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Full Length Research Paper

Phytochemical and biological investigation of *Tanacetum parthenium* (L.) cultivated in Egypt

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Phytochemical investigation of *Tanacetum parthenium* (L.) family Asteraceae cultivated in Egypt resul-ted in isolation and identification of four flavonoids isolated from the aerial part, four sesquiterpene lactones isolated from the leaves, two sterols isolated from the roots. A comparative study of the essential oil content of the leaves and the flower heads was performed using (GC/MS) revealing the presence of 42 and 30 components with the major components 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 analgesic, anti-inflammatory, antipyretic, antispasmodic and uterine-stimulant activities in addition to the *in vitro* cytotoxic effect.

Key words: *Tanacetum parthenium*, ethanol and water extracts, essential oil, analgesic, anti-inflammatory, antipyretic, antispasmodic, uterine stimulant and cytotoxic.

INTRODUCTION

Genus *Tanacetum* includes about 50 species, of those only *Tanacetum santolinoides* (D.C.) grows in Egypt (El-Shazly et al., 2002). *Tanacetum parthenium* (L.) has oth-er synonyms viz.; *Chrysanthemum parthenium* (L.), *Leu-canthemum parthenium* (L.) and *Pyrethrum parthenium* (L.) and many common names viz.; Feverfew, Feather-few, Feather foil, Febrifuge plant, Midsummer daisy and Santa Maria (Berry, 1984; Hobbs, 1989; Ross, 2001). This plant was largely investigated for its traditional uses in medicine viz.; treatment of fever, headache, migraine, stomachache, insect bites, bronchitis, arthritis, cold, abor-tifacient and problems of menstruation (Evans, 2002; Tut-in et al., 1964; Berry, effect on the production of second-dary metabolites, the 1984). Since the environmental conditions have a great present work includes phyto-chemical 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 con-sidered the major active constituent of the plant (Berry, 1984; Hobbs, 1989; Ross, 2001; Bohlmann and Zdero, 1982; Picman, 1986; Knight, 1995).

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The sesquiterpene lactones biosynthetic pathway was influenced by environ-mental conditions (Awang et al., 1991; Abourashed and Khan, 2001; Rateb et al., 2007). In addition, some biolo-gical activities were investigated including the analgesic, anti-inflammatory and antipyretic activities. The antispas-modic and uterine stimulant effects were screened for the first time and the cytotoxic effect was also supplied.

MATERIAL AND METHODS:

General

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 Chemi-cals, Sweden). *P*- anisaldehyde-sulfuric acid spray reagent (Stahl, 1969) for sterols, Sodium methoxide solution, aluminum chloride solution, hydrochloric acid, sodium acetate powder and boric acid powder were used for reagents for UV spectroscopic analysis of fla-vonoids (Mabry et al., 1970), acetylcholine chloride (H and W, Eng-land), nicotine hydrogen tartarate (BDH, England), atropine sulpha-te (BDH, England). The physiological salt solutions (Tyrod's solution and Dejalon's solution) were prepared according to the Staff Members of Pharmacology Department, University of Edinburgh (Staff Members of Department of Pharmacology, 1970) for the iso-lated tissue preparations. Specific gravity bottle for determination of specific gravity of the essential oils; Abbe's refractometer for mea-

ring the refractive indices of the volatile oils samples; Koffler's heating stage microscope for determination of melting points; palmer kynograph and organ bath.

Plant material

100 g seeds of *T. parthenium* (L.) used in this study were imported from Schifeild Company, U.S.A. and cultivated in El-Basateen farm, Beni-Suef, Egypt and was kindly identified by Professor Dr. Abou Dahab M. A. The plant was air-dried, reduced to fine powder and kept in tightly closed amber colored glass container. 10 kg of different plant organs (leaves, flower heads and roots) were air dried, powdered and kept in tightly closed amber colored glass containers and protected from light at low temperature for phytochemical study. 500 g of fresh leaves and flower heads of the plant were collected in April (in flowering) and used just after collection in preparation of its volatile oil content. For biological study, alcoholic and aqueous extracts of different plant organs were prepared and a 20% w/v concentration was supplied, 5% (w/v) parthenolide solu-tion was prepared and a concentration of 100 ug/0.1ml dimethyl sulfoxide for cytotoxic study.

Animals

Albino mice of 20 – 25 g b. wt., adult female albino rats of 100 – 125 g b. wt. and adult albino rabbits of 1 - 2 kg body weight was used for this study.

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) was used. The NMR spectra were recorded in $CDCl_3$ and CD_3OD using TMS as internal standard and chemical shift values were recorded in 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 20 M 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 temperature programming starting with 50ºC increased to 200ºC by rate of 3ºC/min. and split ratio of 100:1).

Statistical analysis: The data of the experimental biology was averaged and statistically submitted to the analysis of variance. The least significant differences (LSD) test was applied for comparison among means according to method described by Steel and Torrie (1980).

Extraction procedures

Investigation of sesquiterpene lactones content (Bohlmann and Zdero, 1982): The air-dried defatted powdered leaves (3 kg collected in the flowering stage) 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 using isocratic elution with 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 (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 recrystallized from petroleum ether:diethylether (1:2) afforded compound L_1 (350 mg; colorless crystals; soluble in chloroform; m.p.115-117^{o}C). Fractions (14 - 16) (95 mg) were pooled and recrystallized from chloroform afforded compound L_2 (15 mg; colorless crystals; soluble in chloroform; m.p.242 - 244^{o}C). Fractions 63 - 67 (90 mg) were pooled and recrystallized from chloroform afforded compound L_3 (20 mg; colorless crystals; soluble in chloroform; m.p. 134-136^{o}C). Fractions 69 - 71 (70 mg) were pooled and recrystallized from methanol afforded compound L_4 (5 mg; colorless sticky substance; soluble in methanol).

Investigation of the flavonoid content (Williams et al., 1999A): 2 kg of fresh leaves (collected in the flowering stage) were dipped for 10 s in 4 l 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 fractions were collected and examined by TLC using (chloroform:methanol 9:1 v/v). 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, evaporated and recrystallized from methanol to yield compound F_1(60 mg, yellow crystals, m.p. 162 - 164°C, soluble in chloroform). 500 g of air-dried defatted powdered flower heads (collected in late April) 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 (15 mg, yellow powder, m.p. 348-350°C, soluble in methanol).

Recrystallization of fractions (21 - 26) from methanol gave compound F_3 (9 mg, yellow powder, m.p. 328-330°C, soluble in methanol). Recrystallization of fractions (30 - 36) from methanol gave compound F_4 (6 mg, yellow powder, m.p. 315 - 317°C, soluble in methanol).

Investigation of the lipid content: The unsaponifiable matter (Finar, 1973) prepared from 500 g 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 (petroleum ether:ethyl acetate 6:4 v/v). 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, evaporated and recrystalized from chloroform to produce compound T_1 (55 mg, white crystals, m.p. 197-199°C, soluble in chloroform). Fractions (22 - 25) were pooled and evaporated to produce compound T_2 (70 mg, white crystals, m.p. 140 - 142°C, soluble in chloroform).

Investigation of the volatile oil composition: 500 g of fresh leaves and fresh flowers (collected in late April) were subjected to hydrodistillation and the two samples of the essential oils obtained were separately dried over anhydrous sodium sulfate and stored in air tight amber colored containers in deep freezer for further GC/MS analysis. Percentage yield, specific gravity and refractive index of

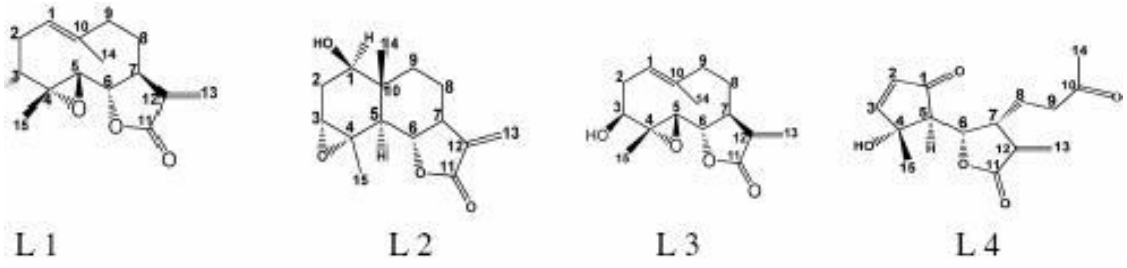

Figure 1. L1-L 4. Parthenolide, Epoxysantamarin, 3 -hydroxyparthenolide and Secotanapartholide.

the oil samples were determined according to the E.P. (1984) (Egyptian Pharmacopoeia, 1984). 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 (Adams, 1995) and quantitative analysis of the identified components was carried out by using area normalization method.

Biological study (each test was performed three times)

Analgesic activity: The analgesic activity was evaluated using acetic acid-induced writhing in mice as described by Taber et al. (1969) and paracetamol (50 mg/kg b.wt. orally) used as a standard. **Antispasmodic activity:** The method described by Staff Members of Pharmacology Department, University of Edinburgh (Staff Mem-bers of Department of Pharmacology, 1970) was used. A piece of 3 cm of rabbit duodenum was suspended in organ bath of containing

One hundred eighty mice of both sexes weighing from 20 - 25 g were divided into 9 groups (20 mice in each). Mice weighing 20 – 25 g were orally administered both the ethanolic and aqueous extracts in a dose of 1 g/kg body weight and parthenolide in a dose of 10 mg/kg body weight. After one hour, each mouse was injected intra-peritoneal (I. P.) with 0.1 ml of 0.6% acetic acid. 10 min later, the number of writhes of each mouse was counted for a period of 15 min. This work was repeated after 2, 3 and 5 h post administration.

Anti-inflammatory activity: Acute anti-inflammatory effect was evaluated using yeast-induced rat paw edema test described by Winter et al. (1962) 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 1 g/kg body weight and parthenolide in doses of 10 mg/kg body weight.

Edema was induced by subcutaneous injection of 0.1 ml brewer's yeast 20% suspension in physiological saline in the skin of the hind paw of all rats after measuring the initial paw thickness in mm using caliber and after 4 h, the paw thickness of all rats was also measured. The thickness of paw of all rats in all groups was measured after 3 and 6 h. Furthermore, the percentage decrease in the mean in comparison with control was estimated.

Antipyretic activity: The antipyretic activity was evaluated using yeast-induced hyperthermia method as described by Teotine et al (1963) 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.

Forty five mature albino rats weighing 100-125 g were divided into 9 groups (5 rats in each). All rats were made hyperthermic by subcutaneous injection of brewer's yeast suspension (15% in physiological saline). The rectal temperature of each rat was then recorded every hour for 4 h.

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.

Uterine stimulant activity: The method described by Dejalon and Dejalon (1945), 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.

***In vitro* cytotoxic activity:** The tested solutions were screened using a single tumor cell (Ehrlich ascitis carcinoma cells, the tumor was maintained in the laboratory by weekly intraperitonial (I.P.) transplantation in female Swiss albino mice) . A set of sterile test tubes were used, where 2.5×10^6 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 37ºC for 24 h. Trypan blue dye exclusion test (Mclimans et al., 1957) was carried out to calculate the percentage of non-viable cells using a dose of 100, 50 and 25 ug/ml of each extract. Concentrations causing less than 30% non-viable cells in the suspension were considered inactive, while those producing more than 70% non-viable cells were considered active and in between were considered of moderate activity.

RESULTS AND DISCUSSION

Phytochemical study

From the comparison with the authentic references and the physicochemical and spectral data published (Bohlmann and Zdero, 1982; Begley et al., 1989; Castaneda et al., 1993; Hewlett et al., 1996; Milbrodt et al., 1997), compounds L_1-L_4 (Figure 1) could be identified as parthenolide, epoxysantamarin, 3 -hydroxyparthenolide and secotanapartholide A, respectively.

From the comparison with the authentic references and the physicochemical and spectral data published (Hurst and Harborne, 1967; Devon and Scott, 1972; Sam et al., 1975; Wilcox and Balafama, 1984; Sachdev and Kulshreshtha, 1985), compounds F_1-F_4 (Figure 2) could be identified as santin, apigenin, luteolin and quercetin, respectively. Compound F_4 was first isolated from *T. partheni-*

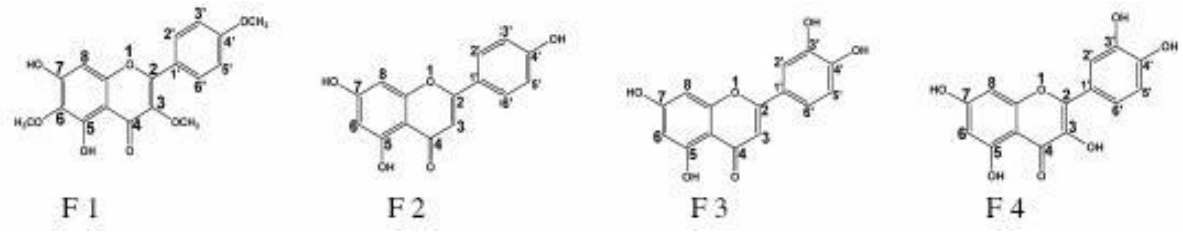

Figure 2. F1-F4 Santin, Apigenin, Luteolin and Quercetin.

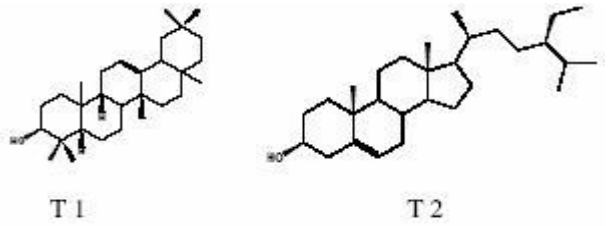

Figure 3. T 1 and T2 -amyrin and -sitosterol.

nium (L.) but isolated from other *Tanacetum* species (Williams et al., 1999B).

From the comparison with the authentic references and the physicochemical and spectral data published (Wilcox and Balafama, 1984; Good and Akisha, 1997), compounds T_1 and T_2 (Figure 3) could be identified as -amyrin and -sitosterol, respectively.

The percentages of essential oil obtained by hydrodistillation from 500 g of each of the two plant samples were 0.49 and 0.58% v/dry wt.; the 2 oil samples acquire camphor like odor and pale yellow color; the refractive indices at 20ºC were 1.4662 and 1.4697 and the specific gravities at 25ºC were 0.8912 and 0.9016 for the oil samples of leaves and flower heads, respectively.

The percentage yield of the oil was slightly different from the previously reported data (Kalodera et al., 1997; Hendriks et al., 1996) . 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 (Adams, 1995) and quantitative analysis of the identified components was carried out by using area normalization method.

Table (1) revealed that the essential oil isolated from the leaves and the flower heads 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 of the essential oil constituting 85.84 and 85% in the oil of leaves and flowers, respectively. The difference between the obtained data and the reported data (Hendriks et al., 1996; Kalodera et al., 1997) 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 (2) 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. These data agree with that reported on the plant in other countries due to presence of high concentration of sesquiterpene lactones, specially parthenolide, and flavornoids (Hewlett et al., 1996; Milbrodt et al., 1997; Jain and Kulkarni, 1999; Ernst and Pittler, 2000; Kwok et al., 2001; Long et al., 2003).

Anti-inflammatory activity: Results presented in Table (3) 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 h) and (about 55 - 60% of the activity of diclofenac sodium after 6 h). All other groups showed non significant anti-inflammatory activity. These data go in harmony with that reported on the plant due to presence of high concentration of sesquiterpene lactones, specially parthenolide, and flavonoids (Hewlett et al., 1996; Milbrodt et al., 1997; Jain and Kulkarni, 1999; Ernst and Pittler, 2000; Kwok et al., 2001; Long et al., 2003).

Antipyretic activity: Results presented in Table (4) 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. These data seem to be the same with that reported on the plant due to presence of high concentration of sesquiterpene lactones, especially parthenolide, and flavonoids (Hewlett et al., 1996; Milbrodt et al., 1997; Ernst and Pittler, 2000).

Table 1. GC/MS analysis of the organs of *T. parthenium* (L.).

Constituents of the oil	Rt. Min	Percentage ± S.D.	
		Flower	Leaf
Cis-2-octene	2.04	0.07±0.04	---
Butyl acetate	2.67	0.88±0.04	0.78±0.03
Tricyclene	3.51	0.37±0.04	0.17±0.03
-pinene	3.67	1.03±0.04	1.35±0.03
Camphene	4.25	8.76±0.04	3.72±0.03
Sabinene	4.87	0.45±0.04	0.17±0.03
-pinene	5.09	0.22±0.04	0.21±0.03
-myrecene	5.86	---	0.06±0.03
P-cymene	5.96	0.12±0.04	0.62±0.03
Limonene	6.71	0.12±0.04	0.29±0.03
-phellandrene	7.19	---	0.54±0.03
1,8-cineole	7.94	0.6±0.04	1.14±0.03
-terpinene	8.66	1.2±0.04	3.1±0.03
Terpinolene	12.37	---	0.13±0.03
P-cymenene	13.72	0.16±0.04	0.09±0.03
Terpin-1-ol	15.54	0.92±0.04	5.14±0.03
Camphor	17.47	48.4±0.04	37.7±0.03
E-chrysanthenyl acetate	18.21	26.3±0.04	33.8±0.03
Pinocarvone	18.68	---	0.49±0.03
Borneol	19.26	0.2±0.04	0.37±0.03
Terpin-4-ol	19.94	0.26±0.04	0.66±0.03
-terpineol	20.43	0.76±0.04	0.64±0.03
Linalool acetate	20.68	0.58±0.04	0.34±0.03
Bornyl acetate	21.75	---	0.15±0.03
Citronellal hydrate	22.18	---	0.62±0.03
Thymol	22.66	1.81±0.04	0.39±0.03
E-pinocarvyl acetate	23.16	0.81±0.04	0.09±0.03
Carvacrol	23.41	---	0.54±0.03
-copaene	23.64	---	0.12±0.03
E-caryophyllene	24.12	0.28±0.04	0.19±0.03
-humulene	24.43	---	0.41±0.03
E-B-farnesene	24.8	---	0.96±0.03
Germacrene D	25.66	0.21±0.04	0.11±0.03
Ar-curcumene	26.33	---	0.18±0.03
Isobornyl-2-methyl butyrate	26.75	---	0.35±0.03
-cadinene	27.89	---	0.18±0.03
Z-chrysanthenyl angelate	28.44	0.68±0.04	0.23±0.03
Bornyl angelate	30.78	1.81±0.04	2.17±0.03
Germacrene D-4-ol	31.72	0.59±0.04	---
-caryophyllene oxide	34.7	0.37±0.04	0.54±0.03
Globulol	41.38	0.45±0.04	0.15±0.03
Veridiflorol	43.03	---	0.27±0.03
E,E-farnesol	43.61	0.26±0.04	0.23±0.03
E,E-farnesyl acetate	45.05	0.19±0.04	0.18±0.03

*Rt = retention time.

Antispasmodic activity: 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

Table 2. Analgesic activity of different extracts and parthenolide of *T. 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 3. Anti-inflammatory activity of different extracts and parthenolide of *T. 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 4. Antipyretic activity of different extracts and parthenolide of *T. 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.

Table 5. *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

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: 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 (5), 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 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. Concerning the cytotoxic effect of the plant, many cited data reported significant citotoxic effect due to its high sesquiterpene lactone content specially Parthenolide which is a germacranolide type sesquiterpene lactone with , - unsaturated- -lactone and C11-C13 exocyclic double bond conjugated to the -lactone which is essential for cytotoxicity and the exocyclic dou-ble bond have more cytotoxic effect than endocyclic dou-ble bond or saturated compound with no double bonds as reported (Guzman and Jordan, 2005; Wu et al., 2006). This might be the reason for the strong cytotoxic activity exerted by the parthenolide isolated from Feverfew herb growing in Egypt.

Conclusion

T. parthenium (L.) growing in Egypt contains many sesquiterpene lactones, with higher concentration of parthenolide (Rateb et al., 2007), lipophilic and polar flavonoids in the leaves and the flower heads. The plant also contains high percentage of sterols and triterpenes in the roots. Moreover, the concentration and the composition of the volatile oil of the plant growing in Egypt differ from that in other countries.

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 and these effects are attributed to leaves and/or flowers mainly due to the presence of sesquiterpene lactones and flavonoids (Hewlett et al., 1996; Milbrodt et al., 1997) while 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 (Ross, 2001). 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 (Berry, 1984; Hobbs, 1989; Ross, 2001).

Parthenolide alone is not the only active ingredient in Feverfew responsible for the vast pharmacological actions of the plant but there are other constituents who could be also responsible for these actions as cited in literature (Williams et al., 1999A; Williams et al., 1999B; Long et al., 2003).

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