Phytochemical and Biological Studies on the Different Organs of *Tanacetum parthenium* L. Cultivated in Egypt

Rateb MEM (Ph.D.)[1]*, El-Gendy AAM (Ph.D.)[2], El-Hawary SS (Ph.D.)[3], El - Shamy AM (Ph.D.)[3]

1- Pharmacognosy Department, Faculty of Pharmacy, Beni-Sueif University, Egypt
2- Pharmacology Department, Faculty of Veterinary Medicine, Beni - Sueif University, Egypt
3- Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt
*Corresponding author: Pharmacognosy Department, Faculty of Pharmacy, Beni-Sueif University, Egypt.
E-mail: mostafa19772002@yahoo.com

Receive: 22 Jan. 2008 **Acceptance:** 4 Mar. 2008

Abstract

Phytochemical investigation of *Tanacetum parthenium* L. family Asteraceae cultivated in Egypt resulted in isolation and identification of four flavonoids. One surface flavonoid was isolated from the fresh leaves and three other flavonoids were isolated from the flower heads. Four sesquiterpene lactones were isolated from the leaves. Two sterols were isolated from the roots. A comparative study of the essential oil content of the leaves and the flower heads was performed using GC/MS. The results revealed the presence of 42 and 30 components in the leaves and the flower heads oil samples, respectively. The major components of both oil samples were camphor (constituted 37.7% and 48.4%) and chrysanthenyl acetate (constituted 33.8% and 26.3%) in the leaves and the flower heads, respectively. Different extracts of *T. parthenium* L. showed significant biological activities. Analgesic, anti-inflammatory, antipyretic, antispasmodic and uterine-stimulant activities were studied in addition to the *in vitro* cytotoxic effect.

Keywords: *Tanacetum parthenium*, Santin, Parthenolide, Camphor, Chrysanthenyl acetate, Analgesic, Anti - inflammatory, Antipyretic, Antispasmodic, Uterine stimulant and cytotoxic

[DOR: 20.1001.1.2717204.2008.7.25.15.1]

Introduction

Genus *Tanacetum* includes about 50 species, of those only *T. santolinoides* D.C. grows in Egypt [1]. Feverfew, *T. parthenium* (L.) has been introduced to Egypt to study some of its traditional uses in medicine viz.; treatment of fever, headache, migraine, stomachache, insect bites, bronchitis, arthritis, cold, abortifacient and problems of menstruation [2-4]. *T. parthenium* L. has other synonyms viz.; *Chrysanthemum parthenium* L., *Leucanthemum parthenium* L. and *Pyrethrum parthenium* L. and many common names viz.; Feverfew, Featherfew, Featherfoil, Febrifuge plant, Midsummer daisy and Santa Maria [4-6]. Feverfew herb originated in the Balkan Peninsula, but found growing in Europe, China and Japan. Feverfew cited to contain many active ingredients and many biological activities [4-7]. Its fresh leaves are included in salad in many countries for its antioxidant, melatonin and other important constituents [4-6]. Since the environmental conditions have a great effect on the production of secondary metabolites, the present work includes phytochemical investigation of sesquiterpene lactones content, flavonoid content, volatile oil and lipid content of different organs of the plant cultivated in Egypt. Feverfew reported to contain many sesquiterpene lactones as a major class of secondary metabolites of which parthenolide is considered the major active constituent of the plant [4-8]. The number and type of the sesquiterpene lactones produced by *T. parthenium* L. differed greatly according to the environmental conditions [9]. In addition, some biological activities were investigated including the analgesic, anti-inflammatory and antipyretic activities. The antispasmodic and uterine stimulant effects were screened for the first time and the cytotoxic effect was also supplied.

Experimental

1. Plant material

Seeds of *T. parthenium* L. used in this study were imported from Schifeild Co., U.S.A. and cultivated in El-Basateen farm, Beni-Suef, Egypt. The plant was air-dried, reduced to fine powder and kept in tightly closed amber colored glass containers.

2. Material for phytochemical study

Different plant organs were air dried, powdered and kept in tightly closed amber colored glass containers and protected from light at low temperature.

A. Material for volatile oil study

The leaves and the flower heads of the plant were collected in April (in flowering) and used just after collection in preparation of its volatile oil content.

B. Material for chromatography:

Silica gel H type 60 for VLC (E. Merck), silica gel (100-200 mesh) for CC, precoated TLC plates (silica gel 60 GF_{254}) from E. Merck (Darmstadt, Germany), Sephadex LH20 (Pharmacia Fine Chemicals, Sweden).

C. Spray reagents:

P-anisaldehyde-sulfuric acid spray reagent [10] for sterols, Aluminum chloride spray reagent [11] for flavonoids.

D. Reagents for UV spectroscopic analysis of flavonoids [11]

Sodium methoxide solution, aluminum chloride solution, hydrochloric acid, sodium acetate powder and boric acid powder.

3. Material for biological study

Plant extracts

a) Alcoholic extracts of different plant organs were prepared and a 20% w/v concentration was supplied.

b) Aqueous extracts of different plant organs were prepared and a 20% (w/v) concentration was supplied.

c) Parthenolide was obtained from the VLC purification of the chloroformic extract of the leaf and a 5% (w/v) solution was prepared.

d) A portion of each of the extracts of different organs of the plant (2 mg) as well as parthenolide was dissolved in 2ml dimethyl sulfoxide to give a concentration of 100ug/0.1ml for cytotoxic study.

Animals

Albino mice of 20-25g b. wt., adult female albino rats of 100-125g b. wt., adult albino rabbits of 1-2 kg body weight. Cancer cell line (Ehrlich ascitis carcinoma) obtained from National Cancer Institute, Kasr El-Ainy, Cairo, Egypt.

Reference drugs and kits

Acetylcholine chloride (H and W, England), nicotine hydrogen tartarate (BDH, England), atropine sulphate (BDH, England).

Perfusion fluids for isolated tissue preparations

The physiological salt solutions (Tyrod's solution and Dejalon's solution) were prepared according to the Staff members of pharmacology department, University of Edinburgh [12].

4. Apparatus:

Jeol mass spectrophotometer, 70 eV. (Finnigan mat SSQ 7000); NMR Jeol GLM: Jeol TMS route instrument (^{1}H-NMR, 300 MHz, ^{13}C, 75 MHz). The NMR spectra were recorded in $CDCl_3$ and CD_3OD using TMS as internal standard and chemical shift values were recorded in δ ppm; UV-visible spectrophotometer: Shimadzu UV 240 (P/N 204-58000) was used for measuring the absorbance in UV range; GC/MS: Hewlett Packard 5890 series II plus GLC instrument with flame ionization detector (FID), equipped with Hewlett Packard MS5970, for analysis of volatile oil samples (Carbowax 20M column with 50 m x 0.2 mm internal diameter and helium as a carrier gas with flow rate of 1ml/min. The Oven temperature was 60ºc, injector temperature was 200ºc, detector temperature was 250ºc and using teperature programming starting with 60ºc increased to 200ºc by rate of 3ºc/min. and split ratio of 100: 1); Specific gravity bottle for determination of specific gravity of the essential oils; Abbe's refractometer for measuring the refractive indices of the volatile oils samples; Koffler's heating stage microscope for determination of melting points; palmer kynograph and organ bath.

Methods

Phytochemical study

1. Investigation of sesquiterpene lactones content [8]

The air-dried defatted powdered leaves (3 Kg) were macerated in 90% ethanol. After stripping- off the solvent, the residue (400 g) was extracted with chloroform, evaporated to a dark residue (60 g) and chromatographed on silica gel VLC. Isocratic elution was performed by chloroform until exhaustion. The combined chloroformic extract (4 g) was chromatographed on a silica gel 60 column using n-hexane and increasing the polarity by chloroform till 100% chloroform then increasing the polarity of chloroform by ethyl acetate. Fractions were collected and examined by TLC using the systems (S: chloroform:ethyl acetate 8:2 v/v) and *p*-anisaldehyde / sulfuric acid as a chromogenic spray. Fractions 51-60 (600 mg) were fractionated on silica gel 60 CC using n-hexane and increasing the polarity using ethyl acetate. Fractions (7 – 12) were pooled and recrystallization from petroleum ether:diethylether (1: 2) afforded compound L_1 as colorless crystals (450 mg). Fractions (14-16) were pooled and recrystallization from chloroform afforded compound L_2 as colorless crystals (25 mg). Fractions 63-67 (120 mg) were purified on silica gel 60 CC where fractions (8 – 12) were pooled and recrystallization from chloroform afforded compound L_3 as colorless crystals (20 mg) Fractions 69-71 (100 mg) were purified on silica gel 60 CC where fractions (15 – 18) were pooled and recrystallization from methanol afforded compound L_4 as colorless sticky substance (5 mg). The isolated compounds were subjected to physico-chemical investigation viz; melting point, IR, ^{1}H-NMR and EIMS.

2. Investigation of the flavonoid content

a. Isolation of leaf surface flavonoids [13]

2 kg of fresh leaves were dipped briefly in 4L of acetone. After stripping-off the solvent under reduced pressure, the yellowish green residue (2 gm) was chromatographed on silica gel 60 column using n-hexane and increasing the polarity using ethyl acetate. The factions were collected and examined by TLC using the solvent system chloroform:methanol (9: 1). Fractions (38 – 45) were pooled and evaporated to a dark yellow residue (120 mg) which was purified on Sephadex LH_{20} column using 80% methanol for elution. Fractions (10-18) were pooled and evaporated to yield compound F_1. Recrystallization from methanol afforded compound F_1 as yellow crystals (90 mg).

b. Investigation of the flavonoid content of the flower heads

500 g of air-dried defatted powdered flower heads was extracted by 90% ethanol and the combined ethanolic extract was evaporated to a dark green residue (45 g) which was extracted with chloroform till exhaustion and evaporated to a semisolid residue (12 g) which was chromatographed on silica gel VLC using chloroform and increasing the polarity by ethyl acetate. Fractions (13-14) were pooled and evaporated to a yellow residue (300 mg) and purified on Sephadex LH_{20} using 90% methanol as eluent. Similar fractions were pooled and evaporated to dryness. Recrystallization of fractions (11-18) from methanol gave compound F_2 (25 mg) as yellow powder. Recrystallization of fractions (21-26) from methanol gave compound F_3 (15 mg) as yellow powder. Recrystallization of fractions (30-36) from methanol gave compound F_4 (6 mg) as yellow powder. All the isolated compounds were subjected to physicochemical investigation viz.; UV spectral data with different shift reagents, ^{1}HNMR and EI/MS.

3. Investigation of the lipid content

The unsaponifiable matter [14] prepared from 500g of air-dried powdered roots (2 g) was chromatographed on silica gel 60 column using n-hexane and increasing the polarity using ethyl acetate. The fractions were collected and examined by TLC using the solvent system petroleum ether:ethyl acetate (6 : 4) and similar fractions were pooled. Fractions (14-20) were pooled and evaporated to a yellowish white residue (250 mg) which was purified on silica gel 60 column using petroleum ether and increasing the polarity using ethyl acetate where fractions (7-12) were collected and evaporated under reduced pressure to produce compound T_1.Recrystalization from chloroform yielded compound T_1 (95 mg) as white crystals. Fractions (22-25) were pooled and left to evaporate at room temperature to produce compound T_2 (70 mg) as white crystals. The structures of these isolated compounds were established on the basis of physicochemical data and EI/MS.

Investigation of the volatile oil content

500g of fresh leaves and fresh flowers were subjected to hydrodistillation and the two samples of the essential oils obtained were separately dried over anhydrous sodium sulfate and stored in refrigerator for further GC/MS analysis. Percentage yield, specific gravity and refractive index of the oil samples were determined according to the E.P. (1984) [15]. Qualitative analysis was carried out for identification of the oil components by their retention times and interpretation of their mass spectra with available reference materials [16] and quantitative analysis of the identified components was carried out by using internal normalization method.

Biological study:

1. Analgesic activity

The analgesic activity was evaluated using acetic acid-induced writhing in mice as described by *Taber, et al* [17] and paracetamol (50 mg/kg b.wt. orally) used as a standard. Mice weighing 20-25g were orally administered both the ethanolic and aqueous extracts in a dose of 1g/kg body weight and parthenolide in a dose of 10mg/kg body weight.

2. Anti-inflammatory activity:

Acute anti-inflammatory effect was evaluated using yeast-induced rat paw edema test described by *Winter, et al* [18] and diclofenac sodium (10 mg/kg body weight) as a standard. Male albino rats, weighing 100-125 g were orally given both the ethanolic and aqueous extracts in doses of 1g/kg body weight and parthenolide in doses of 10mg/kg body weight.

3. Antipyretic activity

The antipyretic activity was evaluated using yeast-induced hyperthermia method as described by *Teotine, et al* [19] and acetyl salicylic acid (50 mg/kg body weight) as a standard. Female albino rats, weighing 100-125 g were orally administered the ethanolic and aqueous extracts in doses of 1g/kg body weight and parthenolide in doses of 10mg/kg body weight.

4. Antispasmodic activity

The method described by Staff members of pharmacology department, University of Edinburgh [12] was used. A piece of 3cm of rabbit duodenum was suspended in organ bath of containing oxygenated Tyrod's solution at 37°C. The normal intestinal motility was recorded. Then different concentrations of alcoholic and aqueous extracts (2-8 mg /ml bath) of different plant organs as well as parthenolide (0.04-0.16 mg/ml bath) were added and the response was recorded for studying their effect on the intestinal motility.

5. Uterine stimulant activity

The method described by Dejalon and Dejalon [20], was used. The animals were killed; a length of 3 cm of uterine horn was mounted in organ bath containing oxygenated Dejalon's solution at 32°C. After recording the normal uterine contractions, the effect of different concentrations of alcoholic and watery extract (2-8 mg /ml bath) of different plant organs as well as parthenolide (0.04-0.16 mg/ml bath) were added and the response was recorded for studying their effect on uterine contractions.

6. *In vitro* cytotoxic activity

The tested solutions were screened using a single tumor cell (Ehrlich ascitis carcinoma cells). A set of sterile test tubes were used, where 2.5×106 tumor cells per ml were suspended in phosphate buffer. One tenth ml of different dilutions of the tested solutions in DMSO was added separately to the suspension and kept at 37°c for 24 hours. Trypan blue dye exclusion test [21] was carried out to calculate the percentage of non-viable cells using a dose of 100ug/ml, 50ug/ml and 25ug/ml of each extract.

Results and Discussion

Phytochemical study

Compound L_1: 350 mg; colorless crystals; soluble in chloroform; m.p.115-117 °C; R_f: system S_1, 0.66; IR (ν_{max} cm^{-1}): 2976-2859 (CH-stretching vibration), 1756 (C=O of lactone ring), 1657 (α,β-unsaturated C=O), 1441, 1291, 1145, 981 and 944; ^{1}H-NMR ($CDCl_3$) δ ppm, 6.28 (1H, d, J=3.6, H_{13}b), 5.95 (1H, d, J=3, H_{13}a), 5.2 (1H, m, H_1), 3.8 (1H, t, H_6), 2.7 (2H, d, J=9, H_5 and H_7), 2.32-2.37 (2H, m, H_2), 2.09-2.15 (6H, m, H_3, H_8 and H_9), 1.69 (3H, s, 14-Me) and 1.28 (3H, s, 15-Me); EIMS m/z, 248 (M^+, 10%), 230 (M^+-H_2O, 35%), 190 (M^+-C_3H_6O, 28%), 159 (M^+-C_3H_4O-CH_3-H_2O, 55%), 145 (50%), 117 (40%), 105 (55%), 79 (80%), 53 ($C_4H_5^+$, 100%).

Compound L_2: 15 mg; colorless crystals; soluble in chloroform; m.p.242-244 °C; R_f: system S_1, 0.58; IR (ν_{max} cm^{-1}): 3448 (OH group), 2933-2864 (CH-stretching vibration), 1756 (C=O of lactone ring), 1656 (α,β-unsaturated C=O), 1463, 1367, 1293, 1141 and 991; ^{1}H-NMR ($CDCl_3$) δ ppm, 6.33 (1H, d, J=3.6, H_{13}b), 5.61 (1H, d, J=3, H_{13}a), 3.8-4 (2H, m, H_1 and H_6), 2.8 (1H, d, J=8.7, H_3), 2.4-2.5 (3H, m, H_2 and H_7), 2.1-2.2 (5H, m, H_5, H_8 and H_9), 1.39 (3H, s, 15-Me) and 1.34 (3H, s, 14-Me); EIMS m/z, 264 (M^+, 5%), 246 (M^+-H_2O, 10%), 230 (35%), 190 (30%), 149 (50%), 123 ($C_9H_{15}^+$, 100%), 105 (55%), 91 (70%) and 53 (85%).

Compound L_3: 20 mg; colorless crystals; soluble in chloroform; m.p. 134-136 °C; R_f: system S_2, 0.42; IR (ν_{max} cm^{-1}): 3451 (hydroxyl group), 2925-2859 (CH-stretching vibration), 1767 (C=O of lactone ring), 1633 (α,β-unsaturated C=O), 1455,1387, 1221, 1139 and 977; ^{1}H-NMR ($CDCl_3$) δ ppm, 6.32 (1H, d, J=3.6, H_{13}b), 5.6 (1H, d, J=3.3, H_{13}a), 5.2 (1H, m, H_1), 4.1 (1H, m, H_3), 3.8 (1H, t, H_6), 2.77 (2H, d, J=9, H_5 and H_7), 2.34-2.39 (2H, m, H_2), 2.15-2.2 (4H, m, H_8 and H_9), 2.1 (3H, s, 14-Me) and 1.3 (3H, s, 15-Me); EIMS m/z, 264(M^+, 20%), 246 (M^+-H_2O, 5%), 230 (10%), 190 (45%), 159 (30%), 105 (55%), 91 (85%) and 55 ($C_4H_7^+$, 100%).

Compound L_4: 5 mg; colorless sticky substance; soluble in methanol; R_f: system S_1; 0.27; IR (ν_{max} cm^{-1}): 3425 (OH group), 2958-2857 (CH-stretching vibration), 1729 (C=O of lactone ring), 1631 (α,β-unsaturated C=O), 1461, 1279, 1126 and 1057; ^{1}H-NMR ($CDCl_3$) δ ppm, 6.2 (1H, d, J=3.3, H_3), 6.1 (1H, d, J=3.3, H_{13}b), 5.7 (1H, d, J=3.3, H_2), 5.5 (1H, d, J=3.3, H_{13}a), 4.06-4.13 (1H, m, H_6), 3.68-3.82 (1H, m, H_7), 2.9 (1H, d, J=9.3, H_5), 2.4-2.6 (2H, m, H_9), 1.8-2 (2H, m, H_8), 1.46 (3H, s, 14-Me) and 1.29 (3H, s, 15Me); EIMS m/z, 278 (M^+, 15%), 260 (M^+-H_2O, 25%), 190 (30%), 159 (55%), 112 (40%), 105 (55%), 94 (90%), 79 (80%) and 55 ($C_4H_7^+$, 100%).

Data of compound F_1: Yellow crystals (60mg), m.p. 162-164°c, soluble in chloroform, R_f = 0.66 in system chloroform: methanol (9:1). UV spectral data: (λ_{max}, nm) (table 1). EI/MS (70 eV. Rel.int.): 344 (M^+,

Table 1. The ultraviolet spectral data (nm) of compounds F_1-F_4

Comp.	MeOH	NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAc.	NaOAc. /H_3BO_3
F_1	269 295 sh 341	274 371	280 355 sh 390	282 354 sh 391	274 298 sh 372	270 302 sh 341
F_2	268 303 sh 335	270 324 390	276 301 sh 348 382	280 300 sh 340 380	274 303 sh 369	268 302 sh 338
F_3	254 267 sh 349	266 329 sh 401	274 301 sh 328 426	275 295 sh 355 385	269 327 sh 384	259 302 sh 370
F_4	255 269 sh 301 sh 370	270 320 sh 415	272 304 sh 333 458	267 301 sh 359 428	257 sh 274 328 392	261 303 sh 388

100%), 301 (M^+-CH_3CO, 60%), 183 (15%), 135 (60%), 165 (30%) and 155 (35%). ^{1}HNMR (δ , $CDCl_3$): 8.06 (2H, d, J=9 Hz, H2' and H6'), 7.05 (2H, d, J=9 Hz, H3' and H5'), 6.5 (1H, s, H8), 4.05 (3H, s, 4'-OMe), 3.9 (3H, s, 3-OMe) and 3.8 (3H, s, 6-OMe).

Data of compound F_2: Yellow powder (15mg), m.p. 348-350°c, soluble in methanol, R_f = 0.48 in system chloroform : methanol (9:1). UV spectral data: (λ_{max}, nm) (table 1). EI/MS (70 eV. Rel.int.): 270 (M^+, 100%), 242 (M^+-CO, 36%), 213 (12%), 152 (30%), 124 (32%), 118 (11%) and 69 (20%).^{1}HNMR (δ , $CDCl_3$): 7.8 (2H, d, J=9 Hz, H2' and H6'), 6.9 (2H, d, J=9 Hz, H3' and H5'), 6.5 (1H, s, H3), 6.4 (1H, d, J=2.1 Hz, H8) and 6.2 (1H, d, J=2.1 Hz, H6).

Data of compound F_3: Yellow powder (9mg), m.p. 328-330°c, soluble in methanol, R_f = 0.4 in system chloroform:methanol (9:1). UV spectral data: (λ_{max}, nm) (table 1). EI/MS (70 eV. Rel.int.): 286 (M^+, 100%), 258 (M^+-CO, 35%), 153 (52%), 134 (25%) and 69 (25%). ^{1}HNMR (δ, $CDCl_3$): 7.3 (2H, m, H2' and H6'), 6.8 (1H, d, J=9 Hz, H5'), 6.5 (1H, s, H3), 6.4 (1H, d, J=2.1 Hz, H8) and 6.2 (1H, d, J=2.1 Hz, H6).

Data of compound F_4: Yellow powder (6mg), m.p. 315-317°c, soluble in methanol, R_f = 0.28 in system chloroform:methanol (9:1). UV spectral data: (λ_{max}, nm) (table 1). EI/MS (70 eV. Rel.int.): 302 (M^+, 100%), 273 (M^+-CHO, 15%), 153 (30%), 137 (34%) and 69 (38%). ^{1}HNMR (δ, $CDCl_3$): 7.6 (2H, m, H2' and H6'), 6.8 (1H, d, J=8.4 Hz, H5'), 6.3 (1H, d, J=1.8 Hz, H8) and 6.1 (1H, d, J=1.8 Hz, H6).

Data of compound T_1: White crystals (55 mg), m.p. 197-199°c, freely soluble in chloroform. R_f = 0.86 in petroleum ether: ethyl acetate (6:4), EI/MS (70 eV, rel. int.) 426 (M^+, 10%), 218 (M^+-"$C_{14}H_{24}O$", 100%), 203 (30%) and 189 (18%), 95 (15%), 69 (18%) and 55 (25%).

Data of compound T_2: White crystals (70 mg), m.p. 140-142°c, freely soluble in chloroform. R_f = 0.65 in petroleum ether: ethyl acetate (6:4), EI/MS (70 eV, rel. int.) 414 (M^+, 29%), 396 (M^+-H2O-2H, 100%), 382 (20%), 275 (18%), 255 (50%), 213 (40%), 159 (55%), 145 (80%) and 55 (93%).

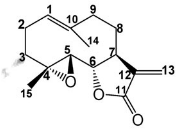
From the physicochemical and spectral data, as well as, comparison with the authentic references and published data [8, 22, 23, 24, 25], compounds L_1-L_4 could be identified as parthenolide, epoxysantamarin, 3β-hydroxyparthenolide and secotanapartholide A, respectively.

From the physicochemical and spectral data, as well as comparison with the authentic references and published data [26-30], compounds F_1-F_4 could be identified as santin, apigenin, luteolin and quercetin, respectively. Compound F_4 was first isolated from *T. parthenium* (L.) but isolated from other *Tanacetum* species [31].

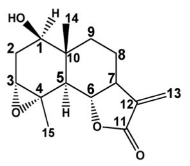
From the physicochemical and spectral data, as well as comparison with the published data [30, 32], compounds T_1 and T_2 could be identified as β-amyrin and β-sitosterol, respectively.

Investigation of the volatile oil content

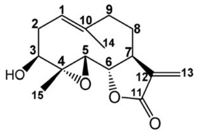
The percentage of essential oil obtained by hydrodistillation from the two plant samples, the physical characters of each sample, and refractive indices of the two tested oils at 20 °c and the specific gravity of each at 25 ° c were compiled in the following table (2). The percentage yield of the oil was slightly different from the previously reported data [33, 34]. Qualitative analysis was carried out for identification of the oil components by their retention times and interpretation of their mass spectra with available reference materials [16] and quantitative analysis of the identified components was carried out by using internal normalization method.



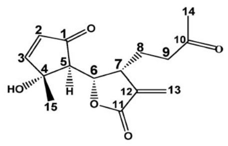
L_1



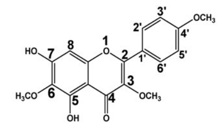
L_2



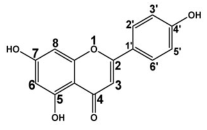
L_3



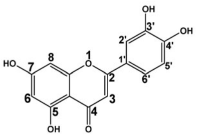
L_4



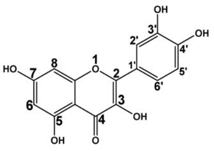
F_1



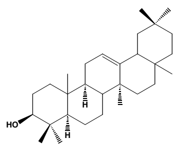
F_2



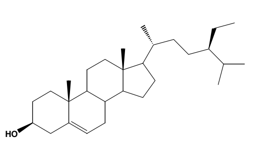
F_3



F_4



T_1



T_2

Table 2. **Physical characters of the isolated essential oil samples**

Plant organ	% yield V/dry W.	Physical constants			
		Color	Odor	Refractive index	Specific gravity
Leaf	0.49	yellow	Camphor like	1.4662	0.8912
Flower	0.58	Pale yellow	odor	1.4697	0.9016

Table (3) revealed that the essential oil isolated from the leaves and the flowers of the plant comprises 42 and 30 identified components constituting 99.56% and 98.87% of the total oil composition in which camphor is the major constituent (37.7% and 48.4%) followed by chrysanthenyl acetate (33.8% and 26.3%) of the total oil composition, respectively. The total oxygenated compounds constituted the highest percentage of the components in the essential oil of different organs of the plant. They constitute 85.84% and 85% in the oil of leaves and flowers, respectively. The difference between the obtained data and the reported data (33,34) concerning the difference in components and relative percentages of the different oil constituents in the investigated essential oil of *T. parthenium* (L.) may be due to environmental conditions under which the plant has grown as well as the variation in conditions of analysis.

Biological study

Analgesic activity: Results presented in table (4) revealed that parthenolide showed significant analgesic activity (about 85-90% of the activity of paracetamol) followed by the alcoholic extracts of flowers and leaves (about 55% and 45% of the activity of paracetamol, respectively). All other groups showed non significant analgesic activity.

Anti-inflammatory activity: Results presented in table (5) revealed that the alcoholic extracts of leaves and flowers and parthenolide showed significant anti-inflammatory activity (about 65-75 % of the activity of diclofenac sodium after 3 hours) and (about 55-60 % of the activity of diclofenac sodium after 6 hours). All other groups showed non significant anti-inflammatory activity.

Antipyretic activity: Results presented in table (6) revealed that parthenolide showed significant antipyretic activity (about 60-70% of the activity of acetyl salicylic acid), followed by the alcoholic extracts of flowers and leaves which showed significant antipyretic activity (40-55% of ASA activity). All other groups showed non significant antipyretic activity.

Antispasmodic activity (fig. 1): The alcoholic extract of the flowers was the most potent antispasmodic producing prolonged inhibition of intestinal motility, followed by the leaf extract. Parthenolide has produced moderate but not complete inhibition even at its high dose. All the aqueous extracts produced spasmogenic effect by increasing the intestinal motility. Concerning the site of action, the alcoholic extracts as well as parthenolide have performed their antispasmodic effect by ganglion blockade, while the aqueous extracts have performed their spasmogenic effect through stimulation of cholinergic nerve fibers.

Uterine stimulant activity (fig. 2): The alcoholic extract of the flower was the most potent uterine stimulant followed by the leaf extract. The root extract was not effective. Parthenolide had mild uterine stimulant effect which was very short. The aqueous extract of the flower was potent uterine stimulant followed by the leaf extract. The root extract was not effective on uterus.

***In vitro* cytotoxic activity:** From table (7), it could be concluded that all extracts showed activity against Ehrlich ascitis carcinoma cell line except the aqueous extract of the root. The alcoholic extract of the flower and parthenolide were the most potent, showed 100% inhibition of cell viability at their

Table 3. GC/MS analysis of the organs of *Tanacetum parthenium* L.

P. No.	Rt. min.	Constituents of the oil	M. Wt.	Percentage	
				Leaf	**Flower**
1	2.04	Cis-2-octene	112	---	0.07
2	2.67	Butyl acetate	116	0.78	0.88
3	3.51	Tricyclene	136	0.17	0.37
4	3.67	α-pinene	136	1.35	1.03
5	4.25	Camphene	136	3.72	8.76
6	4.87	Sabinene	136	0.17	0.45
7	5.09	β-pinene	136	0.21	0.22
8	5.86	β-myrecene	136	0.06	---
9	5.96	*P*-cymene	134	0.62	0.12
10	6.71	Limonene	136	0.29	0.12
11	7.19	β-phellandrene	136	0.54	---
12	7.94	1,8-cineole	154	1.14	0.6
13	8.66	γ-terpinene	136	3.1	1.2
14	12.37	Terpinolene	136	0.13	---
15	13.72	P-cymenene	132	0.09	0.16
16	15.54	Terpin-1-ol	154	5.14	0.92
17	17.47	Camphor	152	37.7	48.4
18	18.21	E-chrysanthenyl acetate	194	33.8	26.3
19	18.68	Pinocarvone	150	0.49	---
20	19.26	Borneol	154	0.37	0.2
21	19.94	Terpin-4-ol	154	0.66	0.26
22	20.43	α-terpineol	154	0.64	0.76
23	20.68	Linalool acetate	196	0.34	0.58
24	21.75	Bornyl acetate	196	0.15	---
25	22.18	Citronellal hydrate	172	0.62	---
26	22.66	Thymol	150	0.39	1.81
27	23.16	E-pinocarvyl acetate	194	0.09	0.81
28	23.41	Carvacrol	150	0.54	---
29	23.64	α-copaene	204	0.12	---
30	24.12	E-caryophyllene	204	0.19	0.28
31	24.43	α-humulene	204	0.41	---
32	24.8	E-B-farnesene	204	0.96	---
33	25.66	Germacrene D	204	0.11	0.21
34	26.33	Ar-curcumene	202	0.18	---
35	26.75	Isobornyl-2-methyl butyrate	238	0.35	---
36	27.89	Σ-cadinene	204	0.18	---
37	28.44	Z-chrysanthenyl angelate	234	0.23	0.68
38	30.78	Bornyl angelate	236	2.17	1.81
39	31.72	Germacrene D-4-ol	222	---	0.59
40	34.7	β-caryophyllene oxide	220	0.54	0.37
41	41.38	Globulol	222	0.15	0.45
42	43.03	Veridiflorol	222	0.27	---
43	43.61	E,E-farnesol	222	0.23	0.26
44	45.05	E,E-farnesyl acetate	264	0.18	0.19

*Rt = retention time, M. Wt. is the molecular weight.

[DOR: 20.1001.1.2717204.2008.7.25.15.1]

Table 4. **Analgesic activity of different extracts and parthenolide of *Tanacetum parthenium* L. on acetic acid induced writhing in mice (n = 5)**

Treatment	Mean number of writhing			
	After 1 hour	**After 2 hours**	**After 3 hours**	**After 5 hours**
Control	15.2 ± 0.16$^{(a)}$	15.2 ± 0.19$^{(a)}$	16 ± 0.2$^{(a)}$	15.6 ± 0.18$^{(a)}$
Paracetamol	0.4 ± 0.13$^{(c)}$ *97 %	1.4 ± 0.11$^{(e)}$ *90 %	1.8 ± 0.16$^{(e)}$ *88 %	4.4 ± 0.15$^{(d)}$ *71 %
Hydroalcoholic extract of leaves	7.4 ± 0.17$^{(b)}$ *51 %	6.6 ± 0.18$^{(c)}$ *56 %	6.9 ± 0.19$^{(c)}$ *57 %	11.4 ± 0.14$^{(b)}$ *27 %
Hydroalcoholic extract of flowers	6.8 ± 0.16$^{(b)}$ *55 %	5.9 ± 0.17$^{(c)}$ *61 %	5.8 ± 0.16$^{(c)}$ *63 %	11 ± 0.14$^{(b)}$ *29 %
Hydroalcoholic extract of roots	15 ± 0.14$^{(a)}$ *1.3 %	15 ± 0.19$^{(a)}$ *1.3 %	15 ± 0.14$^{(a,b)}$ *6.2 %	15.2 ± 0.16$^{(a)}$ *2.5 %
Aqueous extract of leaves	14.6 ± 0.17$^{(a)}$ *3.9 %	13.9 ± 0.16$^{(b)}$ *8.5 %	14.8 ± 0.16$^{(b)}$ *7.5 %	15 ± 0.19$^{(a)}$ *3.8 %
Aqueous extract of flowers	14.7 ± 0.19$^{(a)}$ *3.8 %	13.4 ± 0.22$^{(b)}$ *11.8 %	14.4 ± 0.11$^{(b)}$ *10 %	14.8 ± 0.16$^{(a)}$ *5 %
Aqueous extract of roots	15.1 ± 0.2$^{(a)}$ *0.6 %	15.1 ± 0.16$^{(a)}$ *0.6 %	15.4 ± 0.12$^{(a,b)}$ *3.7 %	15.4 ± 0.11$^{(a)}$ *1.3 %
Parthenolide	1 ± 0.14$^{(c)}$ *93 %	3.2 ± 0.14$^{(d)}$ *79 %	4.3 ± 0.17$^{(d)}$ *73 %	9.4 ± 0.2$^{(c)}$ *40 %
LSD	1.16	1.2	1.13	1.05

(a,b,c,d,e) means with the same letters in each column are not significantly different at $p < 0.05$.
* Percentage decrease in number of writhing in comparison with control (mean ± SE).

Table 5. **Anti-inflammatory activity of different extracts and parthenolide of *Tanacetum parthenium* L. (n = 5)**

Treatment	Mean decrease in thickness of rat paw (mm.)	
	After 3 hours	**After 6 hours**
Control	7.7 ± 0.06$^{(a)}$	7.8 ± 0.04$^{(a)}$
Diclofenac sodium	5.2 ± 0.06$^{(d)}$ *32.5 %	4.5 ± 0.06$^{(d)}$ *42 %
Hydroalcoholic extract of leaves	6.4 ± 0.04$^{(c)}$ *17 %	6.2 ± 0.04$^{(c)}$ *20.5 %
Hydroalcoholic extract of flowers	6.2 ± 0.05$^{(c)}$ *19.5 %	6.1 ± 0.03$^{(c)}$ *21.7 %
Hydroalcoholic extract of roots	7.5 ± 0.07$^{(a)}$ *2.5 %	7.5 ± 0.06$^{(a)}$ *3.8 %
Aqueous extract of leaves	7.5 ± 0.04$^{(a)}$ *2.5 %	7 ± 0.07$^{(b)}$ *10.2 %
Aqueous extract of flowers	7.1 ± 0.03$^{(b)}$ *7.7 %	6.8 ± 0.04$^{(b)}$ *12.8 %
Aqueous extract of roots	7.5 ± 0.06$^{(a)}$ *2.5 %	7.5 ± 0.05$^{(a)}$ *3.8 %
Parthenolide	6.3 ± 0.04$^{(c)}$ *18 %	6 ± 0.03$^{(c)}$ *23 %
LSD	0.38	0.37

(a,b,c,d) means with the same letters in each column are not significantly different at $p < 0.05$.
* Percentage decrease in thickness of paw in comparison with control (mean ± SE).
LSD (least significant difference between means) calculated by one way ANOVA.

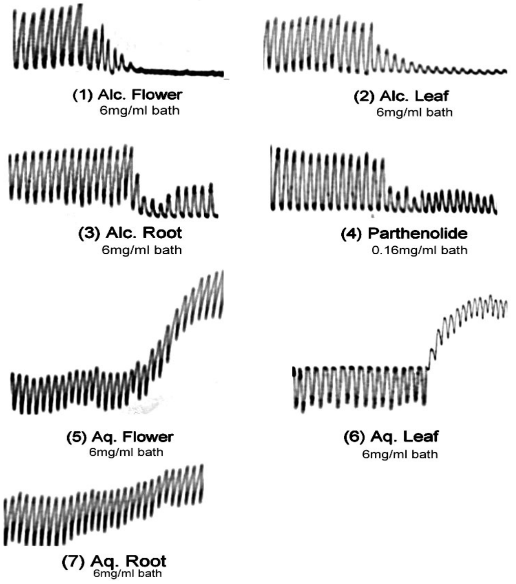
Table 6. **Antipyretic activity of different extracts and parthenolide of *Tanacetum parthenium* L. on yeast induced hyperpyrexia in rats (n = 5):**

Treatment	**Mean decrease in rat rectal temperature (°C)**			
	After 1 hr.	**After 2 hrs.**	**After 3 hrs.**	**After 4 hrs.**
Control	39.7±0.07$^{(a)}$	39.6±0.02$^{(a)}$	39.5±0.06$^{(a)}$	39.5±0.04$^{(a)}$
Acetyl salicylic acid (ASA)	37.7±0.05$^{(d)}$ *5 %	37.3±0.05$^{(f)}$ *5.8 %	37.7±0.05$^{(d)}$ *4.5 %	38.2±0.07$^{(d)}$ *3.3 %
Hydroalcoholic extract of leaves	38.7±0.06$^{(b)}$ *2.5 %	38.7±0.02$^{(c)}$ *2.3 %	38.8±0.04$^{(b)}$ *1.7 %	39.2±0.04$^{(b)}$ *0.7 %
Hydroalcoholic extract of flowers	38.5±0.06$^{(b)}$ *3 %	38.3±0.04$^{(d)}$ *3.3 %	38.7±0.04$^{(b)}$ *2 %	39.1±0.02$^{(b)}$ *1 %
Hydroalcoholic extract of roots	39.5±0.06$^{(a)}$ *0.5 %	39.6±0.04$^{(a)}$ *0 %	39.5±0.04$^{(a)}$ *0 %	39.5±0.06$^{(a)}$ *0 %
Aqueous extract of leaves	39.5±0.04$^{(a)}$ *0.5 %	39.3±0.04$^{(b)}$ *0.7 %	39.5±0.05$^{(a)}$ *0 %	39.4±0.04$^{(a)}$ *0.2 %
Aqueous extract of flowers	39.5±0.04$^{(a)}$ *0.5 %	39.2±0.07$^{(b)}$ *1 %	39.5±0.08$^{(a)}$ *0 %	39.5±0.04$^{(a)}$ *0 %
Aqueous extract of roots	39.6±0.08$^{(a)}$ *0.2 %	39.8±0.04$^{(a)}$ *-0.5 %	39.5±0.03$^{(a)}$ *0 %	39.5±0.06$^{(a)}$ *0 %
Parthenolide	38.1±0.05$^{(c)}$ *4 %	37.9±0.04$^{(e)}$ *4.3 %	38.5±0.02$^{(c)}$ *2.5 %	39±0.05$^{(c)}$ *1.3 %
LSD	0.11	0.17	0.11	0.11

(a,b,c,d) means with the same letters in each column are not significantly different at $p < 0.05$.

* Percentage decrease in rectal temperature in comparison with control (mean ± SE).

LSD (least significant difference between means) calculated by one way ANOVA.



(1) Alc. Flower
6mg/ml bath

(2) Alc. Leaf
6mg/ml bath

(3) Alc. Root
6mg/ml bath

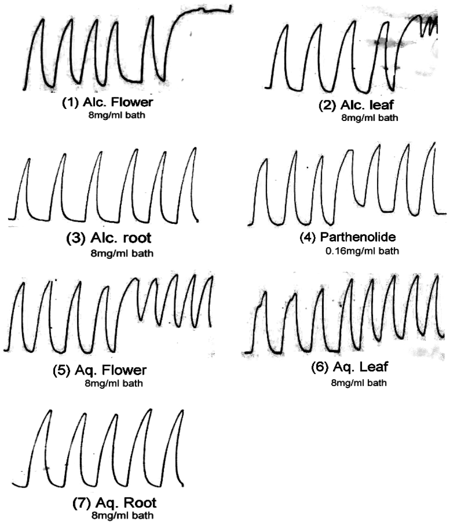
(4) Parthenolide
0.16mg/ml bath

(5) Aq. Flower
6mg/ml bath

(6) Aq. Leaf
6mg/ml bath

(7) Aq. Root
6mg/ml bath

Fig. 1. **Effect of different Feverfew extracts on rabbit intestine**



(1) Alc. Flower
8mg/ml bath

(2) Alc. leaf
8mg/ml bath

(3) Alc. root
8mg/ml bath

(4) Parthenolide
0.16mg/ml bath

(5) Aq. Flower
8mg/ml bath

(6) Aq. Leaf
8mg/ml bath

(7) Aq. Root
8mg/ml bath

Fig 2. **Effect of different Feverfew extracts on rat uterus**

[DOR: 20.1001.1.2717204.2008.7.25.15.1]

Table 7. ***In vitro*** **testing for cytotoxic effect of** ***T. parthenium*** **L. on Ehrlich ascitis carcinoma**

Sample	% inhibition of cell viability		
	25ug/ml	50ug/ml	100ug/ml
Alcoholic extract of leaf	20	75	90
Alcoholic extract of flower	35	85	100
Alcoholic extract of root	0	40	60
Aqueous extract of leaf	0	35	60
Aqueous extract of flower	0	40	65
Aqueous extract of root	0	10	30
parthenolide	30	80	100

highest concentration but the alcoholic extract of the flower was slightly more potent than parthenolide at their lower concentrations. Concerning the aqueous extracts of leaves and flower heads, showed little cytotoxic activity (60% and 65%), respectively at their high concentration.

Conclusion

-The alcoholic extracts of flowers and leaves and parthenolide showed significant analgesic, anti-inflammatory and antipyretic activities which confirmed the folk use of Feverfew herb for treatment of headache, fever, common cold and arthritis.

-The biological activities of Feverfew herb could be attributed to the leaves and/or flowers mainly due to the presence of sesquiterpene lactones and flavonoids [35, 36] while the roots showed no or mild biological activities due to the absence of sesquiterpene lactones and flavonoids.

-The antagonizing results cited when the antispasmodic effect was studied might explain the folk use of different extracts of the same plant as spasmolytic in colic, colitis and gripping and as vermifuge and laxative [6]. The uterine stimulant effect of the plant agreed with the folk uses of the plant as abortifacient, emmenagogue and in certain labor difficulties and also agreed with the warning of the drug producer which indicates the prevention of using feverfew during pregnancy but not agree with the folk use of the drug in threatened miscarriage [4 - 6].

-Parthenolide alone is not the only active ingredient in Feverfew responsible for the vast pharmacological actions of the plant but there are other constituents which could be also responsible for these actions as cited in literature [13, 31].

[DOR: 20.1001.1.2717204.2008.7.25.15.1]

References

1. El-Shazly A, Dorai G, Wink M, Naturforsch Z. 57c, 2002; 620 - 3.

2. Evans WC. "Trease and Evans *Pharmacognosy*", 15th Ed., W.B. Sauners Co. Ltd., London, Philadelphia, Torento, Sydney, Tokyo, 2002.

3. Clapham AR, Tutin TG, Warburg EF. "*Excursion Flora of British Isles*", Cambridge press, 1964.

4. Berry MI. *Pharmaceutical Journal*. 1984; 234, 611 - 6.

5. Hobbs C. *Herbal Gram*, 1989; 20, 26-35.

6. Ross IA. "*Medicinal Plants of the World*", volume 2, 1st Ed., Humana press, Totowa, New jersey, 2001.

7. Picman AK. *Biochemical Systematics and Ecology*, 1986; 14 (3), 255 - 81.

8. Bohlmann, F. and Zdero, C.; *Phytochemistry* 1982; 21 (10), 2543 – 9.

9. Awang DVC, Dawson BA, Kindack DG. *Journal of Natural Products*, 1991; 54 (6), 1516-1521.

10. Stahl E. "*Thin Layer Chromatography*", 2nd Ed., Springer Verlag, Berlin, Heidelberg, New York, 1969.

11. Mabry JT, Markham KR, Thomas MB. "*The Systematic Identification of Flavonoids*", vol. I and II, Springer Verlag, New York, 1970.

12. Staff Members of Department of Pharmacology, University of Edinburgh; "*Pharmacological Experiments on Isolated Preparations*", 2nd Ed., Edinburgh, London, 1970.

13. Williams CA, Harborne JB, Eagles J. *Phytochemistry*, 1999; 52, 1301 - 6.

14. Finar IL. "*Organic Chemistry*", 6th Ed., Longman group Ltd., England, 1973, 445.

15. Egyptian Pharmacopoeia, English text, 3rd Ed., University press, Cairo, 1984.

16. Adams RP. "*Identification of Essential Oil Components by Gas Chromatography / Mass Spectrometry*", Allured Publishing Corp., Carol Stream, Illinois, U.S.A., 1995.

17. Tober RI, Greenhouse DD, Rendell JK, Irwin S. *J. Pharm. Exp. Ther.*, 1969; 169, 29-38.

18. Winter C, Risley E, Nuss GW. Proc. Soc. *Exp. Biol. Med.*, 1962; 3, 544 - 6.

19. Teotine JM, Faris LP, Gadini A, DellaBella D. *J. Med. Chem.* 1963; 6, 248 - 50.

20. Dejalon B, Dejalon S. *Pharmacuture Actual*, 1945; 2, 35.

21. Mclimans WF, Davis EV, Glover FL, Rake GW. *J. Immumol.* 1957; 79, 428.

22. Begley MJ, Hewlett MJ, Knight DW. *Phytochemistry* 1989; 28 (3), 940 - 3.

23. Hewlett MJ, Begley MJ, Groenwegen WA, Heptinstall S, Knight DW, May J, Salan U, Toplis D. *Journal of Chem. Soc*. Perkin Transduction I, 1996; 16, 1979 - 86.

24. Milbrodt M, Schroder F, Konig WA, *Phytochemistry* 1997; 44 (3), 471 - 4.

25. Castaneda J, Fischer NH, Vargas D, *J. Nat. Prod.* 1993; 56 (1), 90 - 8.

26. Devon TK, Scott AI. "*Handbook of Naturally Occurring Compounds*", Academic Press, New York and London, 1972.

27. Sachdev K, Kulshreshtha DK. *Phytochemistry* 1985; 22 (5), 1253 - 6.

28. Hurst HM, Harborne JB. *Phytochemistry* 1967; 6, 1111 – 18.

29. Sam A, Stewart RN, Norris KH. *Phytochemistry* 1975; 14, 1443 - 4.

30. Wilcox N, Balafama HR. *Biochem. Syst. Ecol.* 1984; 12 (4), 357 - 61.

31. Williams CA, Harborne JB, Geiger H. Hoult JRS. *Phytochemistry* 1999; 51 (2), 417 - 23.

32. Good JL, Akisha T. "*Analysis of Sterols*", 1st Ed., Blackie Academic and Professional Press, Champan and Hall, 1997.

33. Kalodera Z, Peplijnjak S, Blazevic N, Petrak T, *Pharmazie* 1997; 52 (11), 885 - 6.

34. Hendriks H, Ros R, Woerdenbag HJ. *Flavor and Fragrance Journal* 1996; 11 (6), 367 - 71.

35. Hewlett MJ, Begley MJ, Groenwegen WA, Heptinstall S, Knight DW, May J, Salan U, Toplis D. *Journal of Chem. Soc.*, Perkin Transduction I, 1996; 16, 1979 – 86.

36. Milbrodt M, Schroder F, Konig WA. *Phytochemistry* 1997; 44 (3), 471 - 4.

[DOR: 20.1001.1.2717204.2008.7.25.15.1]