April 30, 2019

PharmacologyOnLine

Newsletter • 2019 • vol.1 • 27-33

ESTIMATION OF TOTAL PHENOLIC, TOTAL FLAVONOIDS AND TOTAL PROTEIN CONTENT OF HYDROALCOHOLIC EXTRACT OF *ANACYCLUS PYRETHRUM*

Joshi S[1], Parkhe G[2*], Aqueel N[1], Dixit N[1], Jain DK[3]

[1]Sarojini Naidu Government Girls P.G. (Autonomous) College, Bhopal, MP 462023

[*2]Scan Research Laboratories, Sector A H No. 109, J K Road, Indrapuri, Bhopal, MP 462023

[3]Medicinal Chemistry Research Laboratory, SLT Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur, (CG) 495009

Email address: parkhegeeta227@gmail.com

Abstract

Phenolic compounds including flavonoids and phenolic acids are plants secondary metabolites. Due to their ability to act as antioxidant agents, there is a growing interest to use those components in traditional medicine for disease prevention or treatment. The aim of present study was to estimate the total phenolic, flavonoid and protein content of hydro alcoholic extract of *Anacyclus pyrethrum.* Phytochemical screening of the plant showed the presence of glycosides, carbohydrates, phenols, alkaloids, flavonoids, proteins and amino acids and diterpenes. The total phenolic, flavonoid and protein contents were determined by established methods and were found to be 0.609 mg/100mg, 0.566 mg/100mg and 433.9 µg/ml in gallic acid, quercitin and protein equivalents respectively. Relatively high amount of phenolic, flavonoid and protein contents of hydro alcoholic extract of *Anacyclus pyrethrum* make this plant a promising candidate for diseases treatment; however, there is not a direct relationship between the amounts protein components and the efficiency in disease treatment.

Keywords: *Anacyclus pyrethrum, Hydroalcoholic extract, phytochemical screening, total phenolic content, total flavonoid content, total protein content*.

Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that, in order to find active compounds, a systematic study of medicinal plants is very important. There has been an upsurge of interest in the use of plants, as a source in folk medicine to treat various chronic diseases. In western countries many prescriptions correspond to products originating from plants [1-3]. Phenolic compounds are plant secondary metabolites possessing aromatic ring with one or more hydroxyl groups from the aromatic amino acids phenylalanine produced via the phenylpropanoid pathway [4]. The two major classes of phenolic compounds include flavonoids and phenolic acids. These phytochemicals are a diversified group of secondary metabolites that are ubiquitous in the plant kingdom. According to many authors [5-7], phenolic compounds with health-promoting activity are found in dietary plants. Being so, fruits and vegetables represent remarkable sources of bioactive compounds that could play a role in preventing diseases caused as a result of oxidative processes, such as cardiovascular problems, auto-immune complications, type 2 diabetes and age-related dysfunctions [8-10]. It explains the great attention given to phenolic phytochemicals during the last decade and the large number of studies dealing with this subject.

Anacyclus pyrethrum DC roots and leaf have important role in the traditional Ayurvedic and Unani system of holistic health and herbal medicine of the East. Especially the root of *Anacyclus pyrethrum* is reported to have good medicinal values in traditional system of medicine [11]. *Anacyclus pyrethrum* from Asteraceae family and Anacyclus genus is a native plant of India and Arabic countries and its root has therapeutic effects [12]. *Anacyclus pyrethrum* (Linn) De Candolle, commonly known as 'Spanish pyrethrum root' in English, 'Aaqarqarhaa' in Unani, and 'Aaqarqarhaa' in Ayurveda. Traditionally, plant is used as antibacterial, anti-inflammatory and tonic to the nervous system [13]. *Anacyclus pyrethrum* commonly known as pellitory and Akarkara in Hindi local language is perfectly recognized in traditional and herbal medicine and has a positive effect on regulating the immune system [14]. Therefore, the present study consists of investigation of total phenolic, flavonoid and protein content of hydro alcoholic extract of *Anacyclus pyrethrum.*

Materials and Methods

Plant material collection

The plant *Anacyclus pyrethrum* was collected from local area of Bhopal (M.P.) in the month of Jan, 2018.

Storage

Drying of fresh aerial parts was carried out in sun but under the shade. Dried *Anacyclus pyrethrum* was preserved in plastic bags and closed tightly and powdered as per the requirements.

Defatting and extraction of plant material

Anacyclus pyrethrum was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. Dried powdered *Anacyclus pyrethrum* has been extracted with hydro alcoholic solvent using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40 °C.

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods [15, 16].

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10 mg of extract dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The

absorbance was measured at 765 nm using a spectrophotometer [17].

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of extract dissolved in 10 ml methanol and filter. Three (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm [17].

Total protein content estimation

The amount of protein was estimated by Lowry's method.

Reagents A. 2% Na_2CO_3 in 0.1 N NaOH
B. 1% NaK Tartrate in H_2O
C. 0.5% CuSO4.5 H_2O in H_2O
D. Reagent I: 48 ml of A, 1 ml of B, 1 ml C
E. Reagent II- 1 part Folin-Phenol [2 N]: 1 part water.

1 ml of each BSA (Bovine serum albumin) working standard 50-250 µg/ml or test in test tubes. The test tube with 1 ml distilled water was serve as blank. Added 4.5 ml of reagent I and incubated for 10 minutes. After incubation added 0.5 ml of reagent II and incubated for 30 minutes. Measure the absorbance at 660 nm and plot the standard graph [18].

Results and Discussion

Phytochemical screening of the plant showed the presence of glycosides, carbohydrates, phenols, alkaloids, flavonoids, proteins and amino acids and diterpenes. The total phenolic (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.042X+0.002, R^2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance. The total flavonoid (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.06X+0.019, R^2= 0.999, where X is the quercetin equivalent (QE) and Y is the absorbance. The total phenolic, flavonoid and protein contents were determined by established methods and were found to be 0.609 mg/100mg, 0.566 mg/100mg and 433.9 µg/ml in gallic acid, quercitin and protein equivalents respectively. Many flavonoids are found to be strong antioxidants effectively scavenging the reactive oxygen species because of their phenolics hydroxyl groups [19]. Phenolic antioxidants are generally believed to form phenoxyl radical upon donating a hydrogen atom that could quench active free radicals. This has been reported to have multiple biological effects [20].

Conclusion

Anacyclus pyrethrum DC has a great role in treatment of the disease. The hydro alcoholic extract of *Anacyclus pyrethrum* analyzed in this study showed high total phenolic content, total flavonoid content and protein content. The phytochemical screening of hydro alcoholic extract of *Anacyclus pyrethrum* showed the presence of glycosides, carbohydrates, phenols, alkaloids, flavonoids, proteins and amino acids and diterpenes. Results might be helpful for providing the platform for researchers and pharma companies for the development of precious medicines which will be helpful for treatment of various diseases.

Acknowledgements

The authors are grateful to the Scan Research Laboratories, Bhopal for providing a fundamental research facility.

References

1. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. J Nat Prod 2003; 66: 1022-37.
2. Mann J. Natural products in cancer chemotherapy: past, present and future. Nat Rev Cancer 2002; 2: 143-8.
3. Treasure J. Herbal medicine and cancer: an introductory overview. Semin Oncol Nurs 2005; 21: 177-83.
4. Tura D, Robards K. Sample handling strategies for the determination of

biophenols in food and plants. J Chromatogr A 2002; 975: 71-93.
5. Hounsome, N., Hounsome, B., Tomos, D, Edward-Jones, G., Plant metabolites and nutritional quality of vegetables. J Food Sci 2008; 73(4): R48-R65.
6. Naczk, M, Shahidi, F., Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. J Pharma Biomed Ana 2006; 41(5): 1523-1542.
7. McCue P, Shetty K. A model for the involvement of lignin degradation enzymes in phenolic antioxidant mobilization from whole soybean during solid-state bioprocessing by Lentinus edodes. Process Biochem 2005; 40(3- 4): 1143-1150.
8. Morton L, Caccetta R, Puddey I, Croft, K. Chemistry and biological effects of dietary phenolic compounds: relevance to cardiovascular disease. Clin Experim Pharmacol Physiol 2000; 27(3): 152-159.
9. Kaur C, Kapoor H., Antioxidants in fruits and vegetables – the millennium's health, International. Journal Food Science Technology, 36, no. 7, p. 703-725 (2001).
10. Youdim K, Joseph J. A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects. Free Rad Biol Med 2001; 30 (6): 583-594.
11. Kishor K, Lalitha KG. Pharmacognostical studies on the root of Anacyclus pyrethrum DC. Indian J Nat Prod Res 2012; 3(4): 518-526
12. Naderi JN, Niakan M, Khodadadi E. Determination of Antibacterial Activity of Anacyclus pyrethrum Extract against Some of the Oral Bacteria: An in vitro study. J Dent Shiraz Univ Med Sci 2012; 13(2): 59-63.
13. Tyagi S, Ashim MM, Narendra KS, Manoj KS, Bhardwaj P and Singh RK. Antidiabetic Effect of Anacyclus pyrethrum DC in Alloxan Induced Diabetic Rats. European J Biolog Sci 2011; 3(4): 117-120.
14. Sharma V, Thakur M, Chauhan NS and Dixit VK. Effects of petroleum ether extract of Anacyclus pyrethrum DC. on sexual behavior in male rats. Phytotherap Res 2010; 8(8): 767-73.
15. Kokate CK. Practical Pharmacognosy. 4th edition. Delhi: Vallabh Prakashan; 1994.
16. Harborne JB. Phytochemical methods. London: Chapman and Hall; 1973.
17. Mervat M. M. El Far, Hanan A. A. Taie. "Antioxidant activities, total anthocyanins, phenolics and flavonoids contents of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol" Australian J Basic Applied Sc. 2009, 3, 3609-3616.
18. Lowry OH, Rosebrough N.J, Farr AL, Randall R.J. Protein Measurement with the Folin Phenol Reagent. J Biol Chem 1951; 193:265-275.
19. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem. 2006;99(1):191-203.
20. Cao G, Sofic E, Prior RL. Antioxidant and pro-oxidant behavior of flavonoids: Structure activity relationships. Free Rad Biol Med. 2009;22:749-60.

Table 1: Phytochemical screening of hydro alcoholic extract of *Anacyclus pyrethrum*

S. No.	Test	*Anacyclus pyrethrum*
1.	Detection of alkaloids: a) Hager's Test: b) Dragendroff's Test:	 -ve -ve
2.	Detection of carbohydrates: a) Fehling's Test:	 +ve
3.	Detection of glycosides: a) Legal's Test:	 +ve
4.	Detection of saponins a) Froth Test:	 -ve
5.	Detection of phenols a) Ferric Chloride Test:	 +ve
6.	Detection of flavonoids a) Alkaline Reagent Test: b) Lead acetate Test:	 +ve +ve
7.	Detection of proteins and aminoacids a) Xanthoproteic Test:	 +ve
8.	Detection of diterpenes a) Copper acetate Test:	 +ve

+ve = present, -ve = absent

Table 2: Calibration curve of gallic acid

S. No.	Concentration	Absorbance
0	0	0
1	5	0.194
2	10	0.422
3	15	0.637
4	20	0.848
5	25	1.035

Table 3: Calibration curve of quercetin

S. No.	Concentration	Absorbance
0	0	0
1	5	0.352
2	10	0.61
3	15	0.917
4	20	1.215
5	25	1.521

Table 4: Preparation of calibration curve of Protein

S. No.	Concentration	Absorbance
0	0	0
1	50	0.055
2	100	0.111
3	150	0.163
4	200	0.215
5	250	0.268

Table 5: Total phenolic, flavonoid and protien content

S. No.	Extracts	Total phenol (mg/100mg)	Total flavonoid (mg/100mg)	Total protein (µg/ml)
1.	*Hydroalcoholic extract*	0.609	0.566	433.9

Figure 1: Graph of estimation of total phenolic content

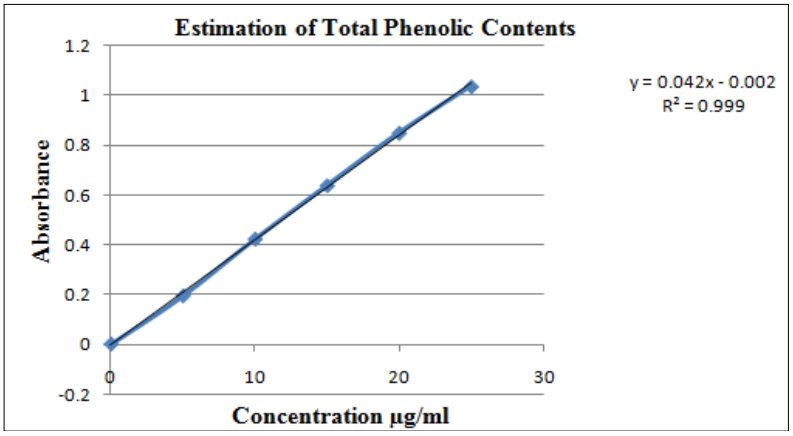

Figure 2: Graph of estimation of total flavanoid content

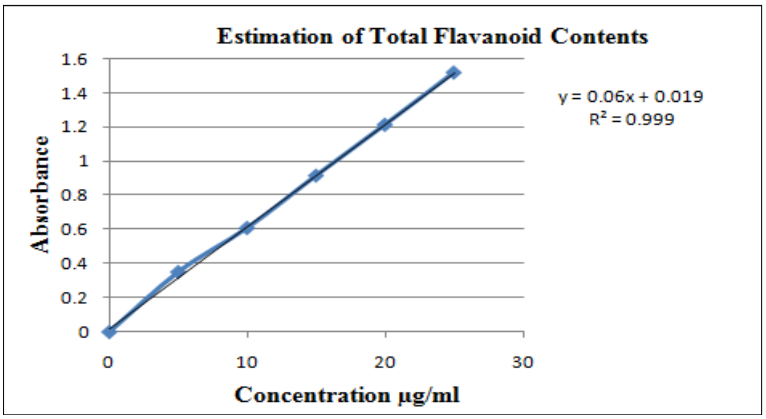

Figure 3: Graph of estimation of total protein content