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Effect of polar and nonpolar solvent on total phenolic and antioxidant activity of roots extracts of *Caralluma europaea*

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ABSTRACT

Caralluma europaea have been used recently as a remedy in Morocco folk medicine, health foods and in numerous other purposes. In the present study, the antioxidant activity of C. Europaea roots extracts from morocco cultivar Zouiatcheikh harvested, using solvents of increasing polarity (hexane, chloroform, dichloromethane, ethyl acetate, acetone, ethanol and methanol) was evaluated using DPPH radical-scavenging assay. The total ascorbic acid, pigment and anthocyanins as well as total phenolics and flavonoids from roots of C. Europaea cultivar were also evaluated. The extracts were found to have different levels in antioxidant proprieties in the test solvent used. The efficiency of the solvents used to extract phenolic compounds from the C. Europaea roots varied considerably. Total polyphenol contents were significantly higher in methanol extract than chloroform and ethanol fractions ($p<0.05$). Ethanol and ethyl acetate extracts in roots was found to be rich in flavonoids (834.7 and 844.2 CEQ/100g DW, respectively). No relationship was observed in the potential antioxidant activity and total phenolic levels of the extracts mean that further investigation on the antioxidant activity of C. Europaea roots was needed.

Key words: Antioxidant, Caralluma europaea, Phenolic, Routs, Fresh.

INTRODUCTION

Cause of a big diffusion of infectious disease and the side effects of modern medicines 50,000 people die worldwide every day[1]. This made scientists and general public use plants as a source of new or complimentary medicinal products[2]. The medicinal value of these plants lies in some chemical active substances like: alkaloids, tannin, flavonoid and phenolic compounds. These products can produce definite physiological action on the human body [3].

Caralluma europaea belong to the genus caralluma of the family Apocynaceae (Asclepiadoideae). There are about 87-133 species Caralluma verities throughout the world, which is a testament to their value to human kind. It is native to Morocco, where it occurs on the slopes of Anti-Atlas, Middle Atlas and the Rif. Distributed in South-Eeastern Spain, southern Italy (Linosa, Lampedusa), Tunisia, Libya, and Egypt to Jordan and on the island of Lampedusa where it was first discovered by Gussone in 1832 [4,5]. *C. Europaea* has been reported to have anticancer activity, in Morocco folk medicine, shows a wide spectrum of action including antitumor activity the patients treat the general cancer by mixed grind parts with honey. In literature there are a few researches regarding this species but several member of genus Caralluma have found medicinal use as anti-diabetic, anti-hyperglycemic, antiparasitic, antitrypanosomal, anti-ulcer, carminative, febrifugal, anti-pyretic, anti-infammatory, anti-nocicepetive, anti-oxidant, anti-obesogenic and anti-artherosclerotic properties [6,7,8,9]

This work was to determine the total phenolic and flavonoid contents of hexane, chloroform, dichloromethane, ethyl acetate, acetone, ethanol and methanol extract from *C. Europaea* roots. Additionally, the antoxidative properties were evaluated by DPPH radical-scavenging assays, in attempt to establish the best solvents for extract the antioxidants compounds.

MATERIALS AND METHODS

2.1 Chemicals and reagents

Folin–Ciocalteu reagent, sodium carbonate anhydrous (Na_2CO_3), gallic acid (GA), aluminium chloride (AlCl3), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), hydrogen chloride (HCl), 2,2-Diphenyl-1- picrylhydrazyl (DPPH), quercetin, ascorbic acid (AA), dinitrophenyl hydrazine (DNPH), thiourea, sulfate pentahydrate ($CuSO_4$. $5H_2O$), sulfuric Acid (H_2SO_4), trichloracetic Acid (TCA). All other chemicals and solvents used were of analytical grade.

2.2 Plant and sample material

Fresh roots of cultivar Zouiatcheikh of *C. Europaea* were collected from the region -Beni-Mellal- Morocco. A voucher specimen of the roots was identified and authenticated at the laboratory of botany at the biology institute.

2.3 Determination of pigment content

The procedure was carried out at 4°C and dark. A roots sample (0.25g) were mashed in a mortar and pestle with 80% acetone (v/v), the extract was filtered through two layers of nylon and centrifuged in sealed tubes at 15,000 x g for 5min .The supernatant was collected and the absorbance was read at 663 and 647nm for chlorophyll a and chlorophyll b, respectively, and at 470nm for carotenoid content. The concentrations for Chlorophyll a, Chlorophyll b, and the sum of roots carotenoids (xanthophyll's and carotenes) were given in µg ml-1 solution according to the equation of Lichtenthaler and Buschmann (2001)[10]:

Chlorophyll a = 12.25A663−2.79A647
Chlorophyll b = 21.50A647−5.10A663

$$\text{Carotenoid} = \frac{1000A470 - 1.82\text{Chla} - 95.15\text{Chlb}}{225}$$

Total chlorophyll= Concentrations were added to give total chlorophyll expressed in µg ml^{-1} of extract.

2.4 Determination of anthocyanin content

Anthocyanin content of roots was analyzed according to Padmavati et al. (1997) [11] modified by Chung et al. (2005)[12]. The roots (1g) were extracted with 25mg/ml of acidified methanol (1% HCl) for 2h at room temperature in the dark, and then centrifuged at 1000 x g for 15min. anthocyanin levels were calculated from the methanolic extract as A530-(0.24 x A653). Total anthocyanin content was determined as mg cyaniding 3-glucoside equivalents (CyE) per 100g of fresh weight, using an extinction coefficient of 26.900 l mol^{-1} cm^{-1} at 530nm and a molar mass (MW) of 449.2 g mol^{-1}[13].

$$\text{Anthocyanin (mg/100g)} = \frac{\text{Ab x MW x V x 100}}{\varepsilon \text{ x G}}$$

2.5 Determination of ascorbic acid content

The ascorbic acid was determined using the method of Benderitter et al. (1998)[14]. Briefly 75µl of DNPH (2g of dinitrophenyl hydrazine, 230mg thiourea and 270mg of $CuSO_4.5H_2O$ in 100ml 5M H2SO4) was added to 500µl reaction mixture (300µl of the different extracts with 100µl 13.3% TCA and water, respectively). The reaction mixture was subsequently incubated for 3h at 37°C, then 0.5ml H2SO4 65% (v/v) was added to the medium, and the absorbance was measured at 520nm. The vitamin content of the sample was subsequently calculated, using an ascorbic acid as standard curve.

2.6 Preparation of plant extracts

Fresh roots were washed with distilled water and cut into cubes with a stainless steel knife, dried in an oven at 37°C for two days after which they were crushed up and ground to get homogeneous fine powder by a grinder. For extraction, seven different solvents in ascending polarity (hexane, chloroform, dichloromethane, ethyl acetate, acetone, ethanol and methanol) were used to fractionate the soluble compounds from the *C. Europaea* roots. The fresh roots sample (5g) was extracted with 50ml of each solvent with simple maceration at room temperature for 24h and then the solvents from the combined extracts evaporated by vacuum rotary evaporator (HEIDOPH VV 2000). After filtration, all extracts obtained were then transferred to vials and kept in the dark at -20°C prior to use.

2.7 Determination of extract yield percentage

The extraction yield is a measure of the solvents efficiency to extract specific components from the original material and it was defined as the amount of extract recovered in mass compared with the initial amount of whole roots. It is presented in percentage (%).

2.8 Determination of polyphenol compounds

Total phenolic contents of fraction were determined according to the method of Singleton et al. (1999)[15] cited by Wolfe et al. (2003)[16]. 50µl of each fraction and 250µl of Folin-Ciocalteu reagent were mixed. After 15 seconds incubation, 500µl of sodium carbonate (20%) was added and the mixture was diluted with 5ml of distilled water. The tubes were kept in the dark for 30min and the absorbance was measured at 727nm. The totalphenolic contents were expressed on a dry weight basis as mg hydroxytyrosol equivalents/100g of sample.

2.9 Determination of total flavonoids

The total flavonoid content (TFC) of the roots extracts were determined according to the colorimetric assay developed by Zhishen et al. (1999)[17]. One ml of properly dilated roots extract was mixed with 5ml of distilled water. At zero time, 300µl of (5%, w/v) $NaNO_2$ was added. After 5min, 300µl of (10%, w/v) $AlCl_3$ was added. At 6min, 2ml of 1M solution of NaOH were added. Finally, the volume of the mixed is immediately adjusted to 10ml with distilled water. The mixture was shaken vigorously and the absorbance was read at 510nm. The results were also expressed on a dry weight basis as mg catechin equivalents (CEQ)/100g of sample.

2.10 Antioxidant Activity (DPPH free radical scavenging activity) determination

The DPPH method[18] was used to determine antioxidant activity of *C.europaea* roots extracts. 10-µl from the stock solution of the sample were dissolved in absolute methanol to a final volume of1ml and the added to 250µl of methanolic DPPH solution (6mg/100ml). The reaction mixture was kept at room temperature. The optical density (OD) of the solution was measured at 515nm, after 30min of incubation. The percentage reduction values were determined and compared to appropriated standards. The optical densities of the samples in the absence of DPPH were subtracted from the corresponding OD with DPPH. The % reduction were determined and compared to appropriate standard. Inhibition of the free radical DPPH, in percent (I %) was calculated using the following equation:

$$I\,\% = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \text{X } 100$$

Where Ablank is the absorbance of control reaction (containing all reagent except the tested compound), and Asample is the absorbance of tested compound.

2.11 Statistical analyses

All assays were run in triplicate. The results are reported as mean values of three analysis and standard deviation. Data were subjected to statistical analysis using the SPSS programme. P less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

3.1 Chlorophyll content

The chlorophyll is one of the antioxidant compounds which widely used as additive or supplements in the food industry, cosmetics, pharmaceutical and livestock feed and also have a positive effect in human health[19]. The table below shows that the content of chlorophyll of *C. Europaea* roots estimated by 5.62µg/ml includes 3.47µg/ml for Chl a and 2.15µg/ml for Chl b regarding carotenoids which play also a huge role in the fields of nutrition and health we found in in our data the value of 0.58µg/ml.

Table (1): Total pigment, anthocyanin and ascorbic acid content of *C. Europaea* roots

Compounds	Values
Chlorophyll a (ug ml^{-1})	3.47 ± 0.04
Chlorophyll b (ug ml^{-1})	2.15 ± 0.07
Total chlorophyll (ug ml^{-1})	5.62 ± 0.11
Carotenoids (ug ml^{-1})	0.58 ± 0.05
Total anthocyanins (mg/100g FW)	0.08 ± 0.01
Ascorbic acid (mg ml^{-1})	5.87 ±1.23

Mean composition of sampled roots from three replications of three individual samples (three plants each) ± standard deviation.

3.2 Ascorbic acid content

Ascorbic acid is a good reducing agent and exhibits its antioxidant activities by electron donation[20]. Moreso; it is widely distributed in plant cells where it plays many crucial roles in growth and metabolism. As a potent antioxidant, ascorbic acid has the capacity to effects on cancer, blood pressure, immunity, drug metabolism and urinary excretion of hydroxyproline[21]. The ascorbic acid content of the *C. Europaea* roots also presented in Table 1. The recorded content is the order of 5.879 ± 1.238 mg/100g, demonstrating the wealth of *C. Europaea* roots of this vitamin.

3.3 Extraction yields percentage

The extraction yield of the *C. Europaea* extracts is shown in Figure 2. In our study, different solvents with increased polarity were used to determine which gave the greatest recovery of phenolic compounds. Based on our results reported here, the extraction yields by various solvents ranged from 0.35%-5.05% and decreased in the following order: methanol> ethanol> chloroform> acetone> dichloromethane> ethyl acetate> hexane. This shows that the extraction yield increases with increasing polarity of the solvent used in extraction. Among the different solvent extractions, the successive methanol solvents found to have higher recovery over other system used.

3.4 Total phenolic compounds

Phenolic compounds in plants constitute a major class of secondary plant metabolites with bioactive potential attributed to antioxidants activity. Figure 3, shows the phenols concentration in the roots extracts, expressed as milligrams of hydroxytyrosol equivalents per 100 grams of dry weight. Result show that the amount of total phenolic contents of the roots extracts by different solvents (hexane, chloroform, dichloromethane, ethyl acetate, acetone, ethanol and methanol) occurred in the range of 149.9-1136.3 mg/100g DW and decrease in the following order: methanol> ethanol> acetone> chloroform> dichloromethane>ethyl acetate>hexane (Fig 3). The recovery of phenols from plant materials is influenced by solubility of the phenolic compounds in the solvent used for the extraction process. Otherwise, solvent polarity will play a key role in increasing phenolic solubility [22]. The result given in Figure 3 showed the amount of phenolic compounds in the methanol and ethanol extracts was higher 1136.3-653.4 mg/100g respectively than in the acetone extract also total phenolic content obtained with ethanol was 2.4 times that of acetone it was by high extraction yield and hence its contribution in the *C. Europaea* roots was significant. For other solvent had similar concentration but comparatively small of total phenolic contents. The results of multivariate dispersion analyses showed that used solvent are significant factors affecting of total phenolic content ($p<0.05$).

3.5 Flavonoids and anthocyanin contents

Anthocyanins comprise of a large group of water-soluble pigments which pertain to the flavonoid class. From the results shown in Table 1 the content of anthocyanin ranged from 0.08±0.01mg/100g. It was reported that some pharmacological activities of polyphenol compounds, like anthocyanosides, may be attributed to their antioxidant properties [23].

Total flavonoid, as one of the most diverse and widespread groups of natural compounds, are probably the most natural phenolics[24]. Total flavonoid contents of various solvent extract from roots are summarized in Figure 4. These extracts were found to have various levels of flavonoids ranging from 255.9-844.2 mg/100g. In fact the content of flavonoids in the roots extract decreased in the order of ethyl acetate>ethanol> acetone >methanol >dichloromethane> chloroform > hexane. The concentration of flavonoids in ethyl acetate extract was 844.2 mg/100g, which was very similar to the value of ethanol extract concentration. The lowest flavonoid concentration was measured in chloroform and hexane extract. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation [25].

Both flavonoid and phenolic compounds are known to have multiple biological effects, including antioxidant and anti-inflammatory properties [26] and may also be responsible for biological actions of *C. Europaea* roots. The results presented herein showed no positive correlation between total phenolic compounds and total flavonoids but from our data it can be deduced that flavonoids are the major fraction relative to total phenols in all extract.

3.6 DPPH radical scavenging activity assay

The DPPH is stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants [27]. In order to evaluate antioxidant activity; DPPH radical scavenging activities was measured from roots extracts and of % inhibition are given in Figure 5. The methanol extracts show the highest antioxidant capacity in the roots extract with concentration of 10ug/ml compare to other system. DPPH scavenging activity ranged from 59.41- 68.70% and followed the order of effectiveness as:

methanol (68.41%)> hexane (67.90)>acetone (66.31%)> ethanol (64.72%)> ethyl acetate (63.39%)> chloroform (59.41%). There are studies in the literature that report a positive correlation between antioxidant activity and the quantity of phenolic compounds[28, 29]. In addition, there are also studies which report that there is no positive relationship[30, 31]. In our study as well, a positive correlation was not observed.

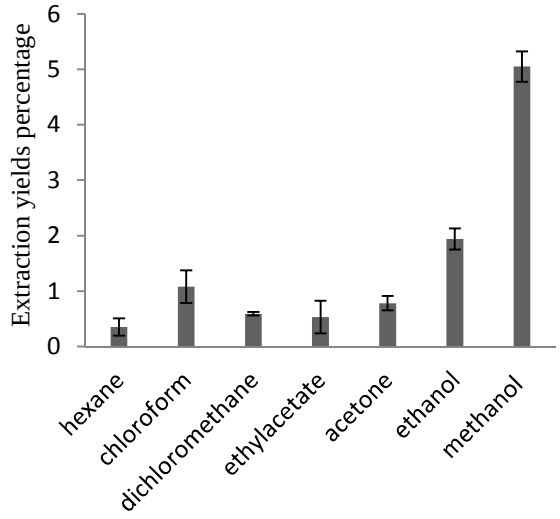

Figure (2): Percentage yield of C.europaea roots extracts in different solvents

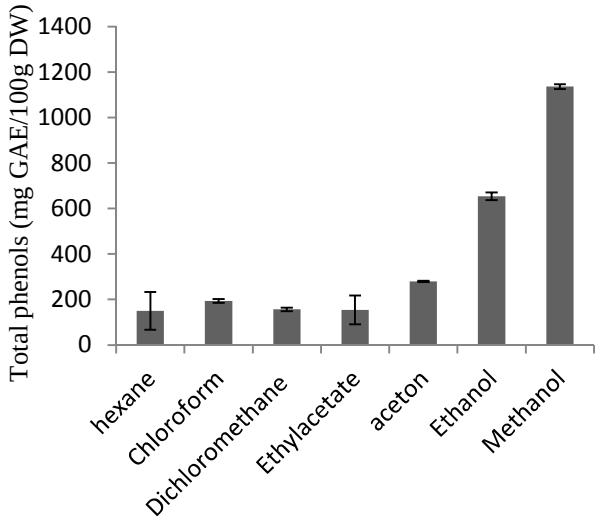

Figure (3): Total phenolic content of extracts of C. europaea roots by different solvent extraction. Results are expressed as hydroxytyrosol equivalents. Values are expressed as means ± standard deviation (n=3).

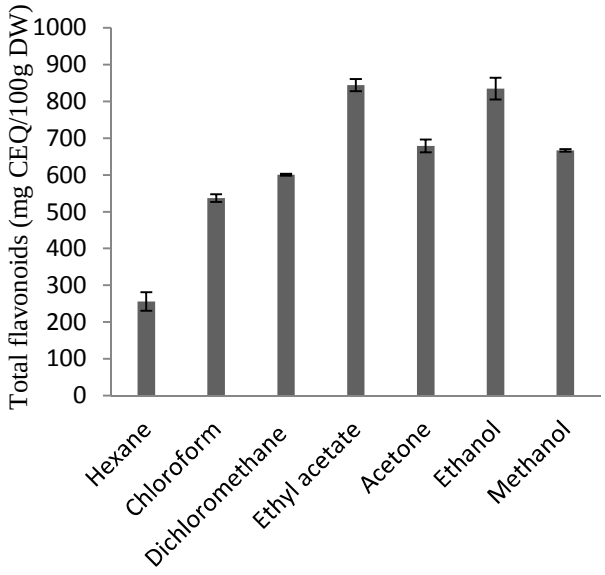

Figure (4): Total flavonoids content (as catechin equivalents) in extracts from C. europaea roots. Results are expressed as hydroxytyrosol equivalents. Values are expressed as means ± SD deviation (n=3).

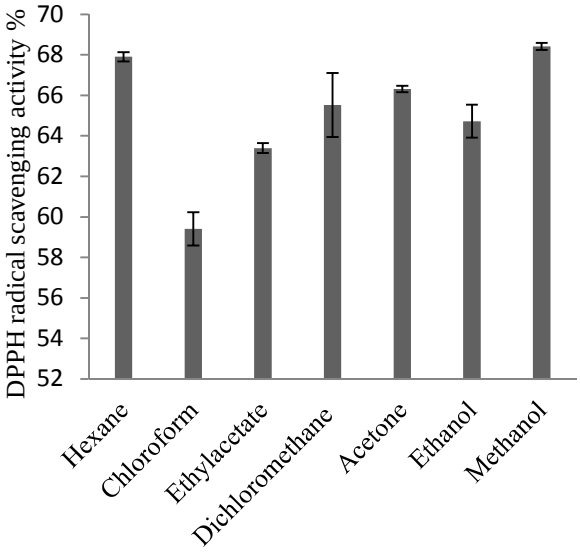

Figure (5): Free radical-scavenging capacities of the extracts measured in DPPH assay. Values are expressed as means ± standard deviation (n=3).

CONCLUSION

In conclusion, our data suggested that the recovery of phenols and flavonoid content in *C. Europaea* roots is mainly affected by the solvent system used. The roots extract are effective in scavenging radicals when assessed by DPPH assay. Accordingly, in this study, the antioxidant activity of roots extract is independent of the amount of total phenols which implies the existence in the extracts of antioxidant molecules in very small

quantities. Therefore, it is suggested that more work could be performed on the isolation and identification of the molecules responsible has this activity and to deduce relationships chemical structure-activity.

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REFERENCES

[1] Polat R., Satıl F.,*J. Ethnopharmacol*, **2012**, 139:626-641.
[2] Premanath R., Lakshmideri, N., *Journal of American Science*, **2011**, 6(10): 736-743.
[3] Aiyelaagbe O.O., Osamudiamen PM.,*Plant Science Research*, **2009**, 2(1):11- 13.
[4] Pietro Z., Maurizio S., Caralluma europaea on Lampedusa Island. Asklepios,**2011**,112:3-14.
[5] Meve U., Heneidak S.,*J. Linn.Soc*, **2005**, 149: 419-432.
[6]Kamalakkannan S., Rajendran R., VenkateshR.v., Clayton P., Akbarsha M.A.,*Journal of Nutrition and Metabolism*, **2010**, 1155:285-301.
[7]Abdel-sattar E., Shehab N.G., Ichino C., Kiyo hara K., Ishiyama A.,Otoguro K., OmuraS.,Yamada H.*Phytomedicine*, **2009**, 16:659-994.
[8]Sreelatha V.R., Rani S.S., Reddy P.V.K., Naveen M., Ugraiah A., Pullaiah T., *Indian Juornal of Biotechnology*,**2009**, 8:236-239.
[9]Zakaria M.N.M., Islam M.W., Radhakrishnan R., Chen H.B., Kamil M., Al-Gifri A.N., Chan K., Al-Attas A.,*Journal of Ethnopharmacology*, **2011**, 76:155-158.
[10] Lichtenthaler H.K., Buschmann C.,Food Analyticial chemistry (CPFA), (supplement 1). Wiley, New York,**2001**.
[11] Padmavati M., sakthivel N., Thara K.V., Reddy A.R.,*Phytochimistry*, **1997**, 46:499-502.
[12] Chung Y.C., Chen S.J., Hsu C.K., Chang C.T., Chou S.T., *Walthar food Chem*, **2005**, 91:419-424.
[13] Giusti M.M., Wrolstad R.E., *Food analytical chemistry*, **2000**, pp.F1.2.1-F1.2.13.
[14] Benderitter M., Maupoil V., Vergely C., Dalloz F., Briot F., Rochette L., *Fundamentals ClinPharmacol*, **1998**,12: 510– 516.
[15] Singleton V.L., orthofor R., Lamuela-Raventos R.M.,*Methods enzymol*,**1999**, 152-177.
[16] Wolfe K., Wu X., Liu R.H.,*Journal of agricultural and food chemistry*, **2003**, 51:609-614.
[17] Zhishen j., Mengcheng T., jianming W.,*Food chemistry*,**1999**, 64:555-559.
[18] Campos M. G., Webby R. F., Markham K. R., Mitchell K. A., Cunha A. P.,*Journal of Agricultural and Food Chemistry*, Washington, **2003**, 51:742-745.
[19] Adamson Shodehinde S., Oboh G., *Asian Pac J Trop Biomed*, **2013**, 3(6): 449–457.
[20] Oboh G., Akindahunsi A.A.,*Nutr. Health*,**2004**,18: 29–36.
[21] José Luis S.B., Maríadel Socorro S.S.,*INTECH*, **2013**,450-485
[22] Naczk M., Shahidi F.,*J.Pham.biomed.Anal*, **2006**, 41:1523-1542.
[23] Martin-agaron S., Basabe B., Benedi, J.M.,Villar,A.M., *Phytother.Res*,**1998**, 12:S104-S106.
[24] Shimoi K., Masuda S., Shen B., Kinae N.,*Mutat.Res*,**1996**, 350:153-161.
[25] Min G., Chun-Zhao L.,**World J. Microb. Biot**,**2005**, 21:1461-1463.
[26] Amarowicz R.,*Eur. Lipid Sci. Technol*, **2007**, 109: 549–551.
[27] Sanchez-Moreno, C., *Food Sci.Technol.Int*, **2002**, 8, 121-137.
[28] Faujan N.H., Noriham A., Norrakiah A.S., Babji A.S., *J. Biotechnol*, **2009**, 8:484–489.
[29] Hesam F., Balali G.R., Tehrani R.T., *AJP*, **2012**, 2:79–85.
[30] Li X., Wu X., Huang L.,*Angelicaesinensis (Danggui) Molecules*,**2009**, 14:5349–5361.
[31] Rafat A., Philip K., Muniandy S., *J. Biotechnol*, **2010**, 5:16–19.