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Phytochemicals, volatile oil and biological activities of *Triumfetta flavescens* (Hochst)

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Abstract

Plant material was collected from wild plant populations of *Triumfetta flavescens* (Hochst) grown in sandy soils in western desert region, Egypt. *Triumfetta flavescens* (Hochst) herb contains high amounts of flavonoids and tannins. The yield of volatile oil (VO) obtained by hydro -distillation from aerial parts of *Triumfetta flavescens* (Hochst) ranged from 0.5 to 0.9%. The major constituents of *Triumfetta flavescens* (Hochst) VO were eudesmol (19.9%), caryophyllene oxide (9.1%), alpha-caryophyllene (5.8%) and pentadecanol (4.7%). The majority of these components were found to belong to the oxygenated sesquiterpenes hydrocarbons class (SCHO), with percentage of 44.5%. *Triumfetta flavescens* (Hochst) extract showed activities against gram positive bacteria, while it was inactive against gram negative bacteria, fungi and yeast.

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Introduction

Plant-derived substances have recently become of great interest owing to their versatile applications. Wild plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al*., 2008). Medicinal plants have important role in primary health care delivery system for improvement of people health, so it needs more attention (Akerele, 1988) It is an essential constituent of human health care especially for the rural communities who solely rely on forest plants for medicine, food, shelter and energy (Hamayun *et al*., 2003). The medicinal values of these plants lie in some active ingredients that produce a definite physiological action on human body; the most important of these bioactive components of plants are alkaloids, tannins, terpenoids, flavonoids and the phenolic constituents (Hill, 1952). Knowledge of organic components of plants is desirable, not only for the discovery of therapeutic agents but also because such information can be of value in disclosing new sources of such economic materials such as tannins, oils, gums, precursors for synthesis of complex chemical substances. The knowledge of chemical composition of plants would further be valuable in discovering the actual value of folkloric remedies (Ghaderi, 2003).

Triumfetta flavescens (Hochst) belongs to Tilaceae family, perennial woody based herb or shrub, up to 2m tall, much branched from the base with yellow flowers. *Triumfetta flavescens* (Hochst) grow wild in Egypt especially at the east desert and Sinai (Tackholm, 1974; Boulos, 1995, 2000). There are a very few studies on the Chemical composition of *Triumfetta flavescens* under the conditions of Egypt, so, the present investigation was carried to study the phyto-chemicals screening, biological activities and volatile oil composition of *Triumfetta flavescens* (Hochst) plants grow wild in Egypt. This study may increase the natural products using in drugs, pharmaceutical and food industries.

Materials and Methods

Plant material

Plant material was collected in March 2013 from wild plant populations of *Triumfetta flavescens* (Hochst) grow in sandy soils in western desert region (Gabal Elba) approximately 1200 km south of Cairo, Egypt. Identification of the species was achieved by Boulos (1995, 2000), voucher specimens are in

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the herbarium of National Research Centre (NRC), Cairo, Egypt.

Phyto-chemical screening

The powdered air-dried aerial parts of *Triumfetta flavescens* (Hochst) were screened for carbohydrates and / or glycosides; sterols and / or triterpenes, flavonoids, tannins, saponins, coumarins and alkaloids. Applying chemical tests according to Harborne (1998).

Volatile oil (VO) isolation

Dried leaves [divided into small pieces (0.5 - 1 cm)] were collected then 500 g from each replicate (three replicates) from many places of western desert region (Gabal Elba) were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus (Clevenger, 1928). The VO content was calculated as a relative percentage (v/w). The samples of VOs were dried over anhydrous sodium sulphate to identify the chemical constituents of the VO.

Gas chromatography (GC)

GC analyses were performed using a Shimadzu GC- 9A gas chromatograph equipped with a DB5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 µm). Oven temperature was held at 40°C for 5 min and then programmed until 250°C at a rate of 4°C/min. Injector and detector (FID) temperature were 260°C; helium was used as carrier gas with a linear velocity of 32 cm/s.

Gas chromatography-Mass spectrometry (GC-MS)

GC-MS analyses were carried out on a Varian 3400 system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.); Oven temperature was 40 to 240°C at a rate of 4°C/min, transfer line temperature 260°C, injector temperature 250°C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, flow rate 1.1 ml/ min, Ionization energy 70 eV; scan time 1 s ; mass range 40-350 amu.

The components of the oils were identified by comparison of their mass-spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices with those of authentic compounds. Kovat's indices (KI) or Retention indices (RI) (Kováts, 1958) were determined by co-injection of the sample with a solution containing a homologous series of n-hydrocarbons, in a temperature run identical to that described above.

Qualitative and quantitative analyses of VO

Identifications were made by library searches (Adams, 1995) combining MS and retention data of authentic compounds by comparison of their GC retention indices (RI) with those of the literature or with those of standards available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C_8–C_{22}) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra from literature. Component relative concentrations were calculated based on GC peak areas without using correction factors.

Biological activity

Plant extraction: The dry herbs of *Triumfetta flavescens* (Hochst) were extracted by 80% alcohol which can extract the whole constituents of the plant (Harborne, 1998). The extract was evaporated under vacuum and the residue was dissolved in alcohol to give concentration of100 µg/ml. Biological activity evaluated by antimicrobial activity test, it is known from the literature that flavonoids and tannins may have antimicrobial activities (Lamb, 2005).

Microbiological techniques

Microbial strains: The antimicrobial activity of the alcoholic extract was tested against five bacterial strains *(Escherichia coli, Proteus vulgaris, Salmonella typha, Bacillus subtilis* and *chromobacter* sp), one fungal strain *(Aspergillus niger)* and one yeast strain *(Candida albicans)*. Test organisms used were obtained from the Faculty of Agriculture, Cairo University.

Agar diffusion method: This method was carried out according to Collins and Lyne (1985). Nutrient agar was used for the cultivation of bacteria and yeast, and Czapek-Dox's medium (Dox 1910) for cultivation of fungal species. In this method, pre-sterilized Whatman no.1 filter paper discs (0.5 mm in diameter) (Whatman International Ltd., Maidstone, England) were impregnated with 100µl of the extract (100 µg/ml) allowed to dry (to get rid of the alcohol) and applied on the surface of agar plates freshly seeded with standard inocula of young cultures, 24-hrs-old bacteria and yeast, and 7-days-old fungi. The plates of test organisms were then incubated at 27°C for 24 hrs for bacteria and yeast and for 48 hrs for fungi. At the end of the incubation period, the inhibition zones were measured (results are the average of triplicate measurements).

Table 1. Phytochemical screening 80 % alcoholic extract

Group	Test
Carbohydrates and / or glycosides	+
Sterols and terpenes	+
Flavonoids	++
Tannins	++
Alkaloids	-
Saponins	-
Coumarins	-
Anthraquinones	±

++ =High amounts, + =Moderate amounts, - =Absent, ± =Traces

Results

Phytochemical screening

Data presented in Table 1 indicated that *Triumfetta flavescens* (Hochst) herb contains high amounts of flavonoids and tannins. On the other hand it contains moderate amounts of terpenes, glycosides, sterols and carbohydrates; while the amounts of anthraquinones were traces. Alkaloids, Saponins and coumarines were absent.

VO composition

The yield of volatile oil (VO) obtained by hydro -distillation from aerial parts of T*riumfetta flavescens* (Hochst) ranged from 0.5 to 0.9% (v/w) on a dry weight. Twenty tow volatile components resulted by GC/MS analysis, amounting to 82.8% of the total volatiles of *Triumfetta flavescens* (Hochst) are summarized in Table 2. These components were found in different percentages. The majority of these components were found to belong to the oxygenated sesquiterpenes hydrocarbons fraction (SCHO), with percentage about 44.5%. SCHO include sapathulenol (4.1%), caryophyllene oxide (9.1%), E-beta-ionone (2.9%), eudesmol (19.9), pentadecanol (4.7%) and nonadecane (4.1%). Another three minor classes of compounds have been detected from *Triumfetta flavescens* (Hochst) VO like sesquiterpene hydrocarbons (SCH) (22.4%), oxygenated monoterpenes (MCHO) (10%) and monoterpene hydrocarbons (MCH) (5.9%) The major constituents of *Triumfetta flavescens* (Hochst) VO were eudesmol (19.9%), caryophyllene oxide (9.1%), alpha-caryophyllene (5.8%) and pentadecanol (4.7%).

Biological activity

Disc diffusion method was adapted to evaluate the antimicrobial activity of *Triumfetta flavescens* (Hochst) in vitro against 7 microorganisms (Table 3). The diameters of the inhibition zones. The data obtained indicated that the extract showed activities against gram positive bacteria, while it was inactive against gram negative bacteria, fungi and yeast.

Table 2. Volatile oil constituents of *Triumfetta flavescens* Hochst (Hayne) identified by RI & MS.

No	Compound	RI	RT	Class	Area %
1	*Alpha*-Pinene	939	6.6	MCH	1.1
2	*Para*-Cymene	1026	7.3	MCH	1.3
3	Linalool	1098	22.9	MCHO	2.5
4	Bornyl acetate	1099	23.3	MCHO	2.6
5	Carvacrol	1137	23.9	MCHO	2.3
6	*Alpha*-Cubebene	1351	23.2	MCH	3.5
7	*Beta*-Elemene	1375	24.5	SCH	2.2
8	Methyl eugenol	1403	25.1	MCHO	2.6
9	*Alpha*-Caryophyllene	1454	25.5	SCH	5.8
10	*E-beta*-Farnesene	1458	26.1	SCH	2.3
11	*Alpha*-Muurolene	1480	26.6	SCH	2.3
12	*Beta*-Bisabolene	1509	27.2	SCH	2.2
13	*Gamma*-Cadinene	1513	28.2	SCH	2.2
14	*Z-gamma*-Bisabolene	1515	29.1	SCH	2.7
16	Sapathulenol	1576	30.9	SCHO	4.1
17	Caryophyllene oxide	1581	35.9	SCHO	9.1
18	*E-beta*-Ionone	1607	31.3	SCHO	2.6
19	Eudesmol	1649	32.7	SCHO	19.9
20	Calamenene	1695	34.2	SCH	2.7
21	Pentadecanol	2280	36.7	SCHO	4.7
22	Nonadecane	2539	37.6	SCHO	4.1
MCH					5.9
MCHO					10.0
SCH					22.4
SCHO					44.5
Total identified					82.8

RI: Confirmed by comparison with Retention index on DB5 column (Adams, 1995), MCH: Monoterpene hydrocarbons, MCHO: Oxygenated monoterpene, SCH: Sesquiterpene hydrocarbons, SCHO: Oxygenated sesquiterpene.

Discussion

Triumfetta flavescens (Hochst) herb contains high amounts of flavonoids and tannins. Flavonoids have an antioxidants and health benefits of anti-inflammatory (Gale otitis *et al.*, 2008). Due to its astringent, tannin has been used to treat tonsillitis, pharyngitis, hemorrhoids, and skin rashes (Kumar and Pandey, 2013). *Triumfetta flavescens* (Hochst) contains moderate amounts of essential oil (terpenes), glycosides, sterols and carbohydrates. Glycosides play many important roles in living organisms. Several plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis which causes the sugar part to be broken off, production the chemical available for use. Many such plant glycosides are used as medications. In humans and animals, poisons are often bound to sugar molecules as part of their elimination from the body (Arias, 2007). Plant sterols, have been shown in clinical trials to block cholesterol absorption sites in the human intestine, thus helping to reduce cholesterol in humans (Ostlund *et al.*, 2003). Carbohydrates make

Table 3. Antimicrobial activities of 80 % (Inhibition zone in mm ±SEM)

Test organism		80 % ethanol extract (100 µg/ml)	Standard (100 µg/ml)
Bacteria (Gram-negative)	*Escherichia coli* (G-)*	NA	16±0.6
	Proteus vulgaris	NA	NT
	Salmonella typh	NA	NT
Bacteria (Gram-positive)	*Bacilllus subtilis*-NRRL–B543) (G+)	12±0.3	24±0.5
	Chromobacter sp	13±0.1	NT
Yeast	*Candida albicans*	NA	NT
Fungi	*Aspargillus niger*	NA	9±0.3

NA: Not active, NT not tested, standard for bacteria; amoxicillin, standard for fungi and yeast: canestin. Each value represents the mean of inhibition zone (mm) of three replicates ± SEM (standard errors of means).

numerous roles in living organisms. Polysaccharides supply for the storage of energy) and as structural components (Anthea *et al*., 1993).

It may be noted that *Triumfetta flavescens* (Hochst) VO contains high amounts of eudesmol. Eudesmol is also known to have various beneficial effects on human health and is considered to be a lead compound for treating epileptic seizures (Chiou *et al.*, 1997), angiogenic diseases (Kimura, 2005) and dementia (Obara, 2006). Eudesmol is known to have unique effects on the nervous system, including blocking the nerve-evoked contraction and markedly alleviating muscle fasciculation, tremor and convulsion (Kimura *et al*., 1991; Chiou *et al.*, 1995). On the other hand eudesmol is found to be a high active compound responsible for resistance of plants to ant attack (Marsaro *et al.*, 2004; Marinho *et al*., 2005), and also has antifungal activities (Itoh *et al*., 2004; Abdulkhade *et al*., 2006).

The antimicrobial activity of the extract may be due to its content of terpenes. It was found that the effect of terpenes on gram positive bacteria is greater than gram negative bacteria (Kêdzia *et al*., 2000). This may be due to differences in composition, change of outer structure of microorganism and permeability. The terpenes may affect the respiration chain and changing the torpedoing of proteins of the cell number (Trombeta *et al.*, 2005).

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