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Analgesic and Antioxidant Activities of Algerian *Retama raetam* (Forssk.) Webb & Berthel Extracts

Samah Djeddi[1,2*], Anastasia Karioti[1,5], Elina Yannakopoulou[3], Kyriakos Papadopoulos[3], Rim Chatter[4] and Helen Skaltsa[1*]

[1]*Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, Zografou, 15771 Athens, Greece*

[2]*Laboratory of Ecobiology of Marine and Littoral Environment, Department of Biology, Faculty of Science, University of Badji Mokhtar BP 12, Annaba 23000, Algeria*

[3]*Institute of Physical Chemistry, N.C.S.R. 'Demokritos', Ag Paraskevi, 15310 Athens, Greece*

[4]*Unity of Biotoxins, Institute Pasteur of Tunis 13, Place Pasteur, BP 74, Tunis 1002 Belvédère, Tunisia*

[5]*Department of Pharmaceutical Sciences, University of Florence, Ugo Schiff 6, Polo Scientifico, Sesto Fiorentino, 50019, Florence, Italy*

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Abstract: Part of this work deals with the isolation and structure elucidation of the main polar secondary metabolites of the aerial parts of *Retama raetam* (Forssk.) Webb & Berthel, as well as the evaluation of their potential analgesic properties, while the rest deals with the antioxidant activities of the aqueous extracts of roots, stem, fruits and flowers of the plant. It was found that the isoflavones genistein **1**, 6-hydroxygenistein **2**, 3'-O-methylorobol **3**, pratensein **4**, biochanin A **8**, the flavones 6-hydroxyapigenin **7** and luteolin **5**, the flavonol kaempferol **6**, as well as the phenolic compound *p*-coumaric acid **9** reduce significantly the pain at a concentration dose of 1 mg/kg. The most active compounds were **3** and **8** (86.19% and 75.23%, respectively). The obtained aqueous extracts of *R. raetam* were also evaluated for their antioxidant activities using two different photometric methods; the results revealed that all extracts exerted very low free radical scavenging activity compared to the well-known butylated hydroxytoluene (BHT) and lower hydrogen peroxide blocking activity than positive control gallic acid.

Keywords: *Retama raetam* (Forssk.) Webb & Berthel; isoflavones; analgesic activity; antioxidant activity.

1. Introduction

The genus *Retama* (syn. *Lygos*) belongs to tribe Genistae of the Fabaceae family and includes four species distributed in the Mediterranean, North Africa and the Canary islands. *Retama raetam* (Forssk.) Webb & Berthel is a spontaneous plant common in the North and East Mediterranean region

* Corresponding author: E-Mail: samah.djeddi@univ-annaba.org; Phone: +213697192143 Fax: 0021338875400 and skaltsa@pharm.uoa.gr; Phone & Fax: +30210 7274593

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[1]. In Algeria, it is located in Sahara and Atlas regions and is used in folk medicine under the common name “R’tam” to reduce the blood glucose and skin inflammations [2], while in Lebanon it is used as folk herbal medicine against joint aches [3] and in Morocco against skin diseases [4]. Previous pharmacological studies on the plant have revealed its various medicinal properties: antibacterial, antifungal, antihypertensive, antioxidant, antiviral, diuretic, hypoglycaemic, hepatoprotective, nephroprotective and cytotoxic effects [5-11]. *Retama* species have been reported to contain flavonoids and alkaloids [12]. To the best of our knowledge, no systematic investigation of the analgesic properties of *R. raetam* constituents, as well as the antioxidant activities of aqueous extracts of *R. raetam* has been studied hitherto. Therefore, we present in this work the analgesic activities of some polar flavonoids isolated from *R. raetam*, as well as the antioxidant properties of aqueous extracts of aerial parts using two different photometric methods.

2. Materials and Methods

2.1. Plant Material

The plant material was collected from Béchar city (965 km South-West of Algiers, Algeria) on May 2005. The plant was authenticated by Mr. Beloued Abd El-Kader (National Agronomic Institute, Algiers) and later by Mr. Zaafour Moncef (Biology Department, Badji Mokhtar University). A voucher specimen has been kept in the Herbarium of the Laboratory of the Biology Department, Vegetable Biology and Environment Laboratory, University of Badji Mokhtar, under the code Ann-BV 2013/001.

2.2. Isolation of flavonoids

The air-dried aerial parts of the plant (440 g) were finely ground and extracted at room temperature with cyclohexane, dichloromethane, methanol and methanol-water (5:1), successively. The methanol extract (16.0 g) was subjected to vacuum liquid chromatography using silica gel (Merck, Art.7736 - fractions of 300 mL) as stationary phase and mixtures of CH_2Cl_2-MeOH of increasing polarity as eluents. 14 fractions combined to six groups (CA-CF) were obtained. Group CE (0.95 g; eluted with CH_2Cl_2-MeOH, 93:7) was subjected to column chromatography over silica gel, (Merck; Art.9385) and yielded 5.7 mg of **1** (genistein), 2.4 mg of **2 (**6-hydroxygenistein), 8.0 mg of **3 (**3´-methylorobol) and 5.1 mg of **9** (*p*-coumaric acid). Group CF (3.6 g; eluted by methanol) was similarly fractionated by column chromatography over silica gel (Merck, Art. 9385) Group CF (3.6 g; eluted by methanol) was similarly fractionated by column chromatography over silica gel Merck; Art.9385; CH_2Cl_2-MeOH mixtures) and afforded 5.7 mg of **4** (pratensein), 93.5 mg **1** (genistein), 1.1 mg of **5** (luteolin) and 1.4 mg of **6** (kaempferol). The methanol-water extract (9.2 g), was similarly fractionated, yielded 1.5 mg of **7** (6-hydroxyapigenin), 2.0 mg of **8** (biochanin A) and 15.4 mg of **1** (genistein) (see structures in Figure 1). The structures of the isolated compounds were established by UV-Vis and 1D and 2 D NMR spectroscopy. ^{1}H, ^{13}C and 2D NMR spectra were recorded in $CDCl_3$ on Bruker DRX 400 and Bruker AC 200 (50.3 MHz for 13C NMR) instruments at 295 K. Chemical shifts are given in δ values and were referenced to the solvent signals at 3.31 ppm and 49.0 ppm for ^{1}H and ^{13}C NMR, respectively UV spectra were obtained on a Shimadzu UV-160A spectrophotometer, according to standard procedures [13].

2.3. Aqueous extracts preparations

Aqueous extracts were prepared by boiling independently 3g of dried stems, fruits or roots and 0.5 g of dried flowers in 100 mL sterile distilled water for 10 min and following filtration of particulate matter. The extracts were evaporated to dryness in vacuum by rotary evaporator. The obtained yields were as follows: 455.0 mg (stems), 218.0 mg (fruits), 547.5 mg (roots) and 307.9 mg (flowers).

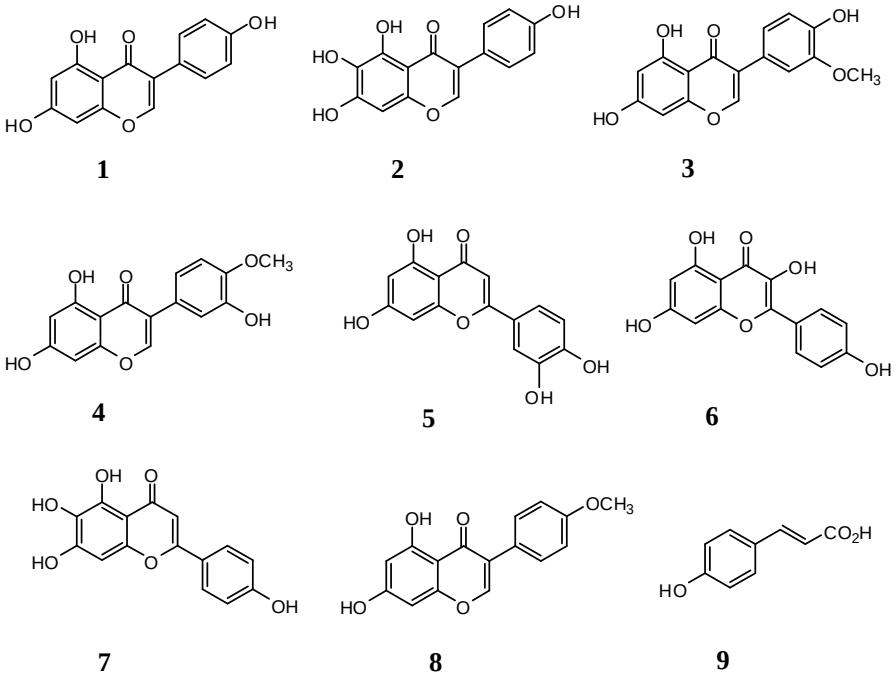

Figure 1. Structures of isolated compounds (**1**-**9**) from *R. raetam*

2.4. Analgesic activity measurements

The analgesic activity was performed according to the method of Koster et *al*. [14]. Swiss mice (18-22 g) were obtained from Pasteur Institute (Tunis, Tunisia). They were housed in polypropylene cages and were kept in a room maintained under controlled conditions. All animals were fed with a standard diet *ad libitum* and had free access to drinking water. Swiss mice were selected one day prior to each test and were divided into three groups of six mice each. One group served as the control and was treated with 10 mL/kg of saline administered through intra-peritoneal injection (i.p.). Aspirin (200 mg/kg) was administered (i.p.) in the second group, while the third group was treated with all compounds (i.p.) at a dose of 1 mg/kg. All animals received 10 mL/kg (i.p.) of 1% acetic acid 30 min after treatment. The number of abdominal writhings was recorded for a period of 30 min. The results of the analgesic activity of compounds **1-4, 8** expressed in percentage were calculated using the formula (1) and are shown in Table 1.

Analgesic activity (%) = [{Wr (Control) - Wr (test compound)} / Wr (Control)] × 100 (**1**)

Wr = Mean number of writhings

All data were represented as mean ± S.EM. Results were statistically evaluated using Student's *t*-test. $P<0.05$ was considered significant.

2.5. Antioxidant activity measurements

2.5.1. DPPH-radical scavenging activity

The free radical-scavenging activity of aqueous extracts of stems, flowers, fruits or roots of *R. raetam* was estimated by using a modified DPPH-method (2,2-diphenyl-2'-picrylhydrazyl) [15]. According to this method, 2 mL of a methanol solution of DPPH of concentration 24 µg/mL were

added to 100 µL methanol solution of extracts of various concentrations (100, 250, 500, 750 and 1000 mg/L) and let stay in the dark for 30 minutes. After this time the absorbance was measured at 516 nm in a Schimadzu 160-UV spectrophotometer. Shorter times have also been reported by some authors, such as 5 min [15] or 10 min [16], but in our experiments the time of 30 min proved to be the optimum (time needed for stable signals). The radical-scavenging activity was calculated using the equation (2).

$$\text{Scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (\mathbf{2})$$

A_0 is the absorbance of the control (sample without extracts) and A_1 the absorbance of samples with extracts.

2.5.2. Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity was estimated using a fluorimetric method reported by Nikokavoura et al [17]. According to this method, 500 µL of methanol sodium hydroxide solution (0.01M) were added into a cuvette containing 500 µL of methanol lucigenin solution (1×10^{-4} M), 500 µL of methanol hydrogen peroxide solution (0.1M) and 1000 µL of a methanol solution containing extracts of various concentrations. After one hour, the fluorescence intensity was measured at 425 nm setting the excitation wavelength at 390 nm. The fluorescence intensity of blank solution (I_0) is recorded by introducing 1000 µL of methanol instead of extract solution into the reaction cell. The final concentrations of studied samples were (100, 250, 500, 750 and 1000 mg/L). The hydrogen peroxide scavenging activity was calculated by the aid of equation (3).

$$\text{Antioxidant activity (\%)} = [(I_0 - I)/I_0] \times 100 \quad (\mathbf{3})$$

I_o is the fluorescence intensity of blank solution (sample without extract) and I the fluorescence intensity of the sample containing the extract. The fluorescence measurements were performed on a JASCO spectrofluorimeter FP-777. Not less than three measurements were taken for each test solution. Stock solution of lucigenin (0.01M) was prepared by weighing the appropriate amount and diluting in 10 mL water. Stock solution of sodium hydroxide (1 M) was prepared in deionised water. Lucigenin and sodium hydroxide working solutions were prepared immediately before use by appropriate dilution of the stock solution with methanol. Hydrogen peroxide working solution (0.1M) was prepared daily by appropriate dilution of a 30% (w/v) hydrogen peroxide solution with methanol. Antioxidant standard solutions were daily prepared by accurately weighing and diluting with methanol.

2.6. Determination of total phenolic content in extracts

The total phenolic content in plant extracts was determined by a modified Folin–Ciocalteu method [17]. According to this method, an aliquot of 10 mL of each extract or 1 mL of gallic acid of various concentrations were diluted with 10 mL distilled water, mixed with 2 mL of Folin–Ciocalteu reagent (2 N) and stirred. 5 mL of sodium carbonate solution (7.5%, w/v) were then added and the mixture was stirred vigorously for a few minutes. The mixture was diluted to 50 mL with distilled water and was allowed to stand for 2 h at room temperature. After this time the absorbance was recorded at 750nm. The results were expressed as mg gallic acid equivalents per gram extracts (mg GAE/g extract) and determined according to the equation (4) prepared from the calibration curve of gallic acid.

$$\text{Absorbance} = 0.0101 + 241.56415 \times C \ (\text{mol l}^{-1}) \quad (\mathbf{4})$$

C is the concentration of gallic acid

3. Results and Discussion

3.1. Chemical constituents of R. raetam

During the last decades, a huge number of chemical compounds have been isolated from the genus *Retama*, including flavonoids, alkaloids, saponins, lignans and others [18, 19]. Among them, the isoflavones are the major constituents of this genus. In the present study, it was revealed that the main secondary metabolites *of R. raetam* from Algeria were genistein **1**, 6-hydroxygenistein **2**, 3'-O-methyloborol **3**, pratensein **4**, luteolin **5**, kaempferol **6**, 6-hydroxyapigenin **7**, biochanin A **8** and the phenolic acid, *p*-coumaric acid **9** (Fig. 2). Previous studies on *R. raetam* from Algeria revealed the presence of vitexin, vicenin-2, genistin and quercetin-3,7-di-*O*-β-glucoside [20]. The chemical profile of *R. raetam* collected at Egypt is totally different compared to that of Algerian origin, as its main constituents are daidzein, vicenin-2, naringenin, apigenin, kaempferol, quercetin and kaempferol-7-*O*-glucoside in the seeds [21], as well as of daidzein, daidzein-7,4′-dimethylether, chrysoeriol-7-*O*-glucoside, apigenin-7-*O*-glucoside, luteolin-7-*O*-glucoside, orientin, orientin-4′-glucoside and vicenin-2 in leaves [22]. Also, luteolin-4′-*O*-neohesperidoside, 5,4′-dihydroxy-(3″,4″-dihydroxy)-2″,2″-dimethylpyrano-(5″,6″:7,8)-flavone and ephedroidin have been isolated from the aerial parts of *R. raetam* collected at Cairo-Suez desert [23]. This difference could be attributed to the different climates in Algeria and Egypt, such as different mean monthly temperatures and different sunshine duration.

3.2. Analgesic properties of flavonoids isolated from R. raetam

The analgesic activity of the main isoflavones (**1-4, 8**) isolated from *R. raetam* was estimated using the writhing test in mice and its effect on the writhing response (Table 1). Compounds **3** and **8** showed significant decrease in pain (86.19% and 75.23 %, respectively) at 30 min post treatments at doses of 1 mg/kg. Biochanin A (**8**) and some other isoflavones have been previously found to possess significant anti-inflammatory activity [24]. The writhing test in mice is very sensitive and able to detect anti nociceptive effects of compounds and dose levels. That may appear inactive in other methods like the tail flick test [25]. Acetic acid causes an increase in peritoneal fluid levels of prostaglandins (PGE2 and PGF2), involving in part peritoneal receptors and inflammatory pain by inducing capillary permeability. Therefore, the effect of the active compounds might inhibit the synthesis and/ or the release of the prostaglandins, suggesting that the molecules are an antagonist of cyclo-oxygenase or other enzymes involved in the arachidonic acid cascade [26, 27].

Table 1. Effect of isoflavones isolated from *R. retam* on the acetic acid induced writhing behaviour in mice.

Flavones* 1mg/kg	**Writhings**	**Inhibition (%)**
genistein (**1**)	59.83 ± 10.4	57.27%
6-hydroxygenistein (**2**)	110 ± 20.28	21.40%
3' methylorobol (**3**)	19.33 ± 3.98	86.19%
pratensein (4' methylorobol) (**4**)	80.33 ± 9.51	42.60%
biochanin A (**8**)	34.66 ± 13.03	75.23%
Aspirin (200mg/kg)	12.89 ± 3.24	92.57%

* Concentration: 1mg/Kg

3.3. Antioxidant activities of aqueous extracts of R. raetam

The free radical-scavenging activity of aqueous extracts of *R. raetam* was determined by measuring the decrease of absorbance of DPPH free radical at 516 nm in the presence of various concentrations of extracts. The initial absorbance of DPPH decreases by increasing extract concentration. This happens due to the high electron or hydrogen donating ability of the phenolic compounds contained in extracts [28]. The results of DPPH radical scavenging activity (%) of *R. raetam* aqueous extracts along with those of known antioxidant BHT (butylated hydroxyltoluene), at

various concentrations are given in Table 2. As shown in Table 2, all *R. raetam* water extracts showed low scavenging ability compared to that of BHT. This result may be due to the fact that radical-scavenging capacity is directly related to the hydrogen atom donating ability of a compound and not correlated to the redox potentials alone, as observed by Lucarini et al. [29] when studying the antioxidant capacity of phenothiazine and other related compounds. In contrary to the above results, the peroxide blocking activity estimated by the spectrofluorimetric method using lucigenin and hydrogen peroxide as an oxidizing reagent revealed that stems and flowers aqueous extracts show higher antioxidant activity (52.75 and 54.44 %, respectively), but lower compared with positive control gallic acid (98.72 %) in the same concentration level. Interestingly, BHT didn't show any peroxide blocking activity (Table 2). The assessed antioxidant activities of each extract were correlated to the corresponding total phenolic content measured by the Follin-Ciocalteu assay. It was found that stems extracts with low free radical scavenging activity (14.06 %), as well as a medium hydrogen peroxide blocking activity (52.57%) (Table 2) correlate relatively well to the low content of phenolic compounds found in the corresponding stems and flowers extracts (25.19 and 51.68 mg GAEs per gram extract, respectively, Table 3). Some authors reported that the flower oils of *R. raetam* cultivated in Tunisia presented higher DPPH radical scavenging activity than the known antioxidant compound BHT, which they have attributed to the relative high percentage of monoterpenes contained in the essential oils [11].

Table 2. DPPH radical scavenging activity (%) from *R. raetam* aqueous extracts and BHT

Concentrations (mg/L)	BHT	Stems extract	Flowers extract	Roots extract	Fruits extract
100	16.70 ± 5.28	04.60 ± 0.17	04.90 ± 0.91	03.13 ± 0.47	00.96 ± 0.66
250	32.46 ± 7.69	07.06 ± 1.15	07.43 ± 2.68	02.66 ± 1.15	02.13 ± 0.75
500	62.83 ± 5.51	09.53 ± 0.05	07.10 ± 1.04	03.30 ± 1.31	02.76 ± 0.86
750	64.73 ± 0.68	11.76 ± 0.68	09.60 ± 1.47	03.76 ± 0.66	04.00 ± 1.66
1000	76.13 ± 3.68	14.06 ± 0.40	11.96 ± 0.49	04.20 ± 1.40	04.26 ± 1.35

Values represent mean ± standard deviation of three replicates.

Table 3. Total phenolic content in 1 gram extract expressed in mg GAEs

Aqueous extracts	Total Phenolic Content (mg GAEs/gram extract)
Stems extract	25.19
Flowers extract	51.68
Roots extract	11.11
Fruits extract	11.11

Table 4. Hydrogen peroxide scavenging activity of *R. raetam* extracts determined fluorimetrically by the reaction of lucigenin with hydrogen peroxide

Concentration (mg/L)	Antioxidant activity (%) Gallic acid	Antioxidant activity (%) stem	Antioxidant activity (%) flowers	Antioxidant activity (%) roots	Antioxidant activity (%) fruits
100	88.90 ± 1.02	06.71 ± 0.18	14.85 ± 6.63	11.26 ± 1.75	09.23 ± 3.95
250	88.27 ± 1.10	10.03 ± 1.32	20.66 ± 7.35	16.81 ± 6.68	13.18 ± 5.79
500	89.85 ± 1.90	25.51 ± 3.50	29.52 ± 11.28	17.06 ± 6.51	20.58 ± 7.74
750	91.91 ± 0.47	40.89 ± 1.88	44.27 ± 9.97	20.69 ± 6.18	26.95 ± 5.02
1000	98.72 ± 0.21	52.75 ± 0.19	54.44 ± 1.88	24.64 ± 5.19	32.59 ± 3.07

5. Conclusion

In frame of this work, we have shown that some isoflavonoids isolated from the methanol extract of *R. raetam* exert analgesic activity, which in the case of 3'-O-methylorobol (86.9%) is almost equal to aspirin. In a further set of experiments it was shown that aqueous extracts of stems, roots, fruits and flowers have very low free radical scavenging activity, but relatively good hydrogen peroxide scavenging activity.

References

[1] P. Quezel and S. Santa (1962). Nouvelle flore de l'Algérie et des régions désertiques méridionales, édition du centre national de la recherche scientifique.

[2] F. Baba Aissa (1999). Encyclopédie des plantes utiles. Flore d'Algérie et du Maghreb. Librairie Moderne, Alger.

[3] M. El Beyrouthy, N. Arnold N, A. Delelis-Dusollier and F. Dupont F. (2008). Plants used as remedies antirheumatic and antineuralgic in the traditional medicine of Lebanon, *J. Ethnopharm.* **120**, 315–334.

[4] J. El-Hilaly, M. Hmammouchi and B. Lyoussi (2003). Ethnobotanical studies and economic evaluation of medicinal plants in Taounate province (Northern Morocco), *J. Ethnopharm.* **86**, 149–158.

[5] M. Maghrani, N. A. Zeggwagh, M. Haloui and M. Eddouks (2005). Acute diuretic effect of aqueous extract of Retama raetam in normal rats, *J. Ethnopharm.* **99**, 31-5.

[6] M. Eddouks, M. Maghrani, L. Louedec, M. Halouji and J. B. Michel (2007). Antihypertensive activity of the aqueous extract of *Retama raetam* (Forssk.) leaves in spontaneously hypertensive rats, *J. Herbal Pharmacotherapy*. **7**, 65-77.

[7] E. Hayet, A. Samia, G. Patrick, M. Ali, M. Maha, G. Laurent, Z. Mighri and L. Mahjoub (2007). Antimicrobial and cytotoxic activity of *Marrubium alysson* and *Retama raetam* grown in Tunisia, *Pakistan J. Biol. Sci.* **10**, 1759-62.

[8] E. Hayet, M. Maha, A. Samia, M. Mata, P. Gros, H. Raida, M. Ali, A. S. Mohamed, L. Gutmann, Z. Mighri and A. Mahjoub (2008). Antimicrobial, antioxidant, and antiviral activities of Retama raetam (Forssk.) Webb flowers growing in Tunisia, *World J. Microbiol. Biotech*. **24**, 2933-40.

[9] K. M. M. Koriem, A. R. H. Farraqm, A. A. Badawy and S. A. El-Toumy (2009). Role of some Egyptian medicinal plants against liver and kidney toxicity induced by cadmium chloride, *Toxicol. Mech. Methods.* **19**, 524-34.

[10] M. M. Algandaby, H. A. Alghamdi, O. M. Ashour, A. B. Abdel-Naim, S. A. Ghareib, E. A. Abdel-Sattar and A. S. Hajar (2010). Mechanisms of the antihyperglycemic activity of *Retama raetam* in streptozotocin-induced diabetic rats, *Food Chem. Toxicol.* **48**, 2448-2453.

[11] H. Edziri, M. Mastouri, I. Chéraif and M. Aouini (2010). Chemical composition and antibacterial, antifungal and antioxidant activities of the flower oil of *Retama raetam* (Forssk.) Webb from Tunisia, *Nat. Prod. Res.* **24**, 789-796.

[12] O. B. Abdel Halim, H. Abdel Fattah and A. F. Halim (1997). Comparative chemical and biological studies of the alkaloidal content of *Lygos* species and varieties growing in Egypt, *Acta Pharm. Hung*. **67**, 241–247.

[13] T. G. Mabry, K..R. Markham and M. B. Thomas (1970). The systematic identification of flavonoides. Sptinger-Verlag: Berlin, Heidelberg, New York.

[14] R. Koster, M. Anderson and E.J. De Boer (1959). Acetic Acid for Analgesic Screening, *Fed. Proc.* **18**, 412.

[15] J. Lebeau, C. Furman, J.L. Bernier, P. Duriez, E. Teissier and N. Cotelle (2000). Antioxidant properties of di-tert-butylhydroxylated flavonoids, *Free Radical Biol. Med.* **29**, 900–912.

[16] K. Schwarz, G. Bertelsen, L. R. Nissen, P. T. Gardner, M. I. Heinonen and A. Hopia (2001). Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds, *Eur. Food Res. Techn*. **212**, 319–328.

[17] A. Nikokavoura, D. Christodouleas, E. Yannakopoulou, K. Papadopoulos and A. C. Calokerinos (2011). Evaluation of antioxidant activity of hydrophilic and lipophilic compounds in edible oils by a novel fluorimetric method, *Talanta.* **84,** 874–880.

[18] J. B Harborne (1969). Chemosystematics of the Leguminosae. Flavonoid and isoflavonoid patterns in the tribe og Genisteae, *Phytochemistry.* **8**, 1449-1456.

[19] J. B. Harborne, D. Boulter and B. L. Tuner (1971). Chemotaxonomu of the Leguminosae, Academic Press, London.

[20] M. L. Lázaro, C. Martín-Cordero, F. Iglesias-Guerra and M. J. Ayuso González (1998). An isoflavone glucoside from Retama sphaerocarpa boissier, *Phytochemistry.* **48** 401-402.

[21] A. E. A. El-Sherbeiny, H. I. El-Sissi, M. A. M. Nawwar and M. A. El-Ansari MA. (1978). The flavonoids of the seeds of Lygos raetam, *Planta Med.* **34,** 335-339.

[22] M.F. Abdalla and N.A.M. Saleh (1983). Flavonoids of Retama raetam, *J. Nat. Prod.* **46**, 757-757.

[23] M. Kassem, S.A. Morsharrafan, N.A.M. Saleh and S.M. Abdel-Wahab (2000). Two new flavonoids from Retama raetam, *Fitoter.* **71,** 649-654.

[24] S. J. Lee, H. J. Baek, C. H. Lee and H. P. Kim (1994). Anti-inflammatory Activity of Isoflavonoids from *Pueraria Radix* and Biochanin A derivatives, *Arch. Pharm. Res.* **17**, 31-35.

[25] H. O. J. Collier, L. C. Dineen, C. A. Johnson and C. Schneider (1968). The abdominal constriction response and its suppression by analgesic drugs in the mouse, *Britich J. Pharm*. **32**, 295- 310.
[26] E. A. Asongalem, H. S. Foyet, S. Ekobo, T. Dimo and P. Kamtchouing (2004). Antiinflammatory, lack of central analgesia and antipyretic properties of *Acanthus montanus* (Ness) T. Anderson, *J. Ethnopharm*. **95**, 63–68.
[27] R. Amann and B. A. Peskar (2002). Anti-inflammatory effects of aspirin and sodium salicylate, *Eur. J. Pharm*. **447**, 1–9.
[28] J. R. Soares, T. C. P. Dinis, A. P. Cunha and L. M. Almeida (1997). Antioxidant activity of some extracts of *Thymus zygis*, *Free Radical Res.* **26**, 469–478.
[29] M. Lucarini, P. Pedrielli, G. F. Pedulli, L. Valginigli, D. Gigmes and P. Tordo (1999). Bond dissociation energies of the N–H bond and rate constants for the reaction with alkyl, alkoxyl, and peroxyl radicals of phenothiazines and related compounds, *J. Am. Chem. Soc*. **121**, 11546–11553.