maximum zone of inhibition (15mm) against the bacteria, *Bacillus subtilis* (Table 3).

Table.1. Phytochemical analysis of *Trichodesma indicum* methanol extract

S.No	Test	Result
1	Alkaloids	+
2	Glycosides	-
3	Triterpenes	-
4	Tannins	+
5	Flavonoids	-
6	Reducing sugars	+
7	Flavonoids	+

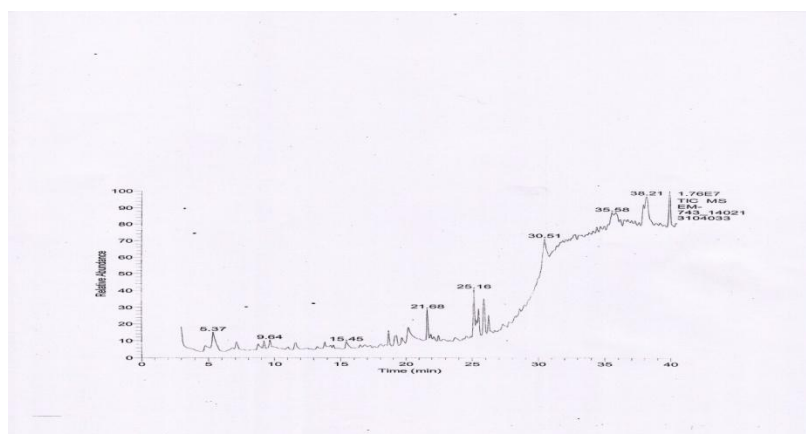
Table.2.GC-MS Analysis of methanolic extract of *Trichodesma indicum*

S.No	Compound	Molecular Formula	Molecular Weight	Probability
1	Triisopropylsilyl 7-(tributylstannyl)hepta-3E,6E-dienoate	C ₂₈ H ₅₆ O ₃ S	588	92.11
2	Tetradecamethyl-cyclo-hepta-siloxane(CAS)	C ₁₄ H ₄₂ O ₇ S ₇	518	91.11
3	Hexadecanoic acid, methyl ester(CAS)	C ₁₇ H ₃₄ O ₂	270	75.40
4	Phytol	C ₂₀ H ₄₀ O	296	66.29
5	2,8-Dichloro-2,4,4,6,6,8,10,10,12,12-decamethyl-5,11-dicarba-cyclohexasiloxane	C ₁₂ H ₃₄ C ₁₂ O ₄ Si ₆	480	45.78
6	Octadecanoic acid, methyl ester(CAS)	C ₁₉ H ₃₈ O ₂	298	44.61
7	4(S)-[à-Methoxy- à-(trifluoromethyl)phenylacetoxy]-2,3-(I sopropylidenedioxy)heptan-1-ol	C ₂₀ H ₂₇ F ₃ O ₆	420	39.17
8	1,7-dibromo-11,12-bis(methoxycarbonyl)-4,4-dimethyl-3,5,9-trioxa-4-silatricyclo[5.3.2.0(2,8)]dodec-11-ene	C ₁₄ H ₁₈ Br ₂ O ₇ Si	484	37.30
9	9,12,15- Octadecanoic acid, methyl ester(CAS)	C ₁₉ H ₃₂ O ₂	292	33.15
10	2-(4-bromophenyl)-3-(4-bromophenylimino)-5-phenyl-4-isoxazoline	C ₂₁ H ₁₄ Br ₂ N ₂ O	468	30.72
11	15-methyltricyclo[6.5.2(13,14).0(7,15)]pentadeca-1,3,5,7,9,11,13-heptene	C ₁₆ H ₁₄	206	26.02
12	9,12,15- Octadecanoic acid, methyl ester(Z,Z,Z)-	C ₁₉ H ₃₂ O ₂	292	24.73
13	2-Hexadecan-1-ol,3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-(CAS)	C ₂₀ H ₄₀ O	296	22.74
14	2-tert-butyl-4-isopropyl-5-methylphenol	C ₁₄ H ₂₂ O	206	19.93
15	Benzaldehyde,4-(octyloxy)-(CAS)	C ₁₅ H ₂₂ O ₂	234	18.88
16	2-methylnaphtho[2'1':4,5]thieno[2,3-c]quinolin-6(5h)-one	C ₂₀ H ₁₃ NOS	315	16.97
17	4-Pyridinamine, N,N-dimethyl-(CAS)	C ₇ H ₁₀ N ₂	122	14.08
18	phythol isomer	C ₂₀ H ₄₀ O	296	11.54
19	Methyl 2-methyl-4H-3,1-benzoxathine-2-carboxylate	C ₁₁ H ₁₂ O ₃ S	224	10.51
20	Borazine,2,4,6-trimethyl-	C ₃ H ₁₂ D ₃ N ₃	123	9.28

Table.3. Antibacterial effect of methanolic extract of *Trichodesma indicum*

S.No	Organism	Concentration of extract and zone of inhibition(mm)		
		50 µl	75 µl	100 µl
1	<i>Escherichia coli</i>	6mm	9mm	11mm
2	<i>Pseudomonas aeruginosa</i>	9mm	12mm	14mm
3	<i>Klebsiella pneumonia</i>	4mm	8mm	9mm
4	<i>Streptococcus pyrogens</i>	7mm	8mm	11mm
5	<i>Bacillus subtilis</i>	11mm	13mm	15mm

FIGURE.1. GC-MS Chromatogram of methanolic extract of *Trichodesma indicum*



4. Discussion

Many of the existing synthetic drugs cause various side effects. Hence, drug development plant based compounds could be useful in meeting this demand for newer drugs with minimal side effects [16,17]. The result of phytochemicals in the present investigation showed that the plant leaves contain components like, steroids, phenolic compounds flavonoids. This study reports the presence of different phytochemicals with biological activity that can be valuable therapeutic index [18,19]. In the present study, we have found that the biologically active phytochemicals were present in the methanolic extracts of few medicinal plants. The antibacterial properties of these extracts may be due to the presence of above mentioned phytochemicals. Purely the phytochemical compounds were responsible for the antibacterial activity of *Trichodesma indicum*. The result also indicated that scientific studies carried out on medicinal plant having traditional claims of effectiveness might warrant fruitful results [20]. Thus this plant could be utilized as an alternative source of useful antimicrobial drugs.

Conflict of interest statement

We declare that we have no conflict of interest

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