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CHEMICAL INVESTIGATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF *TRICHODESMA EHRENBERGII* SCHWEINF. EX BOISS.GROWING WIDELY IN GEBEL ELBA, EGYPT

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Abstract:

In this study, the primary phytochemical and biological screeningof the aerial parts of Trichodesma ehernbergii Schweinf. ex Boiss. (Family; Boraginaceae)has been done. Phytochemical analysis showed the presence of phenols, flavonoids, alkaloids, terpenoids, steroids, tannins, saponins, and sugars in different extracts of the aerial partsof T. ehernbergii .The concentration of phenols, flavonoids, alkaloids and tannins and saponinswere foundin the range 138±0.8mg/g (as gallic acid equivalent), 123±0.6mg/g (as rutin equivalent), 1.3±0.6mg/g, 2.7±0.2mg/g and 6.7±0.2mg/g, respectively.Combined and free amino acidsanalysis showed the presence of 17 amino acids asfree and protein amino acids. Glutamic acid (29.919ppm) and lysine (72.640ppm) represented as the major components of free and protein amino acids, respectively.The antimicrobial activity ofmethanolextractof the aerial parts of T. ehernbergiiagainst 6 bacterial strainsand 5 fungal strainsby a pour plate technique method was determined.

Keywords: *Trichodesma ehernbergii, Gebel Elba, Phytochemical screening, Primary and Secondary metabolites, Eco-physiological analysis, Amino acids, Antimicrobial Activity.*

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INTRODUCTION:

Trichodesma species has been examined for a variety of chemical constituents, viz., monocrotaline, supinine as pyrrolizidine alkaloids [1, 2]; hexacosane, α-amyrin, lupeol[3], non-steroidal [4] and fatty constituents [5]. *Trichodesma* species have hepatotoxic [6], antitumor [7-8], anti-inflammatory [9] and anti-diarrhoeal [10] activities.

Trichodesma ehernbergii Schweinf. ex Boiss. (Family; Boraginaceae), resembles *Trichodesma africanum*, but with cordate leaves. *T. ehrenbergii* is annual or perennating, erect herb, 15-45 cm high, densely short hairy, mixed with tubercle-based bristles. Stem somewhat diffusely branched. Leaves broadly ovate, obtuse to subacute, truncate, rounded or subcordate at base or abruptly narrowed into a petiole; lower leaves petiolate, upper leaves sessile. Inflorescence paniculate, terminal and axillary. Bracts minute; pedicels filiform, much longer than the calyx, densely hispid-pilose. Calyx 4-5 mm long, lanceolate in fruit; fruiting calyx slightly longer, acute. Corolla pale-blue, with five brown spots at throat, campanulaterotate, 5-lobed; lobes long acuminate. Anthers villose. Nutletsscabrid, depressed-ovoid [11].

T. ehrenbergii, grows in sandy and gravelly wadis. Recorded in Egypt, Saudi Arabia, Sudan, Ethiopia, Somalia, Oman and Israel[12-15].

At lower taxonomic level, the species of *Trichodesma* are still poorly investigated, and no recent revisions or monographic treatment, even for single geographic areas, have been yet published. So the present investigation deals with the qualitative and quantitative phytochemical analysis and biological screening of the aerial parts of *Trichodesma ehrenbergii*.

MATERIAL AND METHODS:

Collection of Plant Materials:

*T. ehernbergii*was collected from Gebel Elba region (south eastern corner of the Arabian desert of Egypt)[16], during spring season (2012). The collected plants were identified by Botany Department, Faculty of Science, Zagazig University and by comparison with plant description in flora of Egypt as well as herbarium specimens at Desert Research Center (Egypt). The aerial parts of *T. ehernbergii* were dried under shade and then grinded to fine powder.

PreliminaryPhytochemical Screening:

Preparing of the Extracts:

About1kgof air-dried aerial parts of plant materials was charged into soxhlet apparatus and extraction was carried out with following solvents successively. 1) Petroleum ether (40-60ºC), 2) Chloroform, 3) Ethyl acetate, 4) Methanol. Each time before employing the solvent of higher polarity marc was dried. Each extract was then concentrated using rotary vacuum evaporator at 40-50ºC under vacuum then dried residue was collected in an opaque glass bottles for further studies.

Proximate Analysis:

Proximate analysis of the aerial parts of *T. ehernbergii*such as water content [17], total inorganic matter (ash) and organic matter [18], total crude fibers[19], total carbohydrates (soluble and insoluble)[20], total nitrogen and protein content [21] and total lipid content [22], also, acid value, saponification value [23], ester value [24] and iodine value [25] were determined by standard methods.

Preliminary Phytochemical Screening of the Aerial Parts of *T. Ehrenbergii*:

The concentrated residue from the gradient solvent extracts of the plant material was used to detect the secondary plant metabolites, this include testing for tannins [26], testing for sterols and terpenes[27], testing of alkaloids [28,29], testing for flavonoids and phenolic compounds [30], testing for carbohydrates [26], testing for saponins[26], testing for anthraquinones[29]and testing for volatile oils.

Investigation of total active Constituents:

The total flavonoids were determined spectrophotometrically and calculated as rutin according to the method described by Samatha *et al.*, [31] and Han *et al.*, [32]. Total phenolic acids were estimated according to Makkar *et al.*, [33].Total tannin were determined gravimetrically by copper acetate method according to Ali *et al.*, [34]. This method depends on quantitative precipitation of tannin with copper acetate solution, then ignition of the copper tannate to copper oxide and weighing the residual copper oxide. Saponin content was determined calorimetrically according to Honerlogen and Tretter, [35].Alkaloid subjected to quantitative estimation by gravimetrically according to Woo *et al.*, [28].

Amino Acid Contents:

Free and protein amino acid contents were determined qualitatively and quantitatively by using LKBalpha plus amino acid analyser S433/SYKAMaccording to Steven et al.,[36].

Antimicrobial Assay:

Microbial strains:

Strains were obtained from the bacteria stock present at the research laboratory of bacteriology, Faculty of Science, Al-Azhar University. Gram-positive species (*Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis*), Gram-negative species(*Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa*) and fungi species (*Fusarium oxysporum*, *Rhizoctonia solani*, *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*) were tested. Themethanol extract was dissolved in Dimethylformamide (DMF) for

antimicrobial investigation at the final concentration of (10 mg / 1 ml).

Minimum Inhibitory Concentration (MIC):
To measure the MIC values, various concentrations of the stock, 100, 50, 25, 12.5, 6.25and 3.125mg/ml were assayed against the tested microorganism.

Antibacterial Activity:
In vitro antimicrobial assay of the methanolextract was carried out according to pour plate technique at concentrations (50, 25, 12.5, 6.25 and3.125mg/ml). Culturing and incubated of different bacteria species were carried out at 37°C for 24 hours. After the elapse of incubation periods, the diameter of inhibition zones was measured.

Antifungal Activity:
CzepakDox media was used for cultivation of fungal species. The medium was seeded with different fungal species. After solidification of media on plates, make pores in agar with cup-borer (15 mm) diameter. Fiveconcentrations 100, 50, 25, 12.5 and 6.25mg/ml of the methanol extract were transferred into the well. Dimethylformamide (DMF) was used only as a control. The plates were incubated for 7 days at 28°C. The inhibition zone formed by the extract against the particular test fungal strain determined as the antifungal activities of the extract.

RESULTS AND DISCUSSION:

Eco-Physiological Analysis:
The eco-physiological analysis of the aerial parts of *T. ehernbergii* showed that the moisture content of the sample was (38.5±2.10). This is expected since the sample has been subjected to drying for five days to reduce the moisture content. The results of organic and inorganic matter and crude fibres were summarized in table 1. Also the results of proximate analysis of the aerial parts of *T. ehrenbergii* and chemical properties of lipids were summarized in table 2 and 3, respectively.

Table 1: Certain Pharmacopoeial Constant of the Aerial Parts of *T. ehernbergii*

Parameter	%
In organic matter (total ash)	14.50 ± 0.15
Organic matter	85.50 ± 0.15
Acid insoluble ash	6.90 ± 0.81
Acid soluble ash	7.60 ± 0.50
Water insoluble ash	10.01 ± 0.40
Water soluble ash	4.49 ± 0.30
Crude fibres	12.50 ± 0.30

Table 2: Proximate Analysis of the Aerial Parts of *T. Ehrenbergii*

Parameter	%
Water content	38.50 ± 2.10
Total lipids	1.15 ± 0.07
Total nitrogen	2.20 ± 0.08
Total protein	13.75± 0.50
Total carbohydrates	19.60 ± 0.20
Total soluble carbohydrates	10.20 ± 0.40
Total insoluble carbohydrates	9.40 ± 0.40

Table 3: Chemical Properties (Acid, Ester, Iodine and Saponification Values) of Lipids of the Aerial Parts of *T. Ehrenbergii*

Item	Result
Acid value	19.23 ± 0.40
Iodine value	84.87 ± 0.20
Ester value	104.19 ± 0.25
Saponification value	123.42 ± 0.20

Phytochemical Screening:
Phytochemical screening of the methanol extract of the aerial parts of *T. ehrenbergii* showed the presence of various phytoconstituents like alkaloids, Glycosides and/or carbohydrates, cardiacglycoside , sterols and/or terpenes, tannins, saponins, flavonoids and phenolic compoundsas shown in table 4.

Table 4: Preliminary Phytochemical Screening of the Aerial Parts of *T. Ehrenbergii*

Parameters	Result
Alkaloids	+ve
Glycosides and / or Carbohydrates	+ve
Cardiac Glycosides	+ve
Saponins	+ve
Phenolic Compounds	+ve
Sterols and / or terpenes	+ve
Tannins	+ve
Flavonoids	+ve
Volatile oils	-ve

(+ve) mean present, (-ve) mean absent.

Phenolic, flavonoids, alkaloids, tannins and saponins contents analysis showed that, the aerial part of *T. ehrenbergii* has high percentage of phenolic and flavonoid between active materials; the results weresummarized in table 5.

Table 5: Totalactive Materials of the Aerial Parts of *T. Ehrenbergii*

Secondary Metabolites	Dry Weight (mg/g)
Total flavonoids (rutin)	**123 ± 0.8**
Total phenolic acids (Gallic acid)	**138 ± 0.6**
Total tannins	**2.7 ± 0.6**
Total saponins	**6.7 ± 0.2**
Total alkaloids	**1.3 ± 0.2**

Free and Protein amino acids:
Free and protein amino acid contents of *T. ehrenbergii* have been evaluated. Seventeen amino acids asfree and protein amino acids with different range of concentrations were determined. Glutamic acid (29.919 ppm)and lysine (72.640 ppm) represented as the major components of free and protein amino acids, respectively. While, methionine (1.840ppm)and aspartic acid (0.190ppm)were the minor components in both free and protein amino acids, respectively as shown in table 6.

The remarkable percent of proline may be dueto increase in soil salinity. The salinity inhibited the transmission reactions, then the glutamic acidaccumulated and transformed to other nitrogenous compounds such as proline [37]. High proline concentrations may help to protect cell metabolism and facilities recovery after stress[38]. Also proline improved the growth of salt stressed to cell cultures and that was attributed to the role of proline as an osmoprotectant for enzymes and membranes against salt inhibition [39].

Table 6: Free and Protein Amino Acids (ppm) in Aerial Parts of *Trichodesma Ehrenbergii*

Amino Acids	Free amino Acids		Protein Amino Acids	
	RT	Concentration (ppm)	RT	Concentration (ppm)
Aspartic acid	8.776	22.695	18.869	0.190
Theronine	10.405	9.314	27.280	0.861
Serine	11.293	9.708	29.920	1.001
Glutamicacid	12.435	29.919	32.968	1.002
Proline	15.179	9.327	44.859	8.660
Glycine	20.080	12.935	48.016	4.213
Alanine	21.483	12.694	50.237	6.406
Cysteine	22.088	2.146	58.187	10.311
Valine	23.515	13.046	59.824	6.6556
Methionine	25.525	1.840	63.781	1.350
Isoleucine	27.640	11.045	66.288	4.256
Leucine	28.635	17.603	68.045	2.344
Tyrosine	30.949	3.480	71.677	3.882
Phenyl Alanine	32.179	10.370	75.744	1.907
Lysine	38.936	6.739	97.504	72.640
Histidine	35.419	6.878	108.747	11.173
Arginine	44.515	7.877	125.269	28.794

RT = Retention time

Antimicrobial Screening:
The antimicrobial activity of the methanolextract of *T. ehrenbergii* was evaluated by a pour plate technique method against bacterial species (*Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis*, *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa*) and fungal species (*Fusarium oxysporum*, *Rhizoctonia solani*, *Aspergillus niger*, *Candida flavus* and *Candida albicans*).
The minimum inhibitory concentration MIC was defined as the lowest concentration able to inhibit any visible bacterial growth [40, 41].The results of the MIC of the methanol extract of *T. ehrenbergii* were summarized in tables7 and 8.

Antibacterial Activity:
The results revealed that the maximum inhibitory responses are indicated after the treatment of all bacteria specieswith the concentration 50mg/ml of the methanolextract, while moderating inhibitory activity after treatment of all species with 25 and 12.5mg/ml. On the other hand, there was no activity observed against most bacterial species at concentration 6.5 and 3.125mg/ml, the results were summarizedin table7.

Antifungal Activity:
The methanolextract showed strongly inhibitory activity against all species at 100mg/ml concentration, while at 50mg/ml concentration showed moderate inhibitory activity against most strains. Inaddition, there was no activity observed against all fungal species at concentration 12.5 and 6.25mg/ml as shown in table 8.
MIC = Minimum inhibitory concentration, (-) = No inhibition zone
The antimicrobial effect of methanol extract against these organisms may be due to the ability of the methanol to extract some of the active properties of some plants like phenolic compounds, saponinsand

other secondary metabolites which are reported to be antimicrobial [42,43].

Table 7: Antibacterial Activity of the Methanol Extract of *T. Ehrenbergii*

Bacterial strains	Inhibition zone diameter in mm					MIC
	50 ml	25 ml	12.5 ml	6.25 ml	3.125 ml	
Staphylococcus aureus	19	11	7	-	-	12.5
Bacillus subtilis	23	15	9	-	-	12.5
Enterococcus faecalis	18	12	6	-	-	12.5
Klebsiella pneumonia	30	19	11	6	-	6.25
Escherichia coli	41	29	20	11	-	6.25
Pseudomonas aeruginosa	26	15	8	-	-	12.5

MIC = Minimum inhibitory concentration, (-) = No inhibition zone

Table 8: Antifungal Activity of the Methanol Extract of *T. Ehrenbergii.*

Fungal strains	Inhibition zone diameter in mm					MIC
	100 ml	50 ml	25 ml	12.5 ml	6.25 ml	
Fusariumoxysporum	24	17	9	-	-	25
Rhizoctoniasolani	18	10	-	-	-	50
Aspergillusniger	20	13	8	-	-	25
Candidaflavus	17	8	-	-	-	50
Candida albicans	30	17	9	-	-	25

CONCLUSION:

The aerial parts of *T. ehrenbergii* (family; Boraginaceae) have the potential to act as a source of useful drugs because of presence of various phytochemical components such as carbohydrates, proteins, lipids, phenols, flavonoids, alkaloids, steroids and tannins. The results are very much encouraging but scientific validation is necessary before being put into practice.

REFERENCES:

1. Wassel G, El Menshawi B, Saeed A. Toxic pyrrolizidine alkaloids of certain Boraginaceous plants. Acta Pharm Sin, 1987; 24: 199-204.
2. Bull LB, Culvenor CCJ, Dick AT. 1968. The Pyrrolizidine Alkaloids. North-Holland Publishing Co, Amsterdam, 1-93.
3. Singh B. 2001. Scope of arid zone plants as medicinal agents. Ph.D. Thesis, University of Rajasthan, Jaipur, India.
4. Hassan M, Ahmed S, Mahmood K. Chemical investigation of *Trichodesma indicum* leaves. I. Non-steroidal constituents of the petroleum ether extracts. J Chem Soc Pakistan,1982;4: 281-283.
5. Hosamani KM. Recinoleic acid, cyclopropene acids in *Trichodesma zeylanicum* seed oil. Phytochemistry, 1994; 37: 394-396.
6. Bull LB, Dick AT, Keast JC, Edgar G. Monocrotaline, a hepatotoxic pyrrolizidine alkaloid from Senecio species. Austral J AgricSci, 1956; 7: 281-284.
7. Culvenor CCJ. Antitumor pyrrolizidine alkaloids from Crotalaria spectabilis. J Pharm Sci, 1968; 57: 1122-1125.
8. Kupchan SM, Doskotch RW, Vanervenboven PW. Tumor inhibitors (III). Monocrotaline active principle of Crotalaria spectabilis. J Pharm Sci1964; 53: 343-346.
9. Perianayagam JB, Sharma SK, Pillai KK. Anti-inflammatory activity of Trichodesma indicum root extract in experimental animals. Journal of Ethno-pharmacology,2006;104: 410-414.
10. Perianayagam JB, Sharma SK, PillaiKK. Evaluation of antidiarrhoeal of *Trichodesma indicum* root extract. Methods and Findings in Experimental and Clinical Pharmacology, 2005; 27: 533-537.
11. Alfarhan AH, Al Turky TA, Basahy AY. 2005. Flora of Jizan Region. Final Report Supported by King Abdulaziz City for Science and Technology, 1, 545.
12. Boissier E. 1879. Flora Orientalis4: 281
13. Brand A. Borraginaceae-Borraginoideae-Cynoglosseae. In: ENGLER, A. (ed.), Pflanzenr,1921; 78: 19-44.
14. Täckholm V. 1974. Students' flora of Egypt. 2nd ed. Published by Cairo University. Printed by Cooperative printing company. Beirut. 888.
15. Boulos L. 2000. Fl. Egypt 2: 286-287.
16. Al-Gohary IH. Floristic composition of eleven wadis in gebel elba, Egypt. Int J Agri Biol,2008; 10: 151-160

17. Rowell DL. 1994. Soil Sciences: Methods and Applications. Longman Publishers, Singapore, 350.
18. Askar A, Treptow H. 1993. In "Quality Assurance in Tropical Fruit Processing." Springer Verlag, Berlin, Heidelberg, Germany,238
19. British Pharmacopoeia. 1993. Published on the Recommendation of the Medicines Commission." International Edition. 2: 146 A.
20. Chaplin MF, Kennedy JF. 1994.In "Carbohydrates Analysis-A Practical Approach 2nded." Oxford University Press, Oxford, New York, Tokyo. 324
21. James CS. 1995. In "Analytical Chemistry of Foods." Blackie Academic and Professional Publisher. An Imprint of Chapman and Hall 178
22. Christie WW. 1982. Lipid Analysis 2nd ed., Pergamon Press, Oxford
23. Güenther E. 1972. In "The Essential Oils." Vol. I. Robert, E. Kreiger Publ. Co., Huntinglon, New York., 385
24. Farag RS. 1995. In "Chemical and Physical Analysis of Oils and Lipids". Published by Academic Library, 210
25. Mohamed FE, Amer MA. 1965.In "Oils, Fats, Waxes and Surfactants." Anglo-Egyptian Book Shop, Publisher, Cairo, U.A.R.,259
26. Balbaa SI, 1986. In "Chemistry of Crude Drugs. Laboratory Manual." Faculty of Pharmacy, Cairo University, 195
27. Brieskorn CH, Klinger-Hand Polonius W. Triterpenes and sterols in leaves of Salvia triolobaandPyrusmalus. Arch Pharm, 1961; 294: 380-391.
28. Woo WS, Chi HJ, Yun and Hye S. Alkaloid screening of some Saudi Arabian plants. SaengyakHakhoe Chi (HangukSaengyaKHakhoe), 1977; 8(3): 109-113.
29. Balbaa SI, Hilal SH, Zaki AY. 1981. In "Medicinal Plants Constituents." 3rd Ed. General Organization for Univ. Books, Cairo, Egypt, 644
30. Edeoga HO, Okwu DE, Mbaebic BO. Phytochemical constituents of some nitrogen medicinal plants. African Journal of Biotechnology,2005; 4(7): 685-688.
31. Samatha R, Shyamsundarachary R, Srinivas P, Swamy NR, Kurz L. Total flavonoids content in some plants. American journal of applied sciences, 2012; 5(4): 177-179.
32. Han NM, May CY. Total flavonoids content in some plants of family Solanaceae, American journal of applied sciences, 2012; 9(11): 1862-1867.
33. Makkar HPS, SnghB, Vats SK, SoodRP. Total phenols, tannins and condensed tannins in different parts of Rumexhastatus. Bioresource Technology, 1993; 45(1): 69-71.
34. Ali AA, Ross SA, Mesbah MK, El MoghazySA.Phytochemical study of Limoniumaxillare (Forssk.). Bull Fac Pharm, 1991; 29(3): 59-62.
35. HonerlogenH,TretterHR.Zurroutinemasigenquantitativen Gesamsaponinbestimmung in radix Panax und extrakten. DeutApothZeitung, 1979; 199: 1483-1486.
36. Steven AC, Michael M, Thomas T. 1989. In "Manual of Advanced Tech. for Amino Acids Analysis". The Pico Tag method, Millipore-Coop. USA, 4189.
37. Ali R M, Sawaf NA. Effect of salinity alone and in combination with adipic acid ormethylamine on free amino acid and alkaloids content of Daturainnoxia. J Faculty of Education Ain ShamsUniv,1992; 17: 345-353.
38. Andrade JL, Larque SA, Trejo CL. Proline accumulation in leaves of four cultivars of Phaseolus vulgaris L. with different drought resistance. Phyton,1995; 57: 149-157.
39. Okuma E, SoldaK, Tada M, Murata Y. Exogenous proline mitigates the inhibition of growth of Nicotianatobacum cultured cells under saline conditions. Soil science and plant nutrition, 2000; 46: 257-263.
40. Prescott ML, Harley J, Donald P, Klein A. 1999. In Antimicrobial chemotherapy Microbiology 2nd edition .published by C. Brown Publishers, U.S.A. 325
41. ShahidiBonjar GH. Evaluation of Antibacterial properties of Iranian Medicinal plants against Micrococcus aureus, Serratiamarcescens, Klebsiellapneunomiae and Bordellabronchoseptica. Asian Journal of Sciences, 2004; 3(1):82-86.
42. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Review,1999; 12: 564-582.
43. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology, 2006; 5(4): 257-361.