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ACTIVE CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF *Trichodesma africanum* (L.) R.Br. var. *heterotrichum* Bornm. & Kneuck.

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ABSTRACT

Twenty-four components of the essential oil of *Trichodesma africanum (*L.) R.Br. var. *heterotrichum* Bornm.& Kneuck. (Boraginaceae) were identified using GC-Mass , where the major constituents were caryophyllene oxide (16.05%), 10-epi-γ-eudesmol (13.02%), cadina-1, 4-diene (8.60%), β-gurjunene (7.51%), 2-pentadecanone, 6, 10, 14-trimethyl (5.75%) and β-caryophyllene (5.57%). Ascultin, umbelliferone and scopoletin as coumarin compounds were isolated from ether and ethyl acetate extracts using TLC, while the flavonoid and phenolic acid compounds: luteolin-7-diglucoside, luteolin 7-O-α-L rhamnosyl (1-6) ß-D-glucoside, quercetin, apigenin and gallic acid were separated from methanol extracts using paper chromatography. Antimicrobial effects of the essential oil, petroleum ether and diethyl ether extracts on some selected micro-organisms (bacteria and fungi) indicated positive results.

Kew words: *anti-microbial effects, coumarins, essential oil, flavonoids, Trichodesma africanum.*

1. INTRODUCTION

in Egypt, *Trichodesma africanum* (L.) R.Br. (Boraginaceae) is a perennial richly branched plant, usually with a woody base. Densely beset with white, bulbous-based bristles. Corolla blue, throat yellow with 5 purple spots, where var. *heterotrichum* Bornm. & Kneuck. is characterized with leaves densely hairy between the bristles, thus of a grey appearance (Tâckholm, 1974). Also *Trichodesma africanum* is a desert plant of Africa and Asia, containing beta-amyrin, beta-methyl oleanate, potassium nitrate and a nitrogen-containing toxin, which produce a notable toxic response when injected in laboratory mice and rats, where muscle contraction and a blood pressure reduction caused by the toxin are described. On the other hand, pyrrolizidine alkaloids, *e.g*., trichodesmine, present in other *Trichodesma* species do not show this effect produce. Meanwhile, beta-sitosterol and stigmasterol were identified as the principal sterols in the plant (Omar *et al*., 1983).

The leaves of *Trichodesma africanum* are used as diuretic in Senegal and Nigeria, and in the treatment of diairhoea and as emollient, antipyretic and anti-inflammatory. An infusion of the root is used for treatment of hepatitis (Schmelzer *et al*., 2008).

The major components of the volatile oil of *Echium amoenum* were obtained using GC-Mass, where they were α-cadinene (24.25%), viridiflorol (4.9%), α-muurolene (4.52%), ledene (3.8%) and α-calacorene (3.04% (Ghassemi *et al*., 2003). Meanwhile, the essential oil obtained from the dried aerial parts of *Onosma microcarpum* (Boraginaceae) revealed that fifty components were identified in this oil, their major constituents were thymol (24.1%), carvacrol (9.3%) and n-heptane (9.3%) (Semnani *et al.*, 2005). Fifty-four components of the volatile oil of *Lithospermum erythrorhizon* (Boraginaceae) were identified using capillary GC and GC–MS. The main components were 2-methylbutanoic acid (21.50%), 3-methylbutanoic acid (12.61%), 2-methylpropanoic acid (8.99%), methyl linoleate (8.76%), methyl oleate (6.27%), methyl palmitate (6.06%), and 2-methyl-2-butenoic acid (5.74%) (Kawata *et al*., 2008).The leaves of *Ehretia thyrsiflora* contained six flavonoids and six phenolic acids, isoquercetrin, hyperoside, trifolin, astragalin, kaempferol 3-O-arabinosylgalactoside, quercetin 3-O-arabinosylgalactoside, rosmarinic acid, cinnamic acid, icariside E5, ferulic acid, α-hydroxydihydrocaffeic acid, lithospermic acid B (Li Li *et al*., 2008).

The oil and hexane, chloroform and methanol extracts exhibited antimicrobial activity against

Gram-positive and Gram-negative bacteria and five fungal strains. It was reported that *Sarcina lutea*, *Vibrio cholerae* and *Rhyzoctonia solani* were more sensitive strains to the essential oil effect, while *Trichophyton mentagrophytes* was more sensitive for hexane extract. eleven constituents representing 96.28% of the essential oil were identified using GC and GC-MS as: 4-methyl, 4-ethenyl-3-(1-methyl ethenyl)-1-(1-methyl methanol) cyclohexane (37.34%), β-eudesmol (19.21%), spathulenol (11.25%) and cadina 4(5), 10(14) diene (7.93%) were found to be the major components.

This study aimed to confirm the use of the plant in folk medicine of *Cordia curassavica* in gastrointestinal, respiratory and dermatological diseases (Tzasna *et al*., 2007).

2. MATERIALS AND METHODS

2.1.Plant material

The aerial parts of *Trichodesma africanum* (L.) R. Br. var. *heterotrichum* Bornm. & Kneuch. (Boraginaceae) at full flowering stage were collected from Wadi Hof habitat, Helwan Governorate, Egypt in March 2006. The fresh plant materials were air dried at lab-temperature till constant weight, then ground to fine dry powder and kept to be used for different plant analyses.

2.2. Preliminary phytochemical screening

This includes testing for volatile oil by the steam distillation method, steroids, coumarins, flavonoids, phenolics compounds, alkaloids using Dragendroff's, Mayer's and Wagner's reagents, glycosides and/or carbohydrates and saponins according to Balbaa *et al*. (1981).

2.3. Investigation of volatile oil

2.3.1. Preparation of volatile oil

The fresh aerial parts of *Trichodesma africanum*, var. *heterotrichum*, (500g) were covered with sufficient water in the flask and subjected to steam distillation according to the British Pharmacopoeia (1980).

2.3.2. GC-mass analysis of volatile oil

GC: Trace GC 200. Initial column temperature was 50 ˚C for 3 minutes, then raised to 60 ˚C by rate 3 ˚C/ min. and raised to 260 ˚C by rate 3 ˚C/ min. and held at 260 ˚C for 5 minutes according to Adams (1995), where injector temperature: 220˚C, transforline temperature: 300 ˚C and ion source temperature: 150˚C, while the detector was MS SSQ7000 finniganmat with ionization mode: EI ev70. Meanwhile, the column was 5B-5(5% phenyl) methyl polysilxane (0.25). Capillary column was fused with silica of 30m length. The carrier gas was helium and the flow rate of column head pressure was 13 ps. The injection was splitless.

2.4. Investigation of coumarins and flavonoids

2.4.1. Preparation of extracts

The defatted fine powder of the aerial parts of *Trichodesma africanum* var. *heterotrichum* was extracted with 70 % aqueous ethanol several times, where a brownish sticky residue was obtained and partitioned successively with ether, ethyl acetate and methanol. The solvent of each fraction was evaporated, separately, under reduced pressure to give chloroform fraction, ethyl acetate fraction and methanol fraction.

2.4.2. Chromatographic investigation of ether and ethyl acetate fractions

The residue of ether and ethyl acetate fractions were dissolved in methanol and spotted on several chromatoplates (5×20) coated with silica gel 60 GF, (E-Merch). The colors of the separated spots were visualized under UV light and exposed to ammonia vapor then revisualized, using solvent system:

S_1 - Chloroform: methanol (9:1 v/v)

2.4.3. Isolation of major coumarins

The coumarin constituents were fractionated from ether and ethyl acetate extracts using preparative TLC and system S_1, visualized under UV light and exposed to ammonia vapor then revisualized. The separated bands were eluted then repurified over sephadex column LH-20, where ether extract produced one compound (C_1), while ethyl acetate extract produced two compounds (C_2 and C_3).

2.4.4. Chromatographic investigation of methanol fraction

The residue of methanol fraction was dissolved and spotted on paper chromatography, using the solvent systems:

S_2 - Butanol: acetic acid: water (4:1:5 v/v/v)

S_3 - Acetic acid: water (15:85 v/v)

2.4.5. Isolation of major flavonoids and phenolic acids

Methanol fraction was fractionated using preparative paper chromatography using solvent system S_2. Purification of the separated compounds was performed through sephadex LH-20 column chromatography using MeOH/H_2 O system.

2.4.6. Physiochemical and spectral analysis

The isolated compounds were subjected to physiochemical and spectral analysis UV, ^{1}H-NMR, ^{13}C-NMR, EI-MS.

2.4.6.1.Ultraviolet spectrophotometric analysis

Chromatographically pure materials were

dissolved in pure methanol and subjected to ultraviolet spectrophotometric investigation using Shimadzu UV visible recording spectrophotometer UV-240 in the Desert Research Center , El-Matareya, Cairo, Egypt.

2.4.6.2.^{1}H- and ^{13}C-Nuclear Magnetic Resonance Analysis (NMR)

The NMR measurements were carried out on A JEOL EX-270 NMR spectrometer apparatus (270 MHz for ^{1}H-NMR and 67.5 MHz for ^{13}C-NMR) as described by Mabry *et al*. (1970) in the National Research Center, Dokki, Giza, Egypt.

2.4.6.3. Mass Spectrometric analysis (MS)

The mass spectrum was conducted using finnigan SSQ 7000 and MM 7070 E according to the method of Mabry *et al*. (1970) in the National Research Center.

2.4.6.4. Chemical reactions

2.4.6.4.1.Controlled (Mild) acid hydrolysis

The pure material was hydrolyzed using 0.1N HCl under reflux for 1 h. The resultants were traced chromatographically every 5 min. using comparative paper chromatography as stated by Harborne *et al*. (1975).

2.4.6..4.2.Complete (normal) acid hydrolysis

The pure material was hydrolyzed using 2N HCl under reflux for 1 h. The released a glycone and sugar were subjected to comparative paper chromatography using authentic samples (Harborne *et al*., 1975).

2.4.6.4.3.Enzymatic hydrolysis

The flavonoid glycoside was enzymatically hydrolyzed through an enzyme corresponding to the type of the sugar in the compound in 0.05 acetate buffer (pH=5.1). The mixture was left at 37-40°C for 24 hours. The hydrolysate was examined through comparative paper chromatography against authentic reference markers as described by Harborne *et al.* (1975).

2.5. Anti-microbial effects

The essential oil and six extracts of successive solvents (petroleum ether, diethyl ether, chloroform, ethyl acetate, 96% methyl alcohol and 70% methyl alcohol) were tested for their antibacterial activities against *Pseudomonas aeruginosa, Escherichia coli, Candida albicans, Klebsiella pneumonia, Micrococcus luteus, Scerratia marcesens* and *Staphylococcus aureus* using the disc agar diffusion method (Cruickshank *et al*., 1975). The tested materials were dissolved individually in DMSO at a concentration of (1.0 mg/ml). Discs (5 mm in diameter) were impregnated in the solution of each extract and kept soaked till absorption of all quantity and then dried. The inhibition zone for each material was measured.

3. RESULTS AND DISCUSSION

The present investigation attempted to evaluate the phytochemical constituents of *Trichodesma africanum*, using different micro-analysis, separation methods and spectral analysis.

3.1. Preliminary phytochemical screening of *Trichodesma africanum* var. *heterotrichum*

The preliminary phytochemical screening of the plant showed that it contained volatile oil, steroids, coumarins, flavonoids, phenolics, alkaloids and glycosides and/or carbohydrates. Neither terpenoids nor saponins were detected.

3.2. Investigation of volatile oil

Water distillation of *Trichodesma africanum* var. *heterotrichum* showed that it contained 0.27% (v/w) volatile oil. GC-Mass of the volatile oil showed that it contained twenty-four components accounting for 96.99% of the total oil composition, where the major constituents of the volatile oil were caryophyllene oxide (16.05%), 10-epi-γ-eudesmol (13.02%), cadina-1, 4-diene (8.60%), β- gurjunene (7.51%), 2- -pentadecanone, 6, 10, 14-trimethyl (5.75%) and β-caryophyllene (5.57%) (Table, 1). Anil *et al*. (2007) reported that the major components of the oil of the leaves of *Neolitsea foliosa* var. *caesia* were β-caryophyllene (35.3%), caryophyllene oxide (9.6%), elemol (8.2%) and β-elemene (6.1%), where the leaves of *Neolitsea foliosa* var. *caesia* oil showed moderate antibacterial activity against most of the tested bacteria.

3.3. Investigation of coumarins and flavonoids

Coumarins were extracted with ether and ethyl acetate for their isolation and identification, while flavonoids and phenolic acids were extracted with methanol for their isolation and identification.

3.3.1. Isolation of the major coumarins content

TLC investigation of the ether and ethyl acetate residues revealed that they contained three major spots with R_f 0.47, 0.72 and 0.74, when using the solvent system (S_1).

3.3.1.1. Identification of compound (C_1)

Compound (C_1) was detected as blue color under UV, which changed to intensified blue colour on exposure to ammonia vapor and re-examined under UV light with R_f 0.47, m.p. 267-269 ºC. Examination of compound (C_1) in MeOH using UV spectrophotometer gave two bands at 260 and 350nm, where in addition of NaOAc it gave a pathochromic shift (+40nm) which indicates the presence of free OH at position 7. The obtained UV spectral data of compound (C_1)

were similar to those reported for Ascultin (Murray *et al*., 1982). Meanwhile, ^{1}H-NMR spectrum in DMSO-d_6, showed signals δ 7.9 (1H, d, J=9Hz, H-4), 7.1 (1H, s, H-5), 6.7(1H, S, H-8), and 6.2 (1H, d, J=9Hz, H-3), where the presence of a pair of doublets at δ 7.9 and 6.2 ppm indicates that compound (C_1) is unsubstituted coumarin pyrone ring (Murray *et al.*, 1982). On the other hand ^{13}C-NMR spectrum of compound (1) in DMSO-d_6, showed signals δ 161.2(C2), 110.7 (C3), 144.6 (C4), 112.2 (C5), 148.4 (C6), which appears shifted more down field by 24 ppm due to presence of OH attached to it, 150.3 (C7), which appears also shifted more down field due to presence of OH attached to it (Murray *et al*., 1982), 102.5 (C8), 142.8 (C9) and 110.7 (C10).

The previous obtained data, when compared with published data (Murray *et al*., 1982) concluded that compound (C_1) could be identified as 6, 7- hydroxycoumarin (Ascultin).

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3.3.1.2. Identification of compound (C_2)

Compound (C_2) was obtained as blue color under UV, which changed to bluish violet on exposure to ammonia vapor and re-examined under UV light with R_f 0.72, m.p. 222-269 ºC. Examination of compound (C_2) in MeOH using UV spectrophotometer gave two bands at 220 and 322 nm, where in addition of NaOAc it gave a pathochromic shift (+42nm) which indicates the presence of free OH at position 7. The obtained UV spectral data were characteristic for 7-hydroxy coumarin (Murray *et al*., 1982). Meanwhile ^{1}H-NMR spectrum in DMSO-d_6, it showed signals δ 7.9 (1H, d, J=9.5Hz, H-4), 7.5 (1H,d, J=8.4 Hz, H-5), 7.0 (1H,dd, J=8.4 Hz, H-6), 6.8 (1H, d, J=1.5 Hz, H-8) and 6.2 (1H, d, J=9.5 Hz, H-3). The presence of pair of doublets at δ 7.9 and 6.2 ppm indicates that compound (C_2) is unsubstituted coumarin pyrone ring, this characteristic signals arises from H-4 and H-3,respectively (Murray *et al*., 1982). On the other hand, Mass spectrum showed fragments at m/z 162 (M^+), 134 ([M-CO]$^+$), 105 ([M-CO-CHO]$^+$), 78 ([M-3CO]$^+$).

The previous obtained data, when compared with the published data (Murray *et al*., 1982) showed that compound (C_2) is7- hydroxycoumarin (umbelliferone).

Raoul and Bull (1947) declared that umbelliferone was known to be a growth inhibitor

Table (1) : GC- Mass of the essential oil of the aerial parts of *Trichodesma africanum* var. *heterotrichum.*

Peak No.	Component	Rt (min)	Relative area (%)
1	Butane,1-chloro-2-methyl	8.42	4.46
2	ρ- Cymene	10.50	0.21
3	Longipinene	21.28	3.62
4	Linalool	22.51	1.32
5	Linalyl acetate	23.02	0.09
6	Cyclohexanone, 2-methylene-5- (1- methyl ethyl)	24.22	2.15
7	β- Caryophyllene	25.10	5.57
8	Cis-α- Bisabolene	27.21	2.91
9	Zingiberene	27.68	1.03
10	10- epi γ- Eudesmol	28.43	13.02
11	2- Cyclohexanone, 3- methyl-6- (1- methyl ethyl)	28.58	3.04
12	Cadinene	28.97	0.79
13	β- Sesquiphellandrene	29.61	2.31
14	Trans-geraniol	30.58	1.62
15	β- Damascenone	31.62	3.21
16	Caryophyllene oxide	34.56	16.05
17	Unknown	35.33	2.40
18	Elemol	36.08	3.40
19	Cadina-1,4- diene	36.30	8.60
20	2- Pentadecanone,6,10,14- trimethyl	36.73	5.75
21	2- ethyl dibenzothiophene	38.28	1.65
22	α- Eudesmol	40.12	0.09
23	β- Gurjunene	41.02	7.51
24	Unknown	42.37	5.89

for *Escerichia coli*, *Bacillus* sp. and *Candida albicns*.

3.3.1.3. Identification of compound (C_3)

Compound (C_3) was detected as blue color under UV, which changed to blue fluorescence on exposure to ammonia vapor and re-examined under UV light with R_f 0.74, m.p. 203-204 ºC. Examination of compound (C_3) in MeOH using UV spectrophotometer gave two bands at 225 and 348 nm, where in addition of NaOAc gave a pathochromic shift (+72nm) which indicates the presence of free OH at position 7. The obtained UV spectral data were similar to those reported for 7-hydroxy-6- methoxycoumarin compounds (Murray *et al*., 1982). Meanwhile ^{1}H-NMR spectrum in DMSO-d_6, showed signals δ 7.6 (1H, d, J=9 Hz, H-4), 6.9 (1H, s, H-5), 6.7 (1H, s, H-8), 6.3 (1H, d, J=9 Hz, H-3) and 3.9 (3H, s, OCH3). The presence of a pair of doublets at δ 7.6 and 6.3 ppm indicates that compound (C_3) is unsubstituted coumarin pyrone ring (Murray *et al*., 1982).

Mass spectrum showed fragments at m/z 193 (M^+), 192 (M-1), 178 ([M-CH_3]$^+$), 163 ([M-OCH_3]$^+$), 82([M-4CO]$^+$), 69 ([M-4CO-CH_3]$^+$). The previous obtained data, when compared with published data (Murray *et al*., 1982) concluded that compound (C_3) could be identified as 7-hydroxyl -6- methoxycoumarin (scopoletin).

3.3.2. Isolation of major flavonoids and phenolic acids

Paper chromatography investigation of methanol extracts, revealed the presence of four major spots with R_f 0.24, 0.36, 0.71 and 0.88, when using the solvent system (S_2).

3.3.2.1. Identification of compound (F_1)

Compound (F_1) was detected as brown color under UV changed to yellow on exposure to ammonia vapor and re-examined under UV light with R_f 0.24 and 0.28 in S_2 and S_3 respectively, which revealed that compound (F_1) may be flavonoid glycoside (Mabry *et al.*, 1970). On complete acid hydrolysis of the compound it gave the aglycone luteolin and glucose as sugar moiety (2 moles). UV spectral analysis of the compound (F_1) in methanol gave two bands at 345 and 251 nm, as band I and band II, respectively with a shoulder at 266nm, where in addition of NaOAc it caused no shift in band II, which suggested occupation of 7- position. Meanwhile addition of NaOMe caused bathochromic shift in both band I and II, which proved the presence of a free OH group at 4´- position, while the addition of $AlCl_3$ caused bathochromic shift. On the other hand, addition of $AlCl_3$/HCl decreases the magnitude of the bathochromic shift of $AlCl_3$, proving the presence of 2 catecholic OH groups.

The previous conclusion was confirmed by the addition of H_3BO_3 which gave bathochromic shift in band I (+25 nm) These results are in agreement with Mabry *et al.* (1970).

^{1}H-NMR spectrum of the compound (F_1) in DMSO showed that there were two anomeric protons for glucose sugar moieties present at δ ppm 5.1 J= 7.5 Hz and 4.55 J= 2.5 Hz, respectively, dd at 7.45 for H-2´, H-6´, J= 7.5 Hz and a d, at 6.92 for H-5´ (J= 7 Hz), a S at 6.75 for H-3 and two doublets at δ ppm 6.75 and 6.45 with J=2.5 Hz for H-8 and H-6, respectively which agreed with the conclusion of Mabry *et al.*, (1970). Meanwhile the rest of the two sugar protons was present as m at the range δ 3.1-3.9. The previous obtained data when compared with published data (Mabry *et al.*, 1970) concluded that compound (F_1) can be identified as luteolin -7-diglucoside.

3.3.2.2. Identification of compound (F_2)

Compound (F_2) was detected as brown color under UV, changed to yellow on exposure to ammonia vapor and re-examined under UV light with R_f 0.35 and 0.31 in S_2 and S_3 respectively, this revealed that compound (F_2) may be flavonoid glycoside (Mabry *et al.*, 1970). On complete acid hydrolysis of the compound it gave the aglycone luteolin and two sugar residues identified as glucose and rhamnose (comparative R_f-values with authentic markers). Enzymatic hydrolysis with ß-glucosidase gave no intermediate which confirms that glucose moiety is directly linked to the flavonoid nucleus and that rhamnose is terminally located in this compound.

UV spectral analysis of the compound (F_2) in methanol gave two bands at 351 and 253 nm, as band I and band II, respectively with a shoulder at 265 nm, where in addition of NaOAc it caused no shift in band II, which suggested occupation of 7-position. Meanwhile the addition of NaOMe caused bathochromic shift in both band I and II, which proved the presence of a free OH group at 4´- position, while the addition of $AlCl_3$ caused bathochromic shift. On the other hand, the addition of $AlCl_3$/HCl decreases the magnitude of the bathochromic shift of $AlCl_3$, proving the presence of 2 catecholic OH groups.

The previous conclusion was confirmed by the addition of H_3BO_3 which gave bathochromic shift in band I (+29 nm) This agreed with the conclusion of Mabry *et al.* (1970).

^{1}H-NMR spectrum of the compound (F_2) in DMSO showed that there were two anomeric protons for glucose and rhamonose sugar moieties

present at δ ppm 5.1 J= 7.5 Hz and 4.55 J= 2.5 Hz, respectively, dd at 7.45 for H-2´, H-6´, J= 7.5 Hz and a d, at 6.92 for H-5´ (J= 7 Hz), a S at 6.75 for H-3 and two doublets at δ ppm 6.75 and 6.45 with J=2.5 Hz for H-8 and H-6, respectively which agree with the conclusion of Mabry *et al.*(1970). Meanwhile the rest of the two sugar protons was present as m at the range δ 3.1-3.9, the rhamnosyl CH_3 was detected as d J=6 Hz at δ 0.95.

^{13}C-NMR further confirmed the structure of (F_2) as luteolin 7-O-α-L rhamnosyl (1-6) ß-D-glucoside. The glycosylation at 7-position was proved through the down field shift of C-7 (+2.5) being present at 164.5. Two anomeric sugar carbon signals C-1´´ and C-1´´´ at 100 and 100.3 for glucose and rhamnose, respectively. The rhamnosyl CH_3 was located at 17.9. The two sugar molecules are linked through 1-6 link *i.e.* rutinoside form which was proved through the down field shift of C-6 at 66 characteristic for occupied C-6 glucosyl molecule. The previous obtained data when compared with published data (Mabry *et al.,* 1970) concluded that compound (F_2) can be identified as luteolin 7-O-α-L rhamnosyl (1-6) ß-D-glucoside.

3.3.2.3. Identification of compound (F_3)

Compound (F_3) was detected as yellow color under UV, with no change on exposure to ammonia vapor, and re-examined under UV light with R_f 0.71 and 0.27 in S_2 and S_3 respectively, which revealed that compound (F_3) may be a flavonoid.

UV spectral analysis of the compound (F_3) in methanol gave two bands at 370 and 255 nm, band I and band II, respectively indicating that (F_3) was a flavonol compound with free 3-OH group, where in addition NaOMe formation of a new band at 328 nm which indicated the presence of a free 7- OH group, while addition of NaOAc / H_3BO_3 caused bathochromic shift in band I which indicated the presence of O- dihydroxy group in B-ring. The bathochromic shift of band I in $AlCl_3$ indicates the presence of 3 and 5-OH group, while the hypsochromic shift with $AlCl_3$ / HCl in band I after addition of HCl, indicates the presence of O-dihydroxy group in B-ring where these results were in agreement with Mabry *et al.* (1970).

^{1}H-NMR spectrum in DMSO showed signals at: δ 7.6 (1H, dd, J= 8.5, 2.3 Hz, H2'), 7.5 (1H, dd, J= 8.5Hz, H6'), 6.89 (1H, d, J= 8.5 Hz, H5'), 6.4 (1H, d, J= 2.5 Hz, H6) and 6.2 (1H, d, J= 2.5 Hz, H8) (Mabry *et al.,* 1970). EI-Mass spectrum of compound (F3) showed molecular ion peak M^+ at 301.9 m/z. The previous obtained data when compared with published data (Mabry *et al.,* 1970) concluded that the compound (F_3) can be identified as quercetin.

Quercetin can be used as antioxidant and as an inhibitor of protein tyrosine kinases (Sigma, 1992).

3.3.2.4. Identification of compound (F_4)

Compound (F_4) was detected as purple color under UV changed to yellow green on exposure to ammonia vapor and re-examined under UV light with R_f 0.88 and 0.11 in S_2 and S_3 respectively. These results revealed that the compound may be flavonoid.

UV spectral analysis with methanol gave two bands at 336 and 267 nm, band I and band II, respectively, indicating that (F_4) was a flavone compound, where on addition of NaOMe caused bathochromic shift (+56 nm), thus indicating that the 4´ position to be free hydroxyl group, while addition of NaOAc exhibited a bathochromic shift (+7 nm) so proving that the 7 position to be free hydroxyl. Meanwhile addition of H_3BO_3 caused no shift so proving the absence of catecholic hydroxyl groups. On the other hand, addition of $AlCl_3$ caused a shift of (+47 nm) which was not changed after addition of dilute HCl, so further confirming the absence of catecholic hydroxyl groups This agrees with the conclusion of Mabry *et al*. (1970).

^{1}H-NMR spectrum in DMSO showed signals at: δ (ppm) 7.95(d, J=7.5 Hz for H-2, H-6´), 6.9 (d, J=7.5 Hz for H-3´, H-5´), 6.75 (S, H-3), 6. 5 (d, J=2.5 Hz for H-8) and 6.1 (d, J=2.5 Hz for H-6). EI-Mass spectrum of compound (F4) showed molecular ion peak M+ at 270 m/z and fragments 242, 153, 118, 121. The previous obtained data when compared with the published data concluded that compound (F_4) can be identified as apigenin.

Apigenin has markedly augmented the cytotoxicity of tumor necrosis factor-alpha (TNF) which induced cytotoxicity in murine fibblast L-929 cells and is useful in chemoprevention, plays a role in the prevention of carcinogensis, inhibits the proliferation, and to a lesser degree, the migration of endothelial cells, and capillary formation *in vitro*, independently of its inhibition of hyaluronidase activity (Trochon *et al*., 2000).

Isolation of major phenolic acids

Paper chromatography investigation of methanol extracts, revealed the presence of one major spot having R_f 0.78 using the solvent system (S_2).

3.3.2.5. Identification of compound (P_1)

Compound (P_1) was obtained as a blue color under UV, with no change on exposure to ammonia vapor and re-examined under UV light

with R_f 0.78 and 0.59 in S_2 and S_3 , respectively. These results revealed that the compound has phenolic nature.

UV spectral analysis with methanol gave two bands at 335 and 272 nm, band I and band II, respectively, where on addition of NaOMe caused bathochromic shift. (275 & 345) which proved the presence of free OH. ^{1}H-NMR spectrum in DMSO showed signals at: δ (ppm) 6.98 (s-H2 and H6). EI-Mass spectrum of compound (P_1) showed molecular ion peak M^+ at 169 m/z. The previous obtained data when compared with the published data (Khamis, 2006) concluded that compound (P_1) can be identified as gallic acid.

3.4. Anti-microbial effects

The effects of the essential oil and different successive extracts (petroleum ether, diethyl ether, chloroform, ethyl acetate, 96% methyl alcohol and 70% methyl alcohol) on the inhibition of some micro-organisms (bacteria and fungi) are illusterated in Tables (2 and 3), where the essential oil, petroleum ether and diethyl ether showed a moderate effect on *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans, Klebsiella pneumonia, Micrococcus luteus, Scerratia marcesens* and *Staphylococcus aureus*, while petroleum ether residue has no effect on *Pseudomonas aeruginosa* (Table 2). On the other hand the residues of chloroform, ethyl acetate, 96% methyl alcohol and 70% methyl alcohol extracts had no effect against the tested micro-organisms (bacteria and fungi) (Tables 2 & 3), whereas the essential oil and residues of petroleum ether and diethyl ether extracts of the plant showed a moderate effect against fungi (*Aspragillus niger* and *Yeast*) (Table 3).

It could be concluded from the previous mentioned data that *Trichodesma africanum* var. *heterotrichum* has antimicrobial activity in its essential oil, petroleum ether and diethyl ether extracts. These results are in agreement with Mahady (2002).

Table (2) Antibacterial susceptibility pattern of the essential oil and extracts of *Trichodesma africanum* **var.** *heterotrichum.*

Microorganisms	Inhibition zone (mm)						
	Volatile oil	Petroleum ether	Diethyl ether	Chloroform	Ethyl acetate	96% Methyl alcohol	70% Methyl alcohol
Pseudomonas aeruginosa	9.1	0.1	8.9	0.2	0.3	0.3	2.2
Escherichia coli	8.8	8.0	9.3	0.4	0.4	1.5	1.4
Candida albicans	8.9	8.5	8.8	0.1	0.2	2.1	2.4
Klebsiella pneumonia	9.3	8.7	9.4	0.2	0.3	1.2	1.6
Micrococcus luteus	8.6	8.3	8.9	0.3	0.2	2.4	2.5
Scerratia marcesens	9.0	8.8	9.2	0.3	0.3	1.1	1.3
Staphylococcus aureus	9.3	8.6	9.5	0.5	0.4	0.8	1.2

Table (3): Antifungal susceptibility pattern of the essential oil and extracts of *Trichodesma africanum* **var.** *heterotrichum***.**

Microorganisms	Inhibition zone (mm)						
	Volatile oil	Petroleum ether	Diethyl ether	Chloroform	Ethyl acetate	96% Methyl alcohol	70% Methyl alcohol
Aspragillus niger	9.3	8.5	9.4	0.3	0.4	0.7	1.1
Yeast	9.7	8.2	9.5	0.7	0.5	0.8	1.3

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المكونات الفعالة والتأثير المضاد للميكروبات لنبات شوك الضبع
(*Trichodesma africanum* **(L.) R. Br. var.** *heterotrichum* **Bornm. & Kneuck.)**

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قسم النباتات الطبية والعطرية - مركز بحوث الصحراء- المطرية- القاهرة- مصر

ملخص

تم فصل أربعة وعشرون مركبا من الزيت الطيار لنبات شوك الضبع باستخدام جهاز التحليل الكروماتوجرافى مزود بمطياف الكتلة، وكانت المكونات الرئيسية للزيت هى اوكسيد الكاريوفيللين (16.05%) و 10-ابى-جاما-ايوديسمول (13.02%) وكادينا1،4-دين (8.60%) و بيتا- جيرجونين (7.51%) و 2- بنتاديكانون، 6، 14،10-تراي ميثيل (5.75%) و بيتا كاريوفيللين (5.57%). تم ايضا فصل الاسكولتين و الامبيلليفيرون و الاسكوبولتين كمركبات كومارينية من مستخلص الايثر و الايثيل اسيتات باستخدام كروماتوجرافيا الطبقة الرقيقة ، أما المركبات الفلافونيدية و الاحماض الفينولية: ليتيولين-7- داى جلوكوزيد وليتيولين -7-O- α- L رامنوزيد (1-6) ß -D - جلوكوزيد وكورستين وابيجينين وحمض الجاليك فقد تم فصلها من مستخلص الميثانول باستخدام الورق الكروماتوجرافى. وجد تأثيراً مضاد الميكروبات لكل من الزيت الطيار ومستخلصى الايثر البترولى والايثرفان وكان ايجابيا على بعض أنواع البكتيريا والفطريات المختارة للدراسة.

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