International Journal of Phytomedicine 8 (2016) 62-68
http://www.arjournals.org/index.php/ijpm/index

ISSN: 0975-0185

Original Research Article

Study of phytochemical screening, total Phenolic and flavonoid content and antioxidant activity of *Pulicaria glutinosa* growing in Saudi Arabia

Jehan Al-humaidi [1]

*Corresponding author:

Jehan Al-humaidi

[1]Chemistry Department, College of Science, Princess Nuora Bint Abdulrahman University 84428 Riyadh, Saudi Arabia

Abstract

Phytochemical screening of *Pulicaria glutinosa* different fractions revealed the presence of different classes of secondary metabolites including alkaloids, terpenes, sponins, flavonids, anthraquinone and glycosides. Moreover, total phenolics, total flavonoids, and *in vitro* antioxidant activities (DPPH, ABTS metal chelation effect in the Fe^{2+}-ferrozin test system) of *P. glutinosa* different fractions were determined. Total phenol and flavonoid content of the tested fractions were measured by folin ciocalteu and $AlCl_3$ assays, respectively. The Phenol and flavonoid content of the various fractions varied between 18.33 – 199.44 mg/g and 14.64 – 164.73 mg/g., respectively. Butanol fraction had the highest content of phenols and flavonoids and exhibited the greatest antioxidant activity in scavenging of $DPPH^{\bullet}$ free radical and $ABTS^{\bullet+}$ radical cation assays.

Keywords: *P. glutinosa,* antioxidant, total phenol, total flavonoid; $DPPH^{\bullet}$, $ABTS^{\bullet+}$, metal chelating activity

Introduction

Pulicaria genus belonging to Asteraceae family (coomonly known as Compositeae), comprises more than 100 species [1,2]. Plants belonging to this genus are used in Arabic traditional medicine, especially in Saudi Arabia, as herbal remedies for the treatment of many ailments including inflammation, intestinal disorders, menstrual cramps and as an insect repellent [3-5]. A thorough literature survey reveals that many *Pulicaria* spevies were investigated for their chemical constituents ([6]. Results revealed that these plants contain different classes of terpenoids including diterpenes [7-9], sesquiterpenes [10,11], caryophyllenes and caryophyllane derivatives [12] in addition to simple phenolics [13] and flavonoids [14]. Caryophllene and its 5,6-epoxide has been obtained from *P. crispa* growing wild in Saudi Arabia [15]. However, *Pulicaria glutinosa*, being a rare plant that grows in limited areas in the Arabic world, especially in Saudi Arab is, is seldom investigated. In fact, this genus is least explored in terms of its ethnopharmacological effects and only few reports were concerned with isolating and characterization of its chemical constituents, mainly sesquiterpenoids [16]. The extract obtained from the plant has been used lately in the green chemistry synthesis of different types of nanoparticles. The plant has never been investigated for its antioxidant activity, total phenolics or total flavonoids-content. Lately, *P. glutinosa* has been investigated for its neuro-protective activity in oxidative stress induced neurotoxicity in Zebra fish embryos [17].

In a continuation of an extensive program designed for investigating medicinal plants from the Flora of Saudi Arabia regardless of their claimed ethnopharmacological use, this current investigation has been designed to evaluate the different classes of secondary metabolites in of *P. glutinosa* through chemical methods. Moreover, the total phenolics and total flavonoid contents in the different crude fractions obtained from *P. glutinosa* were determined. In addition, the antioxidant activity of the crude fractions of the plant was evaluated using the DDPH and ABTS and metal chelating assay methods.

Materials and Methods

Plant material

Fresh *Pulicaria glutinosa* was collected from Wadi Nisah, Riyadh-Saudi Arabia. The plant was identified by Dr. Jacob Thomas, Department of Botany, King Saud University, Saudi Arabia. The plant material was grounded into fine powder using a mechanical grinding machine.

Extraction

Freshly collected plant material was air dried in shade until constant weight. A sample weighing 100 g of powdered dried plant material was extracted with 500 mL of petroleum ether in a Soxhlet type apparatus to remove fatty materials. According to the procedure listed in the literature [18,19], the residual plant material was extracted with 500 mL methanol. After evaporating methanol under vacuum, the gummy residue (15.7 g) was partitioned between CHCl3 and H2O (1:1) yielding the chloroform (7.14 g) and water (4.94 g) extracts. The dried CHCl3 extract was partitioned between 10% aqueous methanol and hexane yielding the aqueous methanol (A, 2.17 g) and hexane (H, 3.48 g) fractions. The polar organic compounds were extracted from water by n-butanol yielding the butanol (B, 1.96 g) and water (W, 2.98) fractions.

Phytochemical Screening

All four fractions obtained from *P. glutinosa* upon partitioning the alcoholic gummy residue using solvents of different polarities, were tested for the presence of the different classes of secondary metabolites including flavonoids, alkaloids, tannins, terpenes, saponins, anthraquinones, glycosides and tannins according to the procedures described in the literatures [20-22]. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals (Table 1).

Determination of total phenolic content

Total phenolic content in each fraction of *P.glutinosa* was determined using the Folin-Ciocalteu method according to the procedure described in the literature [23] with slight modifications. Briefly, 0.5 ml sample of each fraction was introduced into a test tube followed by the addition of 2.5 ml of Folin-Ciocalteu's reagent (diluted 10 times with water) and then 2 ml of sodium carbonate solution (7.5% w/v) was added. The mixture was allowed to stand at room temperature for 30 min and the absorbance was then recorded at 765 nm using UV- visible spectrophotometer. Gallic acid standard solutions of different concentrations were used for establishing the calibration curve. All determinations were performed in triplicate. Total phenolic content was obtained from a regression equation, and expressed as mg/g of gallic acid equivalent.

Determination of total flavonoid content

The total flavonoid content (TFC) of each fraction *P. glutinosa* was determined using colorimetric aluminum chloride (AlCl3) assay method according to the procedure listed in the literature [24]. A 0.5 ml aliquot of the tested fraction was added to a test tube. Then, 2 ml of distilled water was added followed by the addition of 0.15 ml of sodium nitrite solution (5% NaNO2, w/v). The reaction was allowed to stand for 6 min, and then 0.15 ml sample of 10 % AlCl3 solution was added. The solution was incubated for 6 min after which, 2 ml of 4 % (w/v) NaOH solution was added and the final volume was adjusted to 5 ml with distilled water. The resulting solution was incubated for 15 min before measuring its absorbance at 510 nm using colorimeter. Distilled water was used as blank. Total flavonoid content (TFC) is expressed in mg of quercetin equivalents per gram of the tested fraction. All the determinations were carried out three times.

DPPH• free radical scavenging activity

The total radical scavenging capacities of the four fractions of *P. glutinosa* were determined according to the procedure described in the literature [25] with slight modifications. Ascorbic acid and -tocopherol were used as positive controls. A solution of DPPH• (0.1 mM) was prepared in methanol. A 1.0 ml sample of various concentrations (0.005-0.10 mg/ml) of each prepared fraction in methanol were added to 2 ml of 0.1 mM methanol solution of DPPH•. The solutions were allowed to stand at room temperature in the dark for 30 min. The absorbances of the resulting solutions were then measured at 517 nm against blank samples using UV- visible spectrophotometer. All determinations were performed in triplicate. The ability to scavenge the DPPH• radical was calculated using the following equation:

DPPH• scavenging effect (%) =Ablank-Asample / Ablank ×100%

The antioxidant activity of all samples was expressed as IC50 which is defined as the minimum concentration (in mg/ml) of extract required to inhibit the formation of DPPH• radicals by 50%. [DPPH•] decreases significantly upon exposure to radical scavengers.

ABTS•+ radical scavenging assay

The total antioxidant activity was determined by radical cation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS•+) decolorization assay according to the method described in the literature with some modifications (Al-Qudah et al., 2014). The ABTS•+ cation radical solution was prepared by reacting similar quantities of 7 mM of ABTS and 2.4 mM of potassium persulfate (K2S2O8) solution for 16 hour at room temperature in the dark. Before use, this solution was diluted with methanol until an absorbance value of 0.75 ± 0.02 at 734 nm was obtained. The reaction mixture comprised 3 ml of ABTS•+ solution and 1 ml of the tested fraction at various concentration (0.005-0.10 mg/ml). The absorbance of the mixture was measured at 734 nm by using UV-visible spectrophotometer. A blank was run in each assay and all measurements were done after at least 5 min. The ABTS•+ scavenging capacity of the each tested fraction was compared to those obtained for the positive controls ascorbic acid and α-tocopherol. The percentage inhibition was calculated according to the equation:

ABTS•+ radical scavenging activity (%) = (A_{blank} – A_{sample} /A_{blank}) × 100

%ABTS•+ scavenging ability was expressed as IC50 (mg. ml-1).

Metal chelating activity

The chelating activity for ferrous ions was measured by the method of [26]. The reaction mixture contained 0.5 ml of various concentrations of tested fraction, 1.6 ml of deionized water and 0.05 ml of 2 mM of $FeCl_2$ solution. After 30 s, 0.1 ml of 5 mM ferrozine solution was added. The Fe^{2+} - Ferrozine magenta complex was very soluble and stable in water. After 10 min at room temperature, the absorbance at 562 nm was measured. EDTA and ascorbic acid were used as the chelating standards. The relative activities of the tested fractions to chelate ferrous iron were expressed as percentage (%) of absorbance disappearance as follows:

Metal-chelating activity (%) = (A_c - A_s) / A_c × 100

Where A_s is the reaction mixture absorbance in the presence of the plant tested fraction; A_c is the reaction mixture absorbance in the absence of the tested plant fraction. Chelating activity was expressed as IC_{50}, the concentration that chelates 50% of Fe^{2+} ions.

Result and Discussion

Phytochemical analysis, total phenolics and total flavonoids content

Phytochemical screening of the four fractions of *P. glutinosa* including the hexane (H), aqueous methanol (A), butanol (B) and water (W) revealed the presence of different classes of secondary metabolites including alkaloids, terpenes, sponins, flavonids, anthraquinone and glycosides (Table 1). Both the aqueous methano and butanol fractions were rich in flavonoids while the water fraction contained tannins. This result was further confirmed when the total phenol and total flavonoid contents were evaluated for the different four fractions of *P. glutinosa* from Saudi Arabia (Table 2).

Table1. Major secondary metabolites detected in the crude fractions of *P. glutinosa* growing wild in Saudi Arabia

Class of secondary metabolite investigated	Different tested fractions of *P. glutinosa*			
	Aq. MeOH	Butanol	Water	n-Hexane
Alkaloids	-	-	-	+
Tannins	+	+	+	-
Glycosides	+	+	+	+
Flavonids	+	+	-	-
Anthraquinone	+	-	+	+
Sponins	-	-	+	-
Terpenes	+	+	-	+

The amount of total phenol was determined with the Folin-Ciocalteu reagent according to the procedure listed in the literature [27]. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: $y = 0.009\ x + 0.03$, $R^2 = 0.969$; y is absorbance at 760 nm and x is total phenolic content in the crude fractions of *P. glutinosa.* On the other hand, The flavonoid content of the different fractions was calculated as quercetin equivalent and the results were expressed as mg/g quercetin equivalent using the standard curve equation: $y = 0.011\ x + 0.01$, $R^2 = 0.987$, y is absorbance at 510 nm and x is total flavonoid content in the crude fractions of *P. glutinosa.* Results of the current investigation (Table 2) clearly indicated that the butanol fraction had the maximum phenolics content (199.44 ± 1.10 mg/g of gallic acid) and flavonoids content (164.73 ± 1.96 mg quercetin equivalent in each g dry powder).

Table 2. Total phenolics and flavanoids content of the tested fractions of *P. glutinosa* from Saudi Arabia (results are the mean value of three independent measurements ± SD)

Tested fraction	Total phenolic (mg/g gallic acid)	Total flavonoid (mg quercetin equivalent/g fraction)
A	68.67 ± 2.71	61.27 ± 1.49
B	199.44 ± 1.10	164.73 ± 1.96
W	54.56 ± 3.87	14.64 ± 3.17
H	18.33 ± 1.29	34.45 ± 1.34

A: aqueous methanol, B: butanol; W: water; H: hexane fractions obtained from *P. glutinosa* from Saudi Arabia.

Antioxidant assay and radical scavenging ability

The antioxidant ability and radical scavenging properties of plants are associated with its medicinal values. In this study, the antioxidant activities of *P. glutinosa* different four fractions were measured using three different assay methods including DPPH, ABTS and metal chelation effect in the Fe2+-ferrozin test system.

The antioxidant activities of eight concentrations (0.005 - 0.100 mg/mL) of the each tested fraction were measured with DPPH• test. This method is a valid and easy method to evaluate the antioxidant activity [28]. The results indicate that the radical scavenging activities of the four fractions were concentration dependent (Table 3). The antioxidant activity increased in the following order: hexane extract < water extract<aq. methanol < α- tocopherol < butanol extract < ascorbic acid. Again the butanol fraction, being rich with phenolics and flavonoids, had the highest DPPH• radical scavenging activity (IC50 0.023 ± 1.87 mg/ml) when compared to all other tested fractions. In fact, the butanol fraction was slightly more active than -tocopherol (IC50 0.027 ± 2.10 mg/ml).

The ABTS assay depends on the antioxidant ability to scavenge ABTS•+ radical. By this assay, the antioxidant capacity of lipophilic and hydrophilic compounds in the same sample can be determined. The free radical scavenging ability of the different four fractions of *P. glutinosa* was also determined. According to the current findings (Table 4), the radical scavenging activity was concentration dependent. At a concentration level of 1.0 mg/ml of *P. glutinosa*, the order of free radical scavenging ability of the tested fraction and the positive controls was: Ascorbic acid (98.65± 0.13%) > Butanol extract (92.77 ± 0.56 %) > α- Tocopherol (79.79 ± 4.86%) > aq. methanol extract (72.94 ± 2.14 %) > water extract (67.76 ± 5.09 %) > hexane extract (27.31 ± 5.88 %).

The chelating agents may inhibit radical generations by stabilizing transition metals consequently reducing free radical damage. In addition, some phenolic compounds exhibit antioxidant activity through the chelating of metal ions. The measurement of the metal-chelating activity of an antioxidant is depend on the absorbance measurement of Fe2+-ferrozine complex after prior treatment of a ferrous ion solution with test material. Ferrozine forms a complex with free Fe2+, but not with Fe2+ bound to other chelators; thus, a decrease in the amount of ferrozine-Fe2+ complex formed after treatment indicates the presence of antioxidant chelators. The ferrozine-Fe2+ complex produced a red chromophore with absorbance that can be measured at λ562 nm (Yamaguchi et al., 2000). Fractions of *P. glutinosa* showed metal chelating activity that was enhanced with increasing concentration (Table 5). The chelating effects of aq. Methanol, butanol and water extracts increased linearly with the increase in concentration, and at the concentration of 1.00 mg/ml, the chelating power was 94.94%, 91.20% and 94.26%, respectively, while for EDTA and ascorbic acid, the corresponding chelating effects were 98.62%, 56.59% at 1.00 mg/ml (Table 5). The chelating ability decreased in the following order: EDTA > aq. Methanol > water > butanol > ascorbic acid > hexane. This result indicates clearly that compounds with strong chelating activity in this herb were polar. No significant correlation was found between the iron chelation ability of the fractions and total phenolics.

Table 3. Antioxidant activity of the of extract fractions of *P. glutinosa* and positive controls (ascorbic acid and α-Tocopherol) on DPPH· assay. Results are the mean ± SD of three independent replicates.

Conc (mg/ml)	I% A	I% B	I% H	I% W	I % Ascorbic acid	I% α- tocopherol
0.005	8.76 ± 1.21	7.80 ± 3.58	1.05 ± 0.89	2.11 ± 2.06	49.36 ± 0.52	7.09 ± 1.86
0.01	14.96 ± 5.41	16.81 ± 1.57	3.22 ± 1.46	2.99 ± 1.52	68.88 ± 0.68	16.81 ± 2.05
0.02	27.11 ± 0.87	43.40 ± 3.99	4.03 ± 2.31	7.92 ± 0.50	94.40 ± 0.15	31.85 ± 1.99
0.04	37.28 ± 3.47	86.56 ± 7.56	6.98 ± 1.31	22.54 ± 3.36	94.59 ± 0.24	81.75 ± 1.59
0.05	48.79 ± 1.54	91.62 ± 0.90	7.31 ± 2.00	33.63 ± 1.30	94.60 ± 0.75	93.20 ± 1.51
0.06	65.09 ± 2.33	92.96 ± 2.24	8.56 ± 4.59	41.73 ± 4.47	95.14 ± 0.40	93.26 ± 1.26
0.08	73.85 ± 1.86	94.07 ± 1.78	9.35 ± 2.97	42.25 ±1.85	95.28 ± 0.33	93.33 ± 0.70
0.1	87.92 ± 1.19	94.71 ± 1.50	10.74 ± 1.24	64.26 ± 1.90	95.74 ± 0.13	93.49 ± 0.90
IC_{50}	0.052± 1.04	0.023 ± 1.87	ND	0.091 ± 164	0.006 ± 0.54	0.027 ± 2.10

A: aqueous methanol, B: butanol; W: water; H: hexane fractions obtained from *P. glutinosa* from Saudi Arabia; ND: not determined

Table 4. Antioxidant activity of the of the extract fractions of *P. glutinosa* and positive controls (ascorbic acid and α-Tocopherol) on $ABTS^{\bullet+}$ assay. Results are the mean ± SD of three independent replicates.

Conc (mg/ml)	I% A	I% B	I% H	I% W	% Ascorbic acid	I% α- Tocopherol
0.005	2.66 ± 2.97	10.02± 1.39	0.65 ± 0.09	17.55 ± 1.55	10.96 ± 0.41	12.21 ± 0.50
0.01	9.23 ± 1.24	23.04 ± 0.93	3.00 ± 2.43	23.98 ± 1.37	17.81± 0.49	15.86 ± 1.46
0.02	33.13 ± 1.34	38.78 ± 8.09	4.95 ± 3.62	29.03 ± 1.36	66.91 ± 1.92	22.47 ± 1.24
0.04	44.83 ± 1.99	65.29 ± 2.50	9.54 ± 1.95	42.60 ± 1.42	97.43 ± 0.60	23.96 ± 2.19
0.05	42.23 ± 1.53	91.57 ± 1.00	11.43 ± 2.15	52.45 ± 1.02	97.47 ± 0.65	39.04 ± 1.95
0.06	55.11 ± 1.54	92.17 ± 0.86	12.60 ± 1.72	52.86 ± 2.56	97.11 ± 0.73	44.45 ± 2.12
0.08	68.79 ± 1.76	92.02 ± 0.59	20.71 ± 2.16	61.28 ± 1.97	98.11± 0.35	56.11 ± 6.28
0.1	72.94 ± 2.14	92.77 ± 0.56	27.31 ± 5.88	67.76 ± 5.09	98.65± 0.13	79.79 ± 4.86
IC_{50}	0.057± 1.74	0.028 ± 1.87	ND	0.046 ± 1.76	0.014 ± 0.73	0.071 ± 1.68

A: aqueous methanol, B: butanol; W: water; H: hexane fractions obtained from *P. glutinosa* from Saudi Arabia.

Table 5. Metal chelating effect on ferrous ions of the extract fractions of *P. glutinosa* and positive controls (EDTA and ascorbic acid). Results are the mean ± SD of three independent replicates.

C(mg/ml)	I% A	I% B	I% H	I% W	I% EDTA	I% Ascorbic acid
0.005	49.26 ± 2.51	30.93 ± 1.95	6.25 ± 2.50	21.60 ± 1.86	53.74 ± 2.70	18.70 ± 3.01
0.01	86.55 ± 2.40	38.00 ± 1.66	7.64 ± 1.80	49.45 ± 1.96	68.46 ± 3.10	32.52 ± 1.20
0.05	89.89 ± 1.15	46.55 ± 1.05	6.74 ± 2.34	51.81 ± 1.13	94.31 ± 0.28	40.41 ± 2.51
0.10	90.72 ± 1.22	54.23 ± 1.22	6.62 ± 4.19	67.34 ± 2.12	97.15 ± 0.84	43.66 ± 2.68
0.50	90.80 ± 1.55	63.91 ± 2.95	7.97 ± 5.62	91.05 ± 4.41	97.64 ± 0.60	46.50 ± 1.03
1.00	94.94 ± 1.23	91.20 ± 0.69	8.04 ±1.07	94.26 ± 2.91	98.62 ± 0.71	56.59 ± 2.26
IC_{50}	0.005 ± 1.24	0.081 ± 1.77	ND	0.009 ± 1.84	0.005 ± 0.54	0.94 ± 2.10

A: aqueous methanol, B: butanol; W: water; H: hexane fractions obtained from *P. glutinosa* from Saudi Arabia.

Conclusion

The results of the current study showed that the butanol fraction, among the different fractions obtained from *P. glutinosa* growing wild in Saudi Arabia, contained high flavonoid and phenolic contents and exhibited the greatest antioxidant activity, as measured by the different assay methods. Moreover, the results also indicate that the aqueous methanol, butanol and water fractions are potential sources of secondary metabolites with interesting antioxidant activity. Further studies are needed to evaluate the in vivo potential of these fractions in animal models and also isolation and characterization of the active antioxidant compounds. Determination of the antioxidant compounds of plant extracts will help to develop new drug supplement for antioxidant therapy.

References

[1]. Alghaithy AA, El-Beshbishy HA, Abdel-Naim AB, Nagy AA, Abdel-Sattar EM Anti inflammatory effects of the chloroform extract of *Pulicaria guestii* ameliorated the neutrophil infiltration and nitric oxide generation in rats. Toxicol. Ind. Health. 2011;27: 899–910.

[2]. Al-Hazimi HMG, Al-Khathlan HZ). Chemistry of Variuos Pulicaria spceies (Asteraceae). Jour. Chem. Soc. Pak. 1992;14: 233-240.

[3]. Al-Jaber HI, Abrouni K, Al-Qudah MA, Abu Zarga MH New terpenes from *Salvia palaestina* Benth. and *Salvia syriaca* L. growing wild in Jordan. J Asian Nat. Prod. Res. 2012;14: 618-25.

[4]. Al-Qudah MA, Al-Ghoul AM, Trawenh IN, Al-Jaber HI, Al Shboul TM, Abu Zarga MH, Abu Orabi ST. Antioxidant activity and chemical composition of essential oils from Jordanian *Ononis Natrix* L. and *Ononis Sicula* Guss. JBAPN. 2014;4:52–61

[5]. Al-Qudah MA, Al-Jaber HI, Abu Zarga MH, Abu Orabi ST Flavonoid and phenolic compounds from *Salvia palaestina* L. growing wild in Jordan and their antioxidant activities. Phytochemistry, 2014;99:115-120.

[6]. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO. Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. Trop. J. Pharm. Res. 2008; 7:1019-1024.

[7]. Banso A, Adeyemo S. Phytochemical screening and antimalarial assessment of *Abutilon mauritianum*, *Bacopa monnifera* and *Datura stramonium*. Biokrmidtri. 2006;18:39-44

[8]. Cai YZ, Sun M, Corke H. Antioxidant activity of betalains from plants of the Amaranthaceae. J. Agric. Food Chem. 2003;51: 2288–2294.

[9]. Chlopicka J, Pasko P, Gorinstein S, Jedryas A, Zagrodzki P. Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. LWT – Food Sci. Tech. 2012;46: 548–555.

[10]. Christine AW, Jeffrey BH, Jenny G, Renee JG, Geoffrey CK, John E. Variation in lipophilic and vacuolar flavonoids among European *Pulicaria* species. Phytochemistry. 2003;64: 275–283.

[11]. Das B, Ravinder RM, Ramu R, Ravindranath N, Harish H, Ramakrishna KV, Koteswar RY, Harakishore K, Murthy US. Clerodane diterpenoids from *Pulicaria wightiana*. Phytochemistry. 2005;66: 633–638.

[12]. Dinis TCP, Madeira VMC, Almeida MLM. Action of phenolic derivatives (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 1994;315: 161–169

[13]. Ezoubeiri A, Gadhi CA, Fdil N, Benharref A, Jana M, Vanhaelen M. Isolation and antimicrobial activity of two phenolic compounds from *Pulicaria odora* L. J. Ethnopharmacol. 2005;99: 287–292.

[14]. Farooq, M. Neuro-protective activity of Pulicaria glutinosa in oxidative stress-induced neurotoxicity in zebrafish embryos. Int. J. Agric. Biol., 2015;17: 193–198

[15]. Ghouila H, Beyaoui A, Jannet HB, Hamdi B, Salah AH, Mighri Z. Isolation and structure determination of pulicazine, a new sesquiterpene lactone from the Tunisian *Pulicaria laciniata* (Coss.et Kral.) Thell. Tetrahedron Letters. 2009;50: 1563–1565.

[16]. Joshi A, Bhobe M, Saatarkar A. Phytochemical investigation of the roots of *Grewia microcos* Linn. J. Chem. Pharm. Res. 2013;5: 80–87.

[17]. Khan M, Khan M, Adil SF, Tahir MN, Tremel W, Alkhathlan Alkhathlan HZ, Al-Warthan A, Siddiqui MRH. Green synthesis of silver nanoparticles mediated by Pulicaria glutinosa extract. Int. J. Nanomedicine. 2013;8: 1507–1516.

[18]. Liu LL, Yang JL, Shi YP. Phytochemicals and biological activities of *Pulicaria* species. Chem. Biodivers.2010;7: 327–349.

[19]. Marco JA, Sanz JF, Albiach R. Caryophyllene derivatives from

Pulicaria dysenterica. Phytochemistry. 1992;31: 2409–2413.

[20]. Mossa JS, Muhammad I, El-Feraly FS, Huffor CD, Mcphail DR, McPhail AT. Bisabolene and guaiane sesquiterpenes from *Pulicaria glutinosa*. Phytochemistry, 1992;31: 575-578.

[21]. Muhammad I, El-Feraly FS, Mossa JS, Ramadan AF. Terpenoids from *Pulicaria glutinosa*. Phytochemistry. 1992;31: 4245–4248.

[22]. Nickavar B, Mojab F. Antibacterial activity of *Pulicaria dysenterica* extracts. Fitoterapia. 2003;74: 390–393.

[23]. Singh P, Sharma MC, Joshi KC, Bolhamann F. Diterpenes derived from clerodanes from *Pulicaria angustifolia*. Phytochemistry.1985;*24*: 190–192.

[24]. Stavri M, Mathew KT, Gordon A, Shnyder SD, Falconer RA, Gibbons S. Guaianolide sesquiterpenes from *Pulicaria crispa* (Forssk.) Oliv. Phytochemistry.2008;69: 1915–1918.

[25]. Williams CA, Harborne JB, Greenham J. Geographical variation in the surface flavonoids of *Pulicaria dysenterica*. Biochem. Syst. Ecol. 2000;28: 679–687.

[26]. Williams CA, Harborne JB, Greenham JR, Grayer RJ, Kite GC, Eagles J. Variations in lipophilic and vacuolar flavonoids among European *Pulicaria species*. Phytochemistry. 2003;64: 275–283.

[27]. Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H. Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. J. Agric. Food Chem. 2000;*48*: 180–185.

[28]. Zhao H, Fan W, Dong J, Lu J, Chen J, Shan L. Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. Food Chemistry. 2008;107: 296.