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RESEARCH ARTICLE

INVESTIGATION OF LIPOIDIAL CONTENTS AND THEIR ANTI MICROBIAL ACTIVITY OF *FORSSKAOLEA VIRIDIS* AND *TRICHODESMA EHRENBERGII* WILDLY DISTRIBUTED IN EGYPT

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ABSTRACT
Objective: The aim of this work was to assess the antimicrobial activity and investigation of lipoidial contents of *F. viridis* and *T. ehrenbergii* wildly distributed in Gebel Elba, Southeast of Egypt for the first time.
Methods: The phytochemical investigation of the ether extracts of *F. viridis* and *T. ehrenbergii* carried out by saponification of two lipoidial extracts and using gas chromatography (GC) with reference standards. The antimicrobial activity of the ether extract was performed as *in vitro* studies by diffusion agar technique for selected +ve and –ve Gram bacterial and fungal strains with reference used drug as a control.
Results: The findings of this study revealed that the two lipoidial extracts have sufficient steroidal and fatty acid methyl ester compounds where *F. viridis* contain (22) hydrocarbons, (6) sterols and (14) fatty acid methyl esters while, *T. ehrenbergii* contain (20) hydrocarbons, (5) sterols and (17) fatty acids where *β*-amyrin, stigmasterol and palmitic and Tricyclic acid were the major concentration of steroid and fatty acid methyl ester contents of *F. viridis* and *T. ehrenbergii* respectively. The lipoidial extract of *F. viridis* and *T. ehrenbergii* exhibited moderate antimicrobial activity against all tested strains as compared to reference used drug.
Conclusion: It can be elicited that the ethereal extracts of two plants have moderate antimicrobial activity against selected strains.
Keywords: Antimicrobial, *F. viridis*, Lipoidial extract, *T. ehrenbergii*.

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INTRODUCTION

Herbal plants have been recognized and used in the human history. Plants make many chemical compounds that have many biological activity, such as protection against insects, fungi. The use of plants as medicine exist before written history of the human. Most of the herbs and spices used by man in food and useful therapeutic compounds[1]. *Forsskaolea* is a small genus in the Urticaceae family, represented by 6 species, distributed in over the world[2,3].
Trichodesma ehrenbergii is a small genus in Boraginaceae family where, is an annual erect herb, 15-45 cm high, densely short hairy[4]. Lipid compounds (sterols, terrenes, free fatty acids, esters of fatty acids) have antimicrobial activity where, the efficacy of these lipids over microorganisms is related to their chemical structure[5]. Where, saturated compounds are effective against microorganisms at lower chain lengths, while unsaturated compounds with longer chain lengths are more active. The position of double bonds is important for long chain fatty acids. The therapeutic use of lipoidial compounds, with particular regard to topical applications for the treatment of bacterial or fungalinfections[5,6]. The survey on the previous studies on the *F. viridis* and *T. ehrenbergii* plants showed no chemical and biological studies performed on it so, current study aimed to investigate the chemical constituents in addition to their biological activity in our previous studies. Because of isolation and identification of some of active chemical constituents and their biological activity of two plants as hepatoprotective, antimicrobial, antitumor and antioxidant activity of different solvents extracts[7-10]. It was decided to complete this study chemical investigations and antimicrobial activity. In this study on two lipoidial fractions of the two plants to obtain a complete chemical and biological profile of two

important plant species of two different families from the same location of Gebel Elba, Haliab, Southeast of Egypt.

MATERIALS AND METHODS

Plant Material

The plant parts of *F. viridis* and *T. ehrenbergii* were collected from their wild habitat in wadi kanthesrob, sarmati, Gebel Elba region, southeast corner of Egypt. The plant specimens were identified by Dr. Omran Ghaly, a researcher of plant taxonomy, department of Plant Ecology and Ranges, authenticated and deposited in the herbarium of Desert Research Center.

Preparation of lipoidial matter

The dried powder of *F. viridis* and *T. ehrenbergii* aerial portions (250 g) were exhaustively extracted separately by petroleum ether: di ethyl ether (1:1) using Soxhlet continuous extraction until exhaustion. The solvent was evaporated at 40˚C under reduced pressure to give 24 g and 26 g residue of lipoidial matter[7,11].

Preparation of the Unsaponifiable Matter

Total 3 g of lipoidial matter of two plants were saponified by refluxing in soxhlet apparatus with 50 ml of 10% alcoholic KOH for 6 hr followed by evaporating the alcohol, diluting with distilled water and extracting with ether exhaustively. The all combined ethereal extracts were cleaned with distilled water till being get rid of alkalinity, then dried over Na_2SO_4, and then concentrated to give 1.5 g unsaponifiable matter (USM) residue[7,11].

Preparation of saponifiable matter (fatty acids)

The remaining saponifiable basic (alkaline) aqueous layer left afterward withdrawal of unsaponifiable matter with ether was acidified with 2N HCl to release the free fatty acids, and then extracted more times with di ethyl ether solvent. Then the ether portions were washed away more times with dist. H_2O until neutralization, dried above anhydrous Na_2SO_4. The residual were kept for analysis the fatty acid contents[12].

Preparation of fatty acid methyl esters

The preparation of methyl esters of free fatty acids (0.6 g) was carried out by refluxing with 100 ml 99.9% MeOH and 5 ml H_2SO_4 for 2 hr. The major part of alcohol was distilled off and the residue was solubilized with distilled water and then extracted more times with ether. The collected fractions were washed with dist. H_2O, till free from any acidity then drying the ethereal layer and the rest part was dehydrated over anhydrous Na_2SO_4 then evaporate the ether extract to give residue of the fatty acid methyl esters and kept for GC analysis[12].

GC analysis of the lipoidial matter conditions:

The saponifiable and unsaponifiable matter of aerial parts of the plant was carried by method described in[13]. Using GC Hewlett Packard hp 6890 Series Agilent Gas Chromatograph. Authentic samples according to the apparatus library from C_{10} to C_{32}. With Capillary column hp-5 (5% diphenyl-95% dimethyl polysiloxane, 150 mm x 4mm), 2 ml/min of chart speed 80/280 ºC for initial/Final time for 25 minutes.

Antimicrobial Activity

Antimicrobial activity of the two lipoidial extracts was determined by diffusion agar technique in Regional Center for Mycology and Biotechnology Al-Azhar university, Cairo, Egypt (RCMB) according to CLSI[13,14]. Bacterial and fungal strains were obtained from the bacteria stock existing at RCMB. Petri dishes comprising on 20 ml of Nutrient (for bacteria) or Malt extract (for fungi), Agar medium was seeded with 1-3 day cultures of microbial inoculums (standardized inoculums 1-2X10^7 cfu/ml 0.5 Mcfarland standard). Wells (6 mm in diameter) were cut off into agar and 100µl of the two plant extracts were tested in a concentration of 5mg/ml and incubated at 37^{o}C for 24 h (bacterial strains) and at 25°C for 7 days (fungal strains). The assessment of antimicrobial activity was built on account of the diameter of the inhibition zone formed around the well. Ketoconazole with MIC 100 mg/ml was used for fungi positive control while, Gentamycin with MIC 4 mg/ml was used for bacteria strains positive control.

RESULTS AND DISSCUSSION

Investigation of saponifiable matter using GC

The data recorded in Table 1: revealed that, there were 22 hydrocarbons beside 6 sterols and 20 hydrocarbons beside five sterols compounds were detected where, *β*-amyrin followed by *β*-sitosterol and stigmasterol followed by cholesterol were represented the major concentration of the sterols for *F. Viridis* and *T. ehrenbergii* ethereal extract respectively, the high concentration of the phytosterols in the lipoidial extracts may be related to their lipid absorption inside the cell membrane of the plant through converting the lipoidial matters to constituents which have sterols chemical structures, where they acts a dynamic role in cell membrane structure and used as a precursor to steroid hormones and fat-soluble vitamins (A, D, E, K)[15]. The high relative percent of *β*-amyrin and stigmasterol earned *F. viridis* and *T. ehrenbergii* plants some medicinal importance, where previous studies showed activity of *β*-amyrin and stigmasterol as human bladder cancer, skin epidermoid, anticancer, anti microbial, anti-inflammatory, and breast cancer[16], antiulcer[17]. Also it can be a probable effective compound for drug development in diabetes and atherosclerosisas *β*-amyrin and stigmasterol have prospective antihyperglycemic and hypolipidemic effects[18]. While, the relatively high percent of *β*-sitosterol and cholesterol in the lipoidial extract of *F. viridis* plays a vital role in therapeutic drugs used for improving sexual activity, relieving symptoms of menopause, lowering of high bad blood cholesterol level and treating benign prostatic hyperplasia by reducing the quantity of cholesterol absorbed by the body. Also, used for improving the immune system and for avoiding colon cancer and in synthesis of cortisone as well as for gallstones[19,20].

Investigation of saponifiable matter using GC:

The fatty acids methyl esters results represented in Table 2: indicated that, there were 14 fatty acid methyl ester, 10 saturated beside 4 unsaturated and 16 fatty acid methyl ester, 13 saturated beside 4 unsaturated of

both plants *F. viridis* and *T. ehrenbergii* saponifiable extracts respectively, the investigation of saponifiable contents showed that the palmitic and oleic acid were major concentrations of saturated and unsaturated fatty acids methyl ethers of *F. viridis* respectively.

Table 1: Hydrocarbons and sterols determined of *F. viridis* and *T. ehrenbergii* using GC.

No. C atom	RT	Name	M. F.	*F. viridis* Area (%)	*T. ehrenbergii* Area (%)
Hydrocarbons					
C13	9.791	n-Tridecane	$C_{13}H_{28}$	0.421	0.596
C14	10.755	n-Tetradecane	$C_{14}H_{30}$	0.793	2.357
C15	12.060	n-Pentadecane	$C_{15}H_{32}$	1.665	12.220
C15:1	12.879	n- Pentadecene-1	$C_{15}H_{30}$	1.048	6.439
C16	13.457	n-Hexadecane	$C_{16}H_{34}$	7.370	14.974
C17	13.884	n-Heptadecane	$C_{17}H_{36}$	4.146	5.519
C17:1	14.386	n-Heptadecene-1	$C_{17}H_{36}$	4.513	14.920
C18	14.869	n-Octadecane	$C_{17}H_{34}$	15.309	3.351
C18:1	15.767	n- Octadecene-1	$C_{18}H_{38}$	4.580	3.003
C19	16.129	n-Nonadecane	$C_{19}H_{40}$	12.599	0.596
C19:1	16.524	n- nonadecene-1	$C_{19}H_{38}$	--	0.829
C20	17.015	n-Eicosane	$C_{20}H_{42}$	2.811	0.409
C21	17.832	n- Heneicosane	$C_{21}H_{44}$	2.959	--
C22	17.975	n-Docosane	$C_{22}H_{46}$	0.956	0.506
C23	18.953	n-Tricosane	$C_{23}H_{48}$	0.707	0.456
C24	21.090	n-Tetracosane	$C_{24}H_{50}$	0.541	0.344
C24-1	21.738	n-Tetracosene-1	$C_{24}H_{48}$	0.562	--
C25	22.086	n-Pentacosane	$C_{25}H_{52}$	0.627	0.563
C26	23.068	n-Hexacosane	$C_{26}H_{54}$	1.741	--
C27	23.616	n-Heptacosane	$C_{27}H_{56}$	1.354	0.563
C28	24.913	n- Octacosane	$C_{28}H_{58}$	4.642	0.174
C28:1	25.464	n- Octacosene-1	$C_{28}H_{56}$	--	0.303
C29	26.729	n- Nonacosane	$C_{29}H_{60}$	2.275	1.004
C30	29.063	n-Triacontane	$C_{30}H_{62}$	4.714	1.359
Sterols					
C:27	30.239	Cholesterol	$C_{27}H_{46}O$	2. 750	6.450
C:28	32.055	Campesterol	$C_{28}H_{48}O$	3.211	2.797
C:29	34.228	Stigmasterol	$C_{29}H_{48}O$	2.612	13.575
C:29	35.138	β -Sitosterol	$C_{29}H_{50}O$	3.956	4.890
C:30	37.168	γ- Amyrin	$C_{30}H_{50}O$	3.652	1.894
C:30	38.734	β- Amyrin	$C_{30}H_{50}O$	4.978	----

RT= Retention time, M.F.= Molecular formula

Table 2: Saponifible matter (fatty acids) of *F. viridis* and *T. ehrenbergii* using GC.

No. of C atom	Systemic name	Trivial name	RT	Area (%)	
				F. viridis	*T. ehrenbergii*
C:10	Decanoic acid	Capric acid	8.562	4.403	---
C:11	Undecanoic acid	Undecylic	8.673	7.680	3.723
C:12	Dodecanoic acid	Lauric	9.398	2.351	15.102
C:13	Tridecanoic acid	Tridecylic	11.018	---	22.140
C:14	Tetradecanoic acid	Myristic	12.657	2.400	6.084
C:15	Pentadecanoic acid	Pentadecylic	14.094	----	1.062
C:16	Hexadecanoic acid	Palmitic	15.605	29.482	16.225
C:17	Heptadecanoic acid	Margaric	17.522	2.060	2.540
C18	Octadecanoic acid	Stearic	18.685	7.190	4.639
C18:1	Cis-9-Octadecanoic acid	Oleic	19.258	21.073	2.589
C18:2	Cis, cis-9, 12-Octadecanoic acid	α-Linoleic	20.440	5.211	3.160
C18:2	Trans, trans -9, 12-Octadecanoic acid	Linoelaidic	21.697	6.350	---
C18:3	All Cis-9, 12, 15-Octadecatrienoic acid	γ-Linoleic	22.523	6.701	3.177
C19	Cis-10-Nonadecylic acid	Nonadecanoic		4.146	----
C20	Eicosanoic acid	Arachidic	23.346	0.512	0.842
C22	Docosanoic	Behenic	24.316	----	4.163
C24	Tetracoanoic acid	Lignocoric	26.985	0.355	4.885
C26	Hexacosanoic acid	Ceric acid	28.293	----	0.655
C27	Heptacosanoic acid	Carboceric	29.605	---	8.485

Tridecylic and γ-Linoleic revealed the major percent for saturated and unsaturated fatty acid of *T. ehrenbergii* respectively. The essential fatty acids have great value where, they give the body healthy value as contrary to what was previously believed where, converted in the body by enzymes into long chain polyunsaturated fatty acids (LCPUFAs). Where *γ*-linolenic acid (*ω*-6) which needed for the maintenance of hormonal balance and healthy skin structure. The presence of essential unsaturated fatty acids in both plants, linoelaidic acid (*ω*-6 trans fatty acid), (*ω*-9) oleic acid, (*ω*-3)*α*- linolenic and (*ω*-6) *γ*-linolenic acid refers to the importance of the two plants as a source of all *ω*-3, ω-6 and *ω*-9 fatty acids as nutritional fats where, each acid of them has a great value in health benefits in the body by right equilibrium between them, where the imbalance between them may cause a number of chronic diseases. Oleic acid (*ω*-9) represented as non-essential fats, subsequently; they can be manufactured by the body. The high relatively percent of (ω-9) can qualify the plant to use as reducing agent of plasma triglycerides by 19% and very-low-density-lipoprotein cholesterol by 22% in patients with diabetes[21], enhanced insulin sensitivity and reduced inflammation[23]. The relatively high percent of (*ω*-3) and (*ω*-6) may give more value of the plants for decreasing, blood pressure, liver fats, a number of symptoms of rheumatoid arthritis, triglycerides and the formation of arterial plaques, promoting of the bone health, preventing asthma[23]. Otherwise, the two plants consists of high percent of saturated fatty acid, palmitic acid which has a vital role in cellular membrane functionality by improving their flexibility and permeability and it forms reversible links to cell membrane proteins, thus being involved in regulating the traffic of molecules in and out of cells and inter cells communication[24]. Palmitic acid is then the precursor of palmitoyl ethanol amide (PEA) compound which formed by the body with anti-inflammatory, analgesic and neuroprotective activities[25].

Table 3: Antimicrobial activity of lipoidial extract of *F. viridis* and *T. ehrenbergii*.

Tested Organism	Inhibition Zone Diameter (mm)		
	Control	*F. viridis*	*T. ehrenbergii*
Gram (+ve) Bacteria Gentamycin (MIC) 4 mg/ml (reference- drug)			
Micrococcus sp. (RCMB 028)s	22	13	11
Streptococcus mutants (RCMB017) (ATCC 25175)	21	12	12
Methicillin-Resistant *Staphylococcus aureus*	15	11	13
Gram (-ve) Bacteria			
Salmonella typhrimurium (RCMB 006) (ATCC 14028)	17	10	10
Escherichia coli (RCMB 010052) (ATCC 25955)	30	13	11
Klebsiella pneumonia (RCMB 003) (ATCC 13883)	21	12	9
Filamentous Fungi Ketoconazole(MIC) 100 mg/ml (reference- drug)			
Aspergillus fumigatus (RCMB 002008)	17	2	7
Penicillium expansum (RCMB 001001)	17	NA	8
Yeasts			
Candida albicans(RCMB 005003) (ATCC 10231)	20	1	NA
Cryptococcus neoformans (RCMB 0049001)	25	16	14

MIC = Minimum inhibitory concentration, NA= No activity, The sample was tested at mg/ml concentration

Antimicrobial activity

The antimicrobial activity of the lipoidial extract of *F. viridis* and *T. ehrenbergii* showed potent antibacterial activity against Gram (+) ve (*Methicillin-Resistant Staphylococcus aureus*)with activity 73% and 86% respectively, moderate activity against *Streptococcus mutants* and *Micrococcus* sp.) with activity 57.3, 57.3 and 59, 50%, respectively when compared with gentamicin as reference used drug. Also, it exhibited weak activity against all tested Gram (-) ve bacteria and there is no activity against tested filamentous fungi while, it exhibited moderate activity against yeasts fungi (*Cryptococcus neoformans*) with activity 64 and 56 % respectively, as compared to ketoconazole as used reference drug. The moderate activity of the lipoidial extract may be due to its phytosterols contents which characterized with antimicrobial activity and fat-soluble vitamins which have ability to inhibit the activity of micro-organisms and acts in cell membrane and DNA of microbial strains[26].

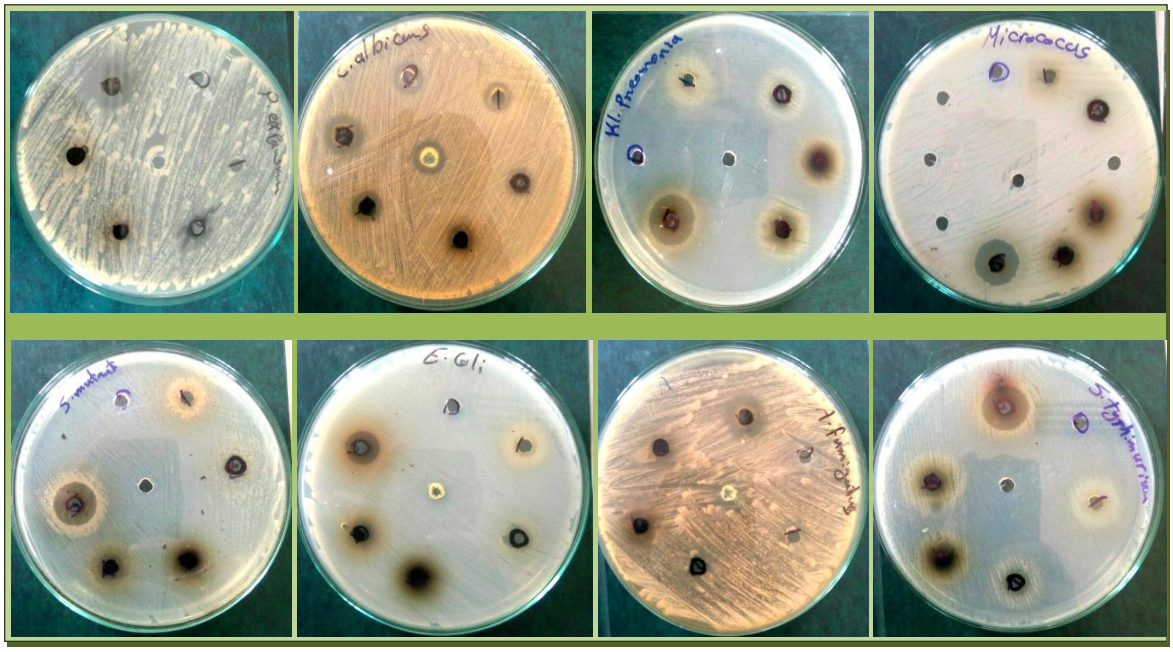

Figure 1: Inhibition zones of microbial activity of lipoidial extract of *F. viridis* and *T. ehrenbergii*.

From the previous obtained data the *F. viridis* show little improvement more than *T. ehrenbergii* as antimicrobial activity this is may be due to little changes in steroidal contents between them where the presence of *β*-Amyrin in *F. viridis* and absence in *T. ehrenbergii*, Also the high percent of stigmasterol in *T. ehernbergii* may be act more activity against *Penicillium expansum* more than *F. viridis.* so we can say as general the two plants extract have moderate activity against some of tested strains as shown in Table 3.

CONCLUSION

The investigation of lipoidial contents of *F. viridis* and *T. ehrenbergii* using (GC) revealed that, of *F. viridis* contain 22 hydrocarbons, 6 sterols and 14 fatty acid methyl ester while *T. ehrenbergii* contain 21 hydrocarbons, 5 sterols and 16 fatty acid methyl esters. The *in vitro* antimicrobial studies showed that moderate antimicrobial activity of two plants against most Gram (-ve and + ve) bacteria while, weak and no activity of fungal strains while, the *F. viridis* showed little improvement than *T. ehrenbergii*.

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CONFLICT OF INTEREST

No conflict of interest associated with this work.

AUTHOR'S CONTRIBUTION

All authors have worked equally for this work.

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