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# PHYTOCHEMICAL INVESTIGATION AND BIOLOGICAL ACTIVITES OF *THYMUS DECUSSATUS* BENTH. GROWING IN EGYPT

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## ABSTRACT

Liquid chromatography coupled with ion spray mass spectrometry in the tandem mode (LC/MS/MS) with negative ion detection was used for identification of a variety of phenolic compounds in methylene chloride fraction of methanol extract of *Thymus decussatus* Benth. growing in Egypt using gradient elution analysis. Standard solutions of phenolic and flavonoid compounds, were studied in negative ion mode where MS/MS product ion scans revealed that 3, 4'-dimethoxychrysin is the major component of methylene chloride fraction of *Thymus decussatus* Benth., in addition to isolation of salvigenin, apigenin, luteolin, chrysoeriol 7-*O*-β-D-glucopyranoside and rutin respectively, moreover, structure elucidation of the isolated compounds was carried out by spectroscopic (UV, IR, 1D-NMR) methods. The antimicrobial study of the methanol extract showed strong effect against the selected microorganisms where screening of cytotoxic activity revealed the significant cytotoxic activity against Ehrlich ascites carcinoma cells (EAC), as well as two human cancer cell lines (U251) brain- tumor and (Hepg2) liver carcinoma cell –lines) respectively.

**KEYWORDS:** *Thymus decussatus* Benth., Egypt, phenolic, liquid chromatography, antimicrobial and cytotoxic.

## INTRODUCTION

Lamiaceae, family includes 230 genera of perennial plants *Thymus* is known for many pharmacological and clinical properties. The genus *Thymus* commonly known as Thyme

comprises hardy herbs and can adapt to extreme climatic conditions. Hence, it is widely distributed throughout the world [Stahl-Biskup 2002, Harley *et.al* 2004]; more than 300 different perennial and subshrub species are mostly distributed in Europe, Asia and North Africa [Tackholm 1974, Stahl-Biskup *et.al* 2002, Bouls 2005].

Thymus genus are herbaceous perennial shrubs commonly used as spices and/or medicinal herbs, with several pharmacological properties, such as antispasmodic, antiseptic, antitussive, expectorant and flatulence-reducing actions [Evans1998]. *Thymus decussatus* Benth. is an erect dwarf shrub with linear leaves, decussatus, densely crowded with margin turned downwards [Tackholm 1974, Stahl-Biskup *et.al* 2002, Bouls 2005]. *Thymus decussatus* Benth. is a chasmophyte growing in rocky area, especially wet site, endemic to Saint Catharine, Sinai, Egypt [Tackholm 1974]. is used for treatment of abdominal colic, bronchitis [Leung *et.al* 1996] dry, spasmodic coughs as well as whooping cough [Weiss *et.al* 1988] carminative, diuretic, urinary tract antiseptic and for kidney problems [Aboutable *et. al* 1986]. Thyme oils are also used in dietary supplementation, as well as in the development of health products, particularly pharmaceuticals. Several studies over the antimicrobial activity of Thymus essential oils have shown their potential against important pathogenic microorganisms as *Staphylococcus aureus* [Bounatirou et al., 2007; Rasooli & Mirmostafa, 2002], *Helicobacter pylori* [Hazzit *et al.,* 2009] and *Candida albicans* [Faleiro *et al.,* 2003; Hazzit *et al.,* 2009] suggesting their ability in foodborne pathogens control.

Thyme improve the organoleptic properties of food products, spices and aromatic plants are also known to contribute to their preservation, in food industry, for example, used as antioxidant, because it contains compounds prevent or delay oxidation reactions, in order to maintain food quality for longer periods and to extend shelf life although some synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary-butylhydroquinone (TBHQ) are being used; however, the use of these synthetic antioxidants is now under discussion due to their questionable safety. Thus, it is highly desirable to find out natural antioxidants able to substitute such compounds [Teresa Delgado *et al* 2014].

*Thymus decussatus* Benth. is used in folk medicine as a spice and flavoring agent, while its infusion is used to treat nausea [Batanonny *et. al* 1999], yet nothing was reported in literatures concerning the phytochemical composition and bioactivities of *Thymus decussatus* Benth. except the composition and bioactivities essential oil [Khodair *et.al* 1993, Elhela

2007], therefore, it deemed of interest to carry out the investigation of the composition and biological activities of *Thymus decussatus* Benth. as a trial to justify the traditional medicinal uses.

## EXPERIMENTALS

### Plant material

*Thymus decussatus* Benth. aerial parts were collected from Saint Catherine in South Sinai Egypt at during flowering stage in April 2014 and were kindly identified by Prof. Dr. Moneer Abd El-Ghany Prof. of Plant Taxonomy, Faculty of Science, Cairo University.

A voucher herbarium specimen had been deposited in the Department of Pharmacognosy, Faculty of Pharmacy Al -Azhar University, Cairo, Egypt.

### Reagents

Reagents were obtained from the following sources; methanol, ethyl acetate and acetonitrile (HPLC grade) from SDS (Peypin, France), hexane (HPLC grade) from Scharlau (Barcelona, Spain), formic acid from Probus (Badalona, Spain) and acetic acid from Merck (Darmstadt, Germany). Ultrapurewater (Milli-Q) was used. Phenolic standards were obtained as follows: protocatechuic acid, coumaric acid and quercetin from Sigma (St. Louis, MO, USA), β-hydroxyl benzoic acid, Alloevodionol, 5-hydroxyverotric acid, Chlorogenic acid, Neptein, 3,4'-Dimethoxychrysin, 3,4'-Dimethoxychrysin, epicatechin, catechin, gallic acid, ferulic acid, caffeic acid, chlorogenic acid, trimethoxy-quercetin, apigenin, hyperoside, kaempferol, kaempferol-3-*O*-glucoside, kaempferol-3-rutinoside, kaempferol-7-*O*-neo-hesperidoside, quercitrin, isoquercitrin, isorhamnetin, luteolin, luteolin-6-*C*-glucoside, luteolin-8-*C*-glucoside, luteolin-7-*O*-glucoside, apigenin, apigenin-7-*O*-glucoside, apigenin-6-*C*-glucoside, apigenin-8-*C*-glucoside, amentoflavone, naringin, naringenin, naringenin-7-*O*-glucoside, isorhoifolin and rutin from Pharmacognosy Dept. (Al- Azhar Univ. Cairo, Egypt), the purity of the standards was 98% and all were prepared as stock solutions at 1 mg in methanol where the working standard solutions were made by diluting the stock solutions with the LC mobile phase.

### Extraction and fractionation of phenols

200 g of *T. decussatus* Benth. were extracted three times with 700 ml of methanol–water (80:20 v/v), the pooled supernatant phases were filtered and concentrated under vacuum to

dryness, the dry powder was re-extracted twice with hexane (500 ml each). The two solid phases (fraction and the insoluble in hexane) were pooled and dried.

Total defatted extract was chromatographed on a Silica gel column (under medium pressure (VLC) at a flow-rate of 10 ml $min^{-1}$ using mixtures of methylene chloride/ methanol as solvent, the fractions were monitored by thin-layer chromatography (TLC) (Alugram Sil G/UV, Macherey–Nagel, D¨uren, Germany) where similar fractions were combined to give seven final fractions (A–E ). The methylene chloride (A) fraction was concentrated under reduced pressure and introduce to LC/MS analyses, the fractions C and D was subjected to a silica gel column eluted with *n*-hexane:ethyl acetate 95:5 to 10:90 to obtain six fractions of A (50mg), B (370mg), C (450mg), D (50mg), E (200g) and F (400mg). Fraction B was re chromatographed on Silica gel CC eluted with *n*-hexane:-ethyl acetate 90:10 to 80:20 and applied to a sephadex LH-20 column (Amersham Pharmacia Biotech, Amersham, UK) column at a flow-rate of 1 ml $min^{-1}$ using methanol as solvent give compound **1** (45mg).

The C fraction was chromatographed on a paper chromatography (PC, descending) Whatman No. 1 and 3mm papers, using solvent systems 15% HOAc (H2O–HOAc, 85:15), BAW (n-BuOH–HOAc–H2O, 4:1:5, upper layer). and Sephadex LH-20 column eluted with $CH2Cl_2$:MeOH 50:50 to give compounds **2** (20mg) and fraction E (200mg), the sub fraction E-1 was further subjected to solid phase extraction (RP-C18) using 50:50-60:40 MeOH: water and applied to sephadex-LH20 column (Amersham Pharmacia Biotech, Amersham, UK) column at a flow-rate of 1 ml $min^{-1}$ using methanol as solvent to obtain compound **3** (25mg).

**Instrumentation**

LC analyses were performed using an Agilent (Germany) Model 1100 quaternary pump equipped with an autosampler and a diode-array detector (DAD). AChemstation HP Rev. A.08.03 was used for data analysis. A Luna C18 column (150 d 2.1 mm i.d., 5 m) (Phenomenex, Torrance, CA, USA) was used. Gradient elution was carried out with water–0.1% formic acid (solvent A) and acetonitrile–0.1% formic acid (solvent B) at a constant flow rate of 400 ml $min^{-1}$.

A linear gradient profile with the following proportions (v/v) of solvent B was applied (*t* (min), %B): (0, 6), (14, 16.5), (16, 17), (18, 17.5), (20, 17.5), (22, 18.5),(24, 18.5), (27, 20), (46, 100), (48, 6), the compounds described were monitored at 280, 320 and 365 nm. An API

3000 triple-quadrupole mass spectrometer (Perkin-Elmer Sciex, Concord, ON, Canada) to obtain the MS and MS/MS data. All the analyses were performed using a Turbo Ions pray source with the following settings: capillary voltage _3500 V, nebulizer gas (N2) 10 (arbitrary units), curtain gas (N2) 12 (arbitrary units), collision gas (N2) 10 (arbitrary units), focusing potential 200 V, entrance potential 10 V, drying gas (N2) heated to 400 $^{o}$C and introduced at a flow rate of 8000 cm3 min$^{-1,}$ $^{t}$he declustering potential (DP) and the collision energy (CE) were optimized for each compound in infusion experiments: individual standard solutions (10 ng ml$^{-1}$) dissolved in 80 : 20 mobile phase (A: B) were infused at a constant flow-rate of 5 ml min$^{-1}$ into the mass spectrometer using a Model 11 syringe pump (Harvard Apparatus, Holliston, MA, USA). Full-scan data acquisition was performed, scanning from *m*/*z* 100 to 800 in profile mode and using a cycle time of 2 s with a step size of 0.1 u and a pause between each scan of 2 ms.MS/MS product ions were produced by collision-activated dissociation (CAD) of selected precursor ions in the collision cell of the triple quadrupoles mass spectrometer and mass analyzed using the second analyzer of the instrument. Additional experimental conditions for MS/MS included collision energy (depending on the compound), CAD gas (nitrogen) at 6 (arbitrary units), and scan range, as necessary for the precursor selected. In all the experiments, both quadrupoles (Q1 and Q3) were operated at unit resolution.

The structure of the compounds was identified by spectroscopic methods including: ultraviolet and visible absorption spectrometer (UV–VIS, Labomed Inc.) for ensuring UV spectral data of the isolated compounds, in the range of 200–500 nm in methanol and with different diagnostic shift reagents.

$^{1}$H-NMR Nuclear magnetic resonance spectrophotometer (NMR, Bruker, 500MHz for determination of $^{1}$H-NMR and 125MHz for determination of $^{13}$C-NMR), ESI-MS [electrospray ionization mass spectrometer (ion trap), Thermo Finnigan] for determination of molecular weight of the compounds.

Column chromatography was carried out on polyamide 6S (Riedel-De-Haen AG, Seelze Haen AG, D-30926 Seelze Hanver, Germany). Paper chromatography (PC, descending) Whatman No. 1 and 3mm papers, using solvent systems 15% HOAc (H2O–HOAc, 85:15), BAW (n-BuOH–HOAc–H2O, 4:1:5, upper layer).

The aglycone and sugar moieties were identified by complete acid hydrolysis for -*O*-glycosides followed by co-chromatography with authentic samples (Sigma) (Mabry *et al.* 1970).

**Testing the antimicrobial activity**

Disc agar diffusion method [Lis- Balchin *et.al* 1995] was used for their antimicrobial activity against certain gram positive and gram negative bacteria and fungi where pure strains of bacteria as *Staphylococcus aureus* ATCC 6538, *Bacillus subtilius* ACCT 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ACCT 27853 and *Candida albicans* were used.

All micro-organisms and antimicrobial screening were done in the Regional Center for Mycology and Biotechnology, Al-Azhar University, Nasr City, Cairo, Egypt. Fractions were the extract was dissolved in Tween 80 1 g % and impregnated on the filter paper (Whitman No. 3, 4 mm. Diameter), which were placed on the surface of nutrient agar seeded with tested microorganisms. The plates were incubated at $37^0$C for 24 hours and at $25^0$C for 72 hours to investigate the antibacterial and antifungal activities respectively, the inhibition zones were measured in mm versus standards (Gentamycine and Nystatin).

**Minimum inhibitory concentration (MIC)**

The total extract of *T. decussatus* Benth. was tested as follows; 0.5% (v/v) in tween 80 was incorporated into the agar after autoclaving to increase solubility of oil, a series dilution of oil (0.06 mg/ ml & 4 mg /ml) was prepared in nutrient agar seeded with tested microorganisms, tween 80 plates were dried at $35^o$C for 30 minutes prior to inoculation with 1-2 µl spots containing approximately $10^6$CFU/ml of each organism, the plates were incubated at $30^0$C for 48 hours. MIC were determined after 24 h for bacteria and 48 hours for *Candida albicanus*.

MICs were determined as the lowest concentration of the oil inhibiting the visible growth of each organism on the agar plate [Hammer *et.al* 1999].

***In vitro* cytotoxic screening**

0.1% w/v solution of essential oil was prepared by dissolution of tested sample in DMSO where a set of sterile test tubes was used for solution. 2.5 x $10^5$ tumor cells (EAC) / ml were suspended in phosphate buffer saline. Different concentration of essential oil (24,50,100 µg / ml) was added to the suspension and kept at $37^o$C for two hours. Tyrpan blue exclusion test [Miclimans *et. al* 1957] was carried out to calculate the percentage of non– viable cell, the results are recorded in table 3 revealed the cytotoxic activity of the extract against Hepg2 and

U 251 human cancer cell lines was preformed adopting Sulforhodamine B stain (SRB) assay [Skehan *et.al*, 1990]. Different concentration (0, 1, 2.5, 5 and 10 µg / ml) was added to the cell monolayer**.** Triplicate well treatments as well as 48 hours were interpolated from graphed- dose response data. Results are represented in table 4. Cytotoxic activities against EAC and 251 human cancer cell lines were carried out in the National Cancer Institute, Cairo University.

## RESULTS

***Salvigenin*** **(11)***:* UV _max (nm): (MeOH) 275, 333, (NaOMe) 295, 369 (sh), (AlCl3) 263, 289 (sh), 301, 357, (AlCl3/HCl) 261, 289 (sh),300, 354, (NaOAc) 280, 328, (NaOAc/H3BO3) 275, 332; 1H NMR (500 MHz, C5D5N): δ 12.77 (s, OH), 7.81 (d, 2H, *J* = 8.8Hz, H-2´,6´), 6.95 (d, 2H, *J* = 8.8 Hz, H-3´,5´), 6.57 (s, 1H, H-3), 6.53 (s, 1H, H-8), 3.93 (s, -OCH3), 3.91 (s, -OCH3), 3.84 (s, -OCH3); 13C NMR (125 Hz, C5D5N): δ 182.8 (C-4), 164.2 (C-2), 162.8 (C-4´), 158.6 (C-8), 153.6(C-9), 153.3 (C-6), 132.2 (C-7), 128.4 (C-2´,6´), 123.4 (C-1´), 114.8 (C-3´,5´), 106.6 (C-5), 104.3 (C-3), 90.8 (C-10), 60.7 (-OCH3), 56.0 (-OCH3), 55.2 (-OCH3); EI-MS *m/z*: [M+H] + 329.

**Table 1: LC/MS/MS characteristics of phenols in the negative mode.**

| Peak No. | $R_t$ | Mw +1 | MS/MS ions (*m/z* (relative abundance, %)) | Compound |
|---|---|---|---|---|
| 1 | 3.2 | 138 | 137(80%), 121(100%), | β –hydroxyl benzoic acid |
| 2 | 5,85 | 180 | 179(100%), 163(25%), 133(30%) | Caffeic acid |
| 3 | 7.85 | 248 | 248(25%), 233(100%), 215(35%) | Alloevodionol |
| 4 | 8,5 | 198 | 193(95%), 183(83%), 127(60%) | 5-hydroxyverotric acid |
| 5 | 9 | 354 | 353 (35), 191 (100) | Chlorogenic acid |
| 6 | 9.9 | 316 | 316(100), 301(80%), 273(50%) | Neptein |
| 7 | 10.2 | 314 | 314(100%), 271(50%), 269(25%) | 3,4'-Dimethoxychrysin |
| 8 | 10.75 | 194 | 193 (30), 149 (55), 134 (100) | Ferulic acid |
| 9 | 11.3 | 328 | 328(70), 287 (100%), 255(30%) | Salvigenin |
| 10 | 12 | 270 | 269 (60), 151 (100) | Apigenin |

***Apigenin*** **(12)**: UV (MeOH): 268, 337 nm; 1H NMR (600 MHz, C5D5N): δ 7.81 (2H, *d*, *J* = 8.8 Hz, H-2´ and H-6´),6.95 (2H, *d*, *J* = 8.8 Hz, H-3´ and H-5´), 6.88 (1H, *d*, *J* = 2.1 Hz, H-6), 6.68 (1H, *d*, *J* = 2.1 Hz, H-8), 6.62 (1H, *s*, H-3); 13C NMR (125 Hz, C5D5N): δ 180.8 (C-4), 164.6 (C-5), 164.2 (C-2), 162.6 (C-4´), 160.7 (C-9), 160.0 (C-7), 129.3 (C-2´ and C-6´),

123.3 (C-1´), 117.0 (C-3´ and C-5´), 109.6 (C-10), 106.5 (C-3), 104.8 (C-6), 98.9 (C-8); EIMS *m/z*: [M+H] + 271.

***Luteolin* (13):** UV (MeOH): 268 and 344 nm; 1H NMR (600 MHz, C5D5N): δ 7.56 (1H, dd, *J* = 9, 2Hz, H-6'), 7.36(1H, d, *J* = 2Hz, H-2'), 6.85 (1H, d, *J* = 9 Hz, H-5'), 6.75 (1H, s, H- 3), 6.46 (1H, d, *J* = 2 Hz, H-8), 6.28 (1H, d, *J* =2 Hz, H-6); 13C NMR (125 Hz, C5D5N): δ 181.8 (C-4), 164.3 (C-7), 164.0 (C-2), 162.1 (C-9), 157.6 (C-5), 149.7 (C-4'), 146.0 (C-3'), 120.8 (C-6'), 119.0 (C-1'), 116.8 (C-5'), 113.2 (C-2'), 103.8 (C-10), 99.2 (C-6), 94.7 (C-8); EI-MS*m/z*: [M+H] + 287.

**Chrysoeriol 7-*O*-B- D-glucopyranoside (14):** Yellow amorphous powder UV *_max* (MeOH) nm: 269, 344; IR *_max* (KBr) cm−1: 3384 (OH), 1714 (ester C=O), 1661 (-pyrone C=O), 1608 and 1508 (Arom. rings); 1H NMR (DMSO-*d6*, 500 MHz) δ δ12.52 (OH), 7.00 (br.s H-3), 6.48 (d.H-6), 6.91(d,H8),7.62 (d, H2;), 6.92( d, H5'), 3.9 (O-CH3); $^{13}$C NMR (DMSO-*d6*, 125 MHz) δ157.73 (C-2), 103.70 (C-3,),182(C-4), 161(C-5) 99.5 ( C-6), 157.07 (C-9),156.78 ( C-9),163 (C-7), 95 (C-8), 157.16 (C-9), 153.64 (C10), 122.01 (C-1'), 110.48 (C2'), 148,(C-3'), 151(C-4'), 116.63 (C-5`), 120.74 (CH, C-6`), 104.99 (C-1").71.2(C-2'), 76.3(C-3"), 67(C-4"), 76.5(C-5"), 60.4(C-6").55.8 (O-CH-3). LC-MS; *m/z* 441.

**Rutin (15):** Yellow amorphous powder [MeOH]; UV λmax (MeOH) nm: 260, 366, λmax (MeONa) nm: 273, 315, 412, λmax (AlCl3) nm: 276, 305, 439, λmax (AlCl3/HCl) nm: 275, 303, 409, λmax (AcONa) nm: 277, 320, 395, λmax (AcONa/boric acid) nm: 261, 389; 1H NMR (DMSO-*d6*, (500 MHz) δ12.52 (OH), 7.53 (1H, br s, H-2`), 7.50(1H, d, *J*=8.0 Hz, H-6`), 6.87 (1H, d, *J*=8.0 Hz, H-5`), 6.43 (1H, br s, H-8), 6.21 (1H, br s, H-6), 5.38 (1H, d, *J*=7.6 Hz, H-1``), 4.36 (1H, d, *J*=2.5 Hz, H-1```), 3.05- 3.36 (10H, m, H-2``-H-6`` of glc and H-2```-H-5``` of rha), 0.95 (1H, d, *J*=6.0 Hz, H-6``); 13C NMR (DMSO-*d6*, 125 MHz) δ177.73 (C, C-4), 164.70 (C, C-7), 161.49 (C, C-5), 157.07 (C, C-2),156.78 (C, C-9),148.84 (C, C-4`), 145.16 (C, C-3`), 133.64 (C, C-3), 122.01 (CH, C-6`), 121.48 (C, C-1`), 116.63 (CH, C-5`), 115.74 (CH, C-2`), 104.99 (C, C-10), 101.54 (CH, C-1``), 101.11 (CH, C-1```), 99.22 (CH, C-6), 94.11 (CH, C-8), 76.80 (CH, C-3``), 76.18 (CH, C-5``), 74.41 (CH, C-2``), 72.23 (CH, C- 4```), 70.92 (CH, C-3```), 70.69 (CH, C-2```), 70.36 (CH, C-4``), 68.61 (CH, C-5```), 67.40 (CH2, C-6``), 18.11 (CH3, C-6```); ESIMS *c* 633 [M+Na]+.

## DISCUSSION

***LC-MS profiling of Polyphenols in T. decussatus Benth growing in Egypt;*** The LC/DAD method previously used was modified to be compatible with the LC/MS system; acetic acid was replaced by the more volatile formic acid and its concentration was reduced to 0.1%. As a consequence, the ionic strength decreased and the signal-to-noise ratio increased in the negative ion mode.

**Table 2: Cytotoxic activity the methanol extract of *T. decussatus* Benth. growing in Egypt on liver carcinoma cell line Hepg2, brain human cell line U251 and Ehrlich ascites carcinoma cell (EAC):**

| | Conc. of extract µg / ml | % inhibition of cell viability |
|---|---|---|
| 1 | 100 | 100 |
| 2 | 50 | 100 |
| 3 | 25 | 100 |

**Table 3: *In vitro* cytotoxic activity *total extract of T. decussatus* Benth. growing in Egypt on human cancer cell – lines.**

| | Cell line | IC 50 values (µg / ml) |
|---|---|---|
| 1 | Hepg2 | 5.3 |
| 2 | U251 | 1.45 |
| 3 | EAC | 3.45 |

**Hepg2, liver carcinoma cell line, U251 brain human cell line and EAC Ehrlich ascites carcinoma cell line**.

The gradient profile used in this work allowed the separation of all the compounds studied with a retention that, in general, followed the expected reversed-phase pattern of flavonoid aglycones ten different polyphenols were identified in the methanol extract of the aerial parts of *T. decussatus* Benth. using LC-ESI/MS displays the chromatogram analysis of the methanol extract at the negative mode. The reconstructed MS spectra from the chromatogram revealed the deprotonated molecular-ions [M-H]- of four flavonoids and sex phenolic acids with the following m/z values; 316, 314, 328, and 270 belong to neptin, 3,4' dimethyl quercitrin, tri methylquercitrin, and apigenin respectively. In addition to m/z values; 138, 180, 248, 198, 354, 194 belongs to β-hydroxyl benzoic acid, caffeic acid, alloevodionol, 5-hydroxyverotric acid, chlorogenic acid and ferulic acid.

The spectra generated for cinnamic and benzoic acids by ions pray in the negative ion mode gave the de protonated molecule [M – H] and some fragments even at relatively low de clustering potentials. For instance, loss of CO2 was observed for caffeic, ferulic and β –

hydroxyl benzoic acid, giving the [M - H - 44] as a characteristic ion, ferulic acid also showed the loss of the CH3 group, providing an [M - 15] ž anion radical at *m*/*z* 178. as a characteristic ion. Ferulic acid also showed the loss of the $CH_3$ group, providing an [M - 15], anion radical at *m*/*z* 178. (P´erez-Magari *et.al* 199) chlorogenic acid showed the deprotonated molecule [M- H] (*m*/*z* 353) and the ion corresponding to the deprotonated quinic acid (*m*/*z* 191).

The flavonoids aglycones gave retro-Diels–Alder fragmentation as described by (Fabre *et al*. 2001) For instance, the *m*/*z* 151 ion is common for all the aglycones studied, the *m*/*z* 117 ion is characteristic for apigenin and its derivatives, whereas the *m*/*z* 119 ion is characteristic for naringenin and derivatives. Isorhamnetin exhibits specific fragmentation with the loss of $CH_3$ radical from the deprotonated aglycone molecule, thus giving *m*/*z* 315 *m*/*z* 300 asdescribed by Justesen [Justesen *et.al* 1998, Justesen 2001, Ferran Sa *et.al* 1998].

Thus, ESI at the negative ionization mode is more sensitive in detecting polyphenols and as lower limits of detection. The LC-MS analyses reveal that the $CH_2Cl_2$ extract of the aerial parts of *T. decussatus* Benth growing in Egypt is rich of 3,4'-Dimethoxychrysin. The molecular-ions of 3,4'-Dimethoxychrysin obtained from combining the data of the chromatograms. 3,4'-Dimethoxychrysin is reported to be the most abundant flavonoid aglycone in the *T. decussatus* Benth. The bioactivity of the methanol extract as an cytotoxic antimicrobial activates of the methanol extract of *T. decussatus* Benth revealed to 3,4'-Dimethoxychrysin.

The structures of the isolated compounds were characterized on the basis of extensive spectral studies and literature review search as: salvigenin, apigenin, luteolin**,** chrysoeriol 7-*O*-β-D-glucopyranoside and rutin [Harborne, 1994; Markham, *et al*., 1978, Wagner, *et al*., 1976, Pereira *et al.*, 2012, Dana Jari *et. al* 2015, Mohamed Ali *et. al* 2015].

The antimicrobial screening of the methanol extract of *T. decussatus* Benth growing in Egypt revealed that, it has significant antimicrobial and antifungal activities against Gram Positive *Staphylococcus aureus*, *Staphylococcus pneumoniae*, *Bacillus subtilius*, Gram negative *E.coli LQC.* and *Candida albicanus* (Table 2).

An antimicrobial activity test carried out with the methanol extract showed that the activity attributed to the presence of phenolic compounds, other constituents of the methanol extract,

such as essential pil and other compounds, could be also taken into account for their possible synergistic effect. In conclusion, this study has proven that *Thymus decussatus* Benth extract could be considered as a natural antimicrobial for treatment of skin, mouth infections cussed by *Staphylococcus aureus* and respiratory tract infection caused by *Staphylococcus pneumoniae*, *Bacillus subtilius*, *E .coli LQC.* and fungl infection caused by *Candida albicans.* This activity is possibly related to cell membrane perforation (Kizil *et.al* 2006).

The methanol extract of *T. decussatus* Benth has in vitro cytotoxic effect against Ehrlich ascites carcinoma cell (EAC), brain human cell line carcinoma (U251) and liver carcinoma cell-line carcinoma Hepg2 (Table 3,4). The activity of essential oil (antimicrobial and / or antifungal against human pathogens and cytotoxic, activity) might be attributed to the major component of the methanol extract of *T. decussatus*, (Nikoli et al. 2014), the methanol extract of *Thymus decussatus* Benth, is recommended for pharmaceutical and cosmetic uses.

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