EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 11
453-457
Year: 2016

RANUNCULUS LAETUS: EVALUATION OF ITS TOTAL PHENOL CONTENT, TOTAL FLAVONOID CONTENT AND REDUCING POWER ASSAY (ANTIOXIDANT PROPERTY).

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Article Received on 19/09/2016 **Article Revised on 10/10/2016** **Article Accepted on 31/10/2016**

ABSTRACT
In the present study total phenol content, total flavonoid content and antioxidant activity was evaluated from the ethanolic leaf extract of *Ranunculus laetus*. Total phenol content was revealed by using Folin-Ciocalteu reagent, total flavonoid content was determined by Aluminum trichloride spectrophotometer method. The ferric reducing power assay was used to evaluate the antioxidant activity of ethanolic leaf extract. Our results showed that ethanolic leaf extract of *Ranunculus laetus* contained a very good amount of phenols, flavonoids and antioxidant property.

KEY WORDS: *Ranunculus laetus*, Total phenol content, total flavonoid content, Ferric reducing power assay.

INTRODUCTION
Right from the primitive period to present day the herbal medicines attain particular position, Greek medicine is found to be the origin of this system, which was adopted by Arabs and it subsequently flourish to India and Europe.[1] In India about 75% of drugs and perfumery products derived from plants are used World-wide.[2] Medicinal plants are widely used as alternative therapeutic tools for the treatment of various diseases. The recent studies have determined that the antioxidant effect of plant products is mainly credited to compounds such as phenolic, flavonoids, tannins etc.[3, 4]

All living beings are provided with antioxidant defense systems to counterbalance harmful effects caused by free radicals. When our endogenous antioxidant defense system fails, antioxidants need to be supplemented from outer sources. Various foods are known to be the best source of antioxidants.[5] Antioxidants help in the prevention of human diseases as they function as free radical scavengers. Antioxidants are frequently used in oils and fatty foods to slow down their auto-oxidation. So, the search for natural antioxidants has greatly increased in recent years.[6] There are about eight thousand naturally occurring plant phenolic compounds and about half of these numbers are flavonoids.[7] Phenolic compounds, the largest group of phytochemicals possess most of the antioxidant activity in plants or plant products.[8] Phenolic compounds acquire a wide range of biochemical activities such as antioxidant, anti-carcinogenic, anti-mutagenic, as well as ability to amend the gene expression.[9] The major antioxidants of our diet are contributed to phenolic compounds.[10] Similarly, Flavonoids act as antioxidants and therefore, giving protection against cardiovascular disease, certain forms of cancer and age related degeneration of cell components. Polyphenolic nature of flavonoids enables them to scavenge free radicals such as super oxide and hydroxyl radicals.[11] Free radicals are responsible for number of disorders, including cancer, neuro-degeneration and inflammation.[12] The antioxidants such as phenolics, flavonoids, tannins and proanthocyanidins provide protection against a large number of diseases.[13] Besides this, various studies showed that these compounds possess other biological effects, including antimicrobial, anti-inflammatory, anti-allergic, anti-platelet, anti-mutagenic, anti-carcinogenic and vasodilatory actions.[14] Various herbal drugs possessing free radical scavenging activity are famous for their therapeutic activity.[15] In this context, various plants have been investigated for evaluation of the total phenolic and total flavonoid content like *Merremia borneensis*[16], *Ageratina adenophora*[17], *Tordylium maximum*[18], *Marrubium peregrinum*[19] etc.

Ranunculus laetus, an important medicinal herb of Kashmir, belongs to family Ranunculaceae. The fresh leaf paste is used to cure skin diseases when applied once

a day for 1-2 days.[20] Also, plant decoction is used to treat ingestion.[21] Since, the plant is widely spread and a few studies have been carried for its pharmacological activities. The major aim of our investigation was to determine the total phenolic and total flavonoid content, and the antioxidant property by reducing power assay using ethanolic leaf extract of *Ranunculus laetus*.

MATERIAL AND METHODS

The plant herb *Ranunculus laetus*, collected in the month of August from village Lar Ganderbal J&K. The plant was identified and authenticated by Dr. Akhtar H. Malik, Centre for Biodiversity and Taxonomy, University of Kashmir. A voucher specimen, bearing No. 2078KASH was deposited in the same department for future reference. Detached leaves were from the plant and were thoroughly washed with distilled water to remove any foreign matter. Leaves were dried under shade for about a week and then powdered with the help of grinder. The powder was then subjected to soxhlation by using 90% ethanol. The extract was then subjected to Rotary vacuum evaporator, the semi-solid material obtained was then stored in storage bottles and kept at - 4^{o}C for future investigation.

Determination of Total phenol content[22]

The total phenolic content of ranunculus leaves was determined by Folin-Ciocalteu reagent. Gallic acid was used as reference for obtaining calibration curve. Various concentrations of Gallic acid (10-100µg/ml) were prepared in methanol. Also, test sample (100µg/ml) was prepared in methanol. The 0.5ml test sample was mixed with 2 ml of Folin-Ciocalteu reagent (1:10 in deionized water). The reaction mixture was neutralized by adding 4ml of Sodium Carbonate solution (7.5% w/v). The resulting solution was incubated for 30 minutes at room temperature with intermittent shaking to develop colour. Absorbance of blue colour solution was taken at 765nm by using double beam spectrophotometer (UV-Vis Analyst- 0001), methanol was used as blank. Standard curve of different concentrations of Gallic acid was prepared and line of regression was obtained [Fig 1]. Total phenolic content of the herb was calculated and expressed as µg/mg Gallic acid equivalent by putting the absorbance value of test sample in line of regression of Gallic acid.

Determination of Total Flavonoid Content[23]

Total flavonoid content of the herb was determined by Aluminum trichloride spectrophotometer method using Rutin as a standard. Different concentrations of Rutin (10-100µg/ml) were prepared in methanol. Test sample (100µg/ml), prepared in methanol. 0.5ml of test sample was diluted with 2ml of distilled water followed by addition of 0.15ml of $NaNO_2$ solution (5%). After 6 minutes, 0.15ml of $AlCl_3$ (10%) was added and allowed to stand for 6 minutes. Then 2ml of NaOH solution (1mM) was added to mixture. Finally the reaction mixture was diluted with distilled water to bring the final volume to 5ml and allowed to stand for 15 minutes. Absorbance was taken at 510nm (methanol as blank). The total flavonoid content was calculated from a calibration curve and the result was expressed as µg/mg Rutin equivalent [Fig 2].

Determination of Antioxidant Property

Antioxidant property of test herb was determined by Ferric Reducing Power Assay.[24] Different concentrations of test sample were prepared. To a volume of 0.5ml of different concentrations of sample, 0.5ml phosphate buffer (0.2M, pH 6.6) was added, followed by adding 0.5ml of Potassium Ferricyanide (0.5ml, 1%w/v). The reaction mixture was incubated at 50^{o}C for 20 minutes. After cooling, 1.5ml of Trichloroacetic acid solution (10% w/v) was added to the mixture to terminate the reaction. This was followed by adding 0.5ml ferric chloride (0.1% w/v) and absorbance was taken at 700nm. Increased absorbance of mixture shows increase in reducing power, thus high antioxidant property.

RESULTS AND DISCUSSION

The dried leaves of *Ranunculus laetus* upon soxhlation showed 22% yield. The Organoleptic evaluation of ethanolic extract showed that it was blackish in colour, Hay in odour, very sour in taste and fine in texture.

Phenol content

The total phenol content of ethanolic leaf extract was standardized with Gallic acid. The total phenolic content calculated from the calibration curve (R^2= 0.983), was 12.95 ± 0.95 µg Gallic acid equivalent [Table 1]. Same results have been shown, *Arisaema jacquemontii*[25], *Taraxcum officinale*.[26] The secondary metabolites like alkaloids, flavonoids, glycosides, tannins, steroids etc. attribute to diverse pharmacological actions of medicinal plants. Various plants act as a source of natural antioxidants, helping to reduce the risk and progression of severe and chronic diseases such as heart disease, cancer and stroke by scavenging free radicals.[27] The redox properties of phenolic compounds allow them to act as superb antioxidants.[28]

Table 1: Total Phenolic Content of Ethanolic Leaf Extract of *Ranunculus Laetus*.

S.No	Concentration (µg/ml)	Absorbance (nm)	TPC in µg/100 µg Gallic acid equivalent
1	100	0.193	12.12
2	100	0.198	12.75
3	100	0.208	14.00
Mean ± S.D			12.95±0.95

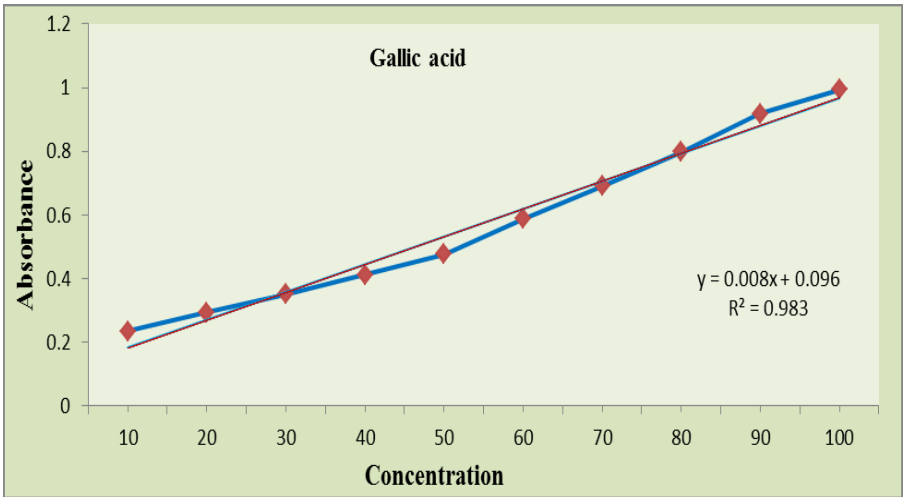

Fig 1: Standard Curve of Gallic Acid

Flavonoid Content

The total flavonoid content, calculated from standard curve of Rutin (R^2 = 0.991), was 29.44±5.91 µg rutin equivalent [Table 2]. Flavonoids possess vital antioxidant activity, scavenging singlet oxygen and quenching free radicals, chelate metals which increase the catalysis of oxidation process.[29] Chronic health problems at high levels of ROS in cells are eliminated by natural antioxidants.[30] The antioxidant activity of flavonoids including flavones, flavanols and condensed tannins, which are secondary metabolites, depends on the presence of free OH groups (3-OH). Plant flavonoids exhibit both *in vitro* and *in vivo* antioxidant activity.[31]

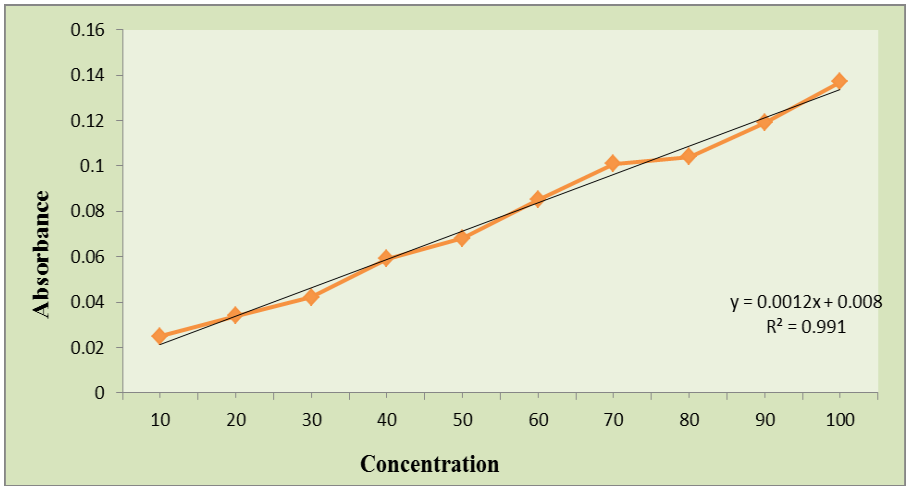

Fig 2: Standard Curve of Rutin

Table 2: Total Flavonoid Content of Ethanolic Leaf Extract of *Ranunculus Laetus.*

S.No	Concentration (µg/ml)	Absorbance (nm)	TFC in µg/100 µg Rutin equivalent
1	100	0.037	24.16
2	100	0.042	28.33
3	100	0.051	35.83
Mean ± S.D			29.44±5.91

Ferric Reducing Power Assay

In the present study our results revealed that absorbance values increase with the increase in concentration of reaction mixture. Our results showed a minimum absorbance value of 0.518 nm for 100 µg/ml concentration while as, the maximum value of absorbance, 1.770 nm was recorded for 500 µg/ml concentration [Table 3]. Same kind of results were found for the leaves of *Hypericum hookerianum*[32], *Eurya japonica*.[33] Reducing power is directly linked with antioxidant activity, thus it serves as an important indication of the antioxidant activity.[34] Most of investigations have shown a relationship among the antioxidant activity and amount of total phenolics or total flavonoids.[35]

Table 3: Ferric Reducing Power Assay of Ethanolic Leaf Extract of *Ranunculus Laetus.*

S.No	Concentration (µg/ml)	Absorbance (nm)
1	100	0.518
2	200	1.054
3	300	1.634
4	400	1.682
5	500	1.770

CONCLUSION

The present study showed that the *Ranunculus laetus* possess a good quantity of phenolics and flavonoids, which in turn possess potential antioxidant activity. Since, natural antioxidants are safer than the synthetic ones. Furthermore, phytochemical investigation is required to evaluate the wide spectrum drugs of pharmacological importance.

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