Original Research Article

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

Abstract:

Objective: The objective of this study 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 &stigmasterolandpalmitic &Tricyclicacid 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*showed potent 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 and need further studies to study the possibility of using the plant extracts as some strains of anti-bacterial and fungi-fighting drugs.

Keywords: Lipoidial extract, antimicrobial, *F. viridis* and *T. ehrenbergii*

INTRODUCTION:

Medicinal plants have been identified and used throughout human history. Plants make many chemical compounds that are for biological functions, including defense against insects, fungi and herbivorous mammals. The use of plants as medicine predates written human history. Many of the herbs and spices used by humans to season food also yield useful medicinal compounds. The use of herbs and spices in cuisine developed in part as a response to the threat of food-borne pathogens[1]. *Forsskaolea*is a small genus in the Urticaceae family, represented by 6 species, distributed inover the world[2,3].*Trichodesma ehernbergii*is a small genus in Boraginaceae family where, is an annual erect herb, 15-45 cm high, densely short hairy [4].

The survey on the previous studies on the *F. viridis* and *T.ehrenbergii*plants showed no chemical and biological studies performed on it so, we aimed to investigate the active chemical constituents in addition to their biological activity [5,6,7,8]. In this study we concerned to focus our study on the lipoidial extract of the two plant extracts and its antimicrobial activity to obtain about complete chemical and biological profile of two important plant species of two different families from same location Gebel Elba, Haliab, Southeast of Egypt.

MATERIALS AND METHODS

Plant Material

Aerial parts of *F.viridis* and *T. ehrenbergii* were collected from their wild habitat in wadikanthesrob, sarmati, Gebel Elba region, southeast corner of Egypt. The plant specimens were identified, authenticated and deposited in the herbarium of Desert Research Center (CAIH).

Preparation of lipoidial matter

The air-dried powder of *F. viridis* and *T. ehrenbergii*aerial parts (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 [6,9].

Preparation of the Unsaponifiable Matter

3 g of lipoidial matter of two plants were saponified by refluxing with 50 ml of 10% alcoholic potassium hydroxide solution for 6 hr followed by evaporating the alcohol, diluting with distilled water and extracting with ether exhaustively. The collected ethereal extract was washed with distilled water till being free from alkalinity, dried over anhydrous sodium sulphate, and then evaporated to give 1.5 g unsaponifiable matter (USM) residue [6,9].

Preparation of saponifiable matter (fatty acids)

The remaining saponifiable alkaline aqueous layer left after extraction of unsaponifiable matter with ether was acidified with hydrochloric acid (2 N) to liberate the free fatty acids, followed by extraction several times with ether. The ether extracts were washed three times with distilled water until neutralization, dried over anhydrous sodium sulfate. The residual were kept for studying the fatty acid contents [10].

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 of absolute methanol and 5 ml sulphuric acid for 2 hr, The major part of alcohol was distilled off and the residue was solubilized with distilled water and then extracted several times with ether. The combined ether extracts were washed with distilled water, till the wash was free from any acidity then drying the ethereal layer concentrated and the residue was dried over anhydrous sodium sulfate followed by evaporation of ether to give residue of the fatty acid methyl esters (FAME) and kept for GC analysis [10].

GC analysis of the lipoidial matter conditions:

The saponifiable and unsaponifiable matter of aerial parts of the plant was carried by method described in[11]. Using 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 was determined by diffusion agar technique in Regional Center for Mycology and Biotechnology Al-Azhar university, Cairo, Egypt (RCMB) according to CLSI [11,12]. Strains were obtained from the bacteria stock present at RCMB. Petri plates containing 20 ml of Nutrient (for bacteria) or Malt extract (for fungi), Agar medium were seeded with 1-3 day cultures of microbial inoculums (standardized inoculums 1-2 X 10^7cfu/ml 0.5Mcfarland standard). Wells (6 mm in diameter) were cut off into agar and 100µl of plant extracts were tested in a concentration of 5mg/ml and incubated at 37°C for 24 h (bacterial strains) and at 25°C for 7 days (fungal strains). The assessment of antimicrobial activity was based on measurement of the diameter of the inhibition zone formed around the well. Positive control used for fungi was ketoconazole with MIC 100 mg/ml, while positive control used for bacteria strains was gentamycin with MIC 4 mg/ml.

RESULTS AND DISSCUSSIONS

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 compoundswere detected where,the highest concentration of the sterols was *β*-amyrin followed by *β*-sitosterol and stigmasterol followed by cholesterol of*F. viridis* and *T. ehernbergii*ethereal extractrespectively,the high concentration of the phytosterols in the lipoidial extracts may be due to their lipid metabolism inside the cell membrane of the plant through converting the lipoidial matters to compounds which have chemical structures of sterols, where they acts a vital role in cell membrane structure and utilities as a precursor to fat-soluble vitamins (A, D, E, K) and steroid hormones [13]. The highest percent of *β*-amyrin and stigmasterol reflected to the medicinal importance of the two plantsrespectively, where studies showed activity of*β*-amyrin and stigmasterol as anti-inflammatory, antimicrobial, human bladder cancer, breast

and skin epidermoid anticancer [14] and as antiulcer [15]. prospective antihyperglycemic and hypolipidemic effects of *β*-amyrin and stigmasterol suggested that, it could be a probable compound for drug development effective in diabetes and atherosclerosis [16] While, The relatively high percent of *β*-sitosterol and cholesterol in the lipoidial extract of *F. viridis* plays a vital role in pharmaceutical drugs used for enhancing sexual activity, treating benign prostatic hyperplasia, relieving symptoms of menopause, lowering of high bad blood cholesterol level by reducing the amount of cholesterol absorbed by the body. Also, used for boosting the immune system and for preventing colon cancer, synthesis of cortisone as well as for gallstones [17,18].

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 ofboth plants*F. viridis* and *T. ehernbergii*saponifiable extractsrespectively, 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,and the 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 existence of essential unsaturated fatty acids in both plants; oleic acid (*ω*-9), linoelaidicacid (*ω*-6 trans fatty acid), *α*- linolenic (*ω*-3) and *γ*- linolenic acid (*ω*-6) refers to the importance of all *ω*-3, ω-6 and *ω*-9 fatty acids as dietary fats where, each one of them has a number of health benefits for your body by right balance between them, where the imbalance may contribute to a number of chronic diseases. Oleic acid (*ω*-9) fats are non-essential fats, since they can be produced by the body. The high relatively percent of oleic acid (ω-9) can qualify the plant in utilization for reducing plasma triglycerides by 19% and very-low-density-lipoprotein cholesterol by 22% in patients with diabetes [19], improved insulin sensitivity and decreased inflammation [20]. The relatively high percent of *α*- linolenic (*ω*-3) and *γ*- linolenic acid (*ω*-6) reflected to the importance of the plant for reducing triglycerides, blood pressure and the formation of arterial plaques, decreasing liver fat, promoting bone health, preventing asthma and reducing a number of symptoms of rheumatoid arthritis [21]. On the other hand, the two plants contains high percent of saturated fatty acid, palmitic acid which has a critical role in cellular membrane functionality by affecting 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[22]. Palmitic acid is then the precursor of palmitoyl ethanol amide (PEA) compound which produced by our body with neuroprotective, anti-inflammatory and analgesic activities[23].

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

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 activity73%and 86% respectively, moderate activity against *Streptococcus mutants* and *Micrococcus* sp.) with activity 57.3, 57.3 and 59, 50%, respectively as compared with gentamicin as reference used drug. Also, it showed weak activity against all tested gram (-) ve bacteria and no activity against the tested filamentous fungi while, it showed moderate activity against yeasts fungi (*Cryptococcus neoformans*) with activity 64and 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 [24]. 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 *Penicilliumexpansum*more than *F.viridis*. so we can say as general the two plants extract have moderate activity against some of tested strains as showen in table 3.

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

Tested Organism	Inhibition Zone Diameter (mm)		
Gram (+ve) Bacteria	**Control**	***F.viridis***	***T. ehrenbergii***
Gentamycin (MIC) 4 mg/ml (reference- drug)			
Micrococcus sp. (RCMB 028)s	22	13	11
Streptococcus mutants (RCMB 017) (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)			
Aspergillusfumigatus (RCMB 002008)	17	2	7
Penicilliumexpansum(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 5 mg/ml concentration

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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AUTHOR'S CONTRIBUTION All authors have worked equally for this work.

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