J. Appl. Environ. Biol. Sci., 4(11S)141-145, 2015

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Phytochemical Analysis, Total Phenolic and Flavonoid Content, and Antioxidant Activity from Aerial Parts of *Hymenocrater calycinus (Boiss)*

Maryam Soodmand[1], Ali Mohamadi Sani[2*], Mohammad Reza Jalilvand[3]

[1]M. Sc Student, Department of Food Science & Technology, Quchan Branch, Islamic Azad University, Quchan, Iran
[2]Department of Food Science & Technology, Quchan Branch, Islamic Azad University, Quchan, Iran
[3]North Khorasan University of Medical Sciences, Bojnurd, Iran

Received: October 29, 2014
Accepted: December 31, 2014

ABSTRACT

Hymenocrater is an important genus of Lamiaceae family. *Hymenocrater calycinus (Boiss)* is one of species in this genus and this plant is endemic to Iran and it is growing wildly in the north east of Iran. In this study, antioxidant activities of dichloromethane, methanol and ethyl acetate extracts of *Hymenocrater calycinus (Boiss.) Benth* were evaluated with 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power tests. Methanol extract gave the highest extraction yields. Total phenolic and flavonoid content of methanol extract was more than ethyl acetate and dichloromethane extracts. The strong antioxidant index (AI) was observed from the methanol extract in both DPPH and FRAP method and antioxidant activity of all of extracts were lower than the vitamin C and butylated hydroxytoluene (BHT). Reducing power of the extracts increased by increasing their concentration. A good Correlation was found between the antioxidant activity and total phenolic content R^2 = 0.915, 0.912 and also in the antioxidant and total flavonoid content R^2 = 0.950, 0.948 (both of DPPH and FRAP methods). The results indicated the aerial parts of this plant are a potential source of natural antioxidants, phenolic and flavonoids.

KEYWORDS: *Hymenocrater Calycinus*, DPPH, FRAP, Phenolic and flavonoid.

1. INTRODUCTION

Antioxidants have been widely used as food additives to provide protection against free radicals, and the side effects of synthetic antioxidants such as butylated hydroxytoluene (BHT) have been documented [1]. Plant antioxidants can protect the body from free radical [2] and there are many published studies reporting the antioxidant activities of plants. Plant extracts used as antioxidants by hydrogen-donating properties with their hydroxyl groups and by electrons -donating to stop free radical chain reactions [3].

Hymenocrater is an important genus of Lamiaceae family and named Gol-e-Arvaneh in Persian. it is observed in Iran, Iraq, Pakistan and Afghanistan, and nine of them reported in Iran [4, 5] and four species including H. longiflorus, H. calycinus, H. yazdianus, and H. incanus are endemic to Iran and they are found in north-east of Iran [6]. H. calycinus (Boiss.) Benth is endemic to Iran and it is growing wildly in the north east of Iran [5]. Hymenocrater genus has been previously investigated such as identification of essential oils compounds [7] and anti-microbial effects [8].

From the extracts of *Hymenocrater calycinus*, four compounds such as ß-sitosterol, ursolic acid, quercetin-3-O- rutinoside and rosmarinic acid were isolated [9]. The main compounds of *Hymenocrater longiflorus Benth* were: α- Pinene ,β-Caryophyllene , β- Eudesmol , α-Copaene ,γ-Elemene , δ-Cadinene [10].

The present study describes phytichemical investigation and antioxidant activity of various extracts of H. calycinus (Boiss.) Benth that is endemic to Iran.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Methanol (CHROMASOLV, ≥99.9%, Sigma–Aldrich), Folin-Ciocalteau reagent (F9252, Sigma–Aldrich), Na2CO3(451614,anhydrous powder, 99.999%, Sigma–Aldrich), Gallic acid(91215, Fluka), Aluminum Chloride (563919, anhydrous powder,99.999%, Sigma–Aldrich), 2,2-diphenyl-1-picrylhydrazyl (257621,Sigma–Aldrich), Quercetin (Q4951, ≥95%, Sigma–Aldrich),vitamin C (A1300000, European Pharmacopoeia (EP) Ref-erence Standard, Fluka), Butylated Hydroxy Toluene (BHT)(W218405 ≤ 99, Sigma–Aldrich), 2,4,6-tripyridyl-s-triazine (TPTZ)(T1253 for spectrophotometric, ≥98%, Sigma), FeSO4.7H2O (21542,≥99.0%, Sigma–Aldrich)

*** Corresponding Author:** Ali Mohamadi Sani, Department of Food Science & Technology, Quchan Branch, Islamic Azad University, Quchan, Iran. E-mail: mohamadisani@yahoo.com

2.2. Preparation of H. calycinus (Boiss.) Benth

The aerial parts of H. calycinus (Boiss.) Benth were collected from Bojnurd, North Khorasan province of Iran in Jun 2014. The plant was identified from the Research Center of Natural Products Health Herbarium and the Voucher specimen (No. 36.3.2.) was deposited in the Herbarium of North Khorasan University of Medical Sciences.

2.3. Preparation of the extracts

100 g of dried plant was macerated in methanol, dichloromethane, and ethyl acetate for 48 h separately and solvent was evaporated under vacuum at 40◦C [11].

2.4. Phytochemical analysis

In order to test the presence of flavonoids, concentrated methanol extract (1 g) was dissolved in 10 ml of water. And then magnesium powder (100 mg) was added to 2–3 ml of the solution and then, 0.5 ml of HCl was added to the mixture. The presence of flavonoids is characterized by the formation of a pale pink to red colour within 2 min [12].

2.4.1. Test for phenol

2 ml of extract added to 2 ml of methanol and ferric chloride solution and its color observed [13].

2.4.2. Test for saponin

Methanol extract (1 g) was diluted with 10 ml distilled water in a test tube, after shaking for 2 min, the presence of saponins was confirmed by the formation of froth which is stable for at least 30 min. The height of the stable froth is proportional to the saponin content of extract [14].

2.4.3. Test for tannin

The presence of tannins was evaluated by dissolving methanol extract (1 g) in 10 ml distilled water and then the solution was divided into 2 portions. NaCl 10% and aqueous gelatin 1% solutions were added to the first and second parts. The presence of tannins was observed by produce of sediment in the gelatin containing tube or both tubes. As a confirmation test, one drop of extract was placed on a paper and sprayed with 5% ferric chloride solution. Formation of blue or dark green spots is indicative of the presence of tannins [15].

2.5. Determination of total phenolic content

Total phenol content of *H*ymenocrater *calycinus* was determined by Folin-Ciocalteu test. 100 µL from the extracts were added with 500 µL of Folin–Ciocalteu reagent and 20% Na_2CO_3 (1.5 mL) and the mixtures were shaken. After 2 h, the absorbance of samples was read at 760 nm. The gallic acid calibration curve was plotting by 5 to 500 mg/mL of gallic acid and total phenol content expressed as milligrams of gallic acid (GAE) per gram of extract [16].

2.6. Determination of total flavonoid content

0.5 mL from each extract were mixed with0.1 mL of 10% aluminum chloride and 1.5 mL of methanol, 0.1 mL of potassium acetate (1 M) and 2.8 mL of distilled water and then they were incubated at room temperature for 30 min and then the absorbance of them was measured at 415 nm. The calibration curve was prepared using 12.5–100 µg/mL of Quercetin in methanol and the flavonoid content was expressed in terms of Quercetin per dry weight of extract [17].

2.7. DPPH radical scavenging assay

The antioxidant activity of the extracts was measured in terms of hydrogen donating or radical scavenging ability [18]. 0.1 mM of DPPH was prepared in methanol. Extracts were dissolved in methanol to several concentrations (8, 4, 2, 1, 0.5, 0.25 mg/mL) and 0.1 mL of the extracts and 3.9 mL of DPPH solution were mixed and were kept in darkness for 30 min and the absorbance was read at 517 nm. The experiment was done in triplicate. The percentage of radical scavenging activity was calculated from this equation: % DPPH radical scavenging = [(Absorbance of blank − Absorbance of Sample)/ (Absorbance of blank)] × 100. Methanol was used as blank, ascorbic acid and butylated hydroxyl toluene (BHT) used as positive controls. Absorbance Inhibitor (AI) was calculated as IC_{50}values were calculated using Biodatafit online software [19].

2.8. Ferric reducing antioxidant power (FRAP) assay

The FRAP reagent was made from a mixture of acetate buffer (0.3 M) with pH =3.6, 2, 4, 6-tripyridyl-S-triazine (TPTZ) 10 mmol/L in HCl (40 mmol/L) and ferric chloride (20 mmol/L) (10:1:1, v/v/v). 100 µL of extracts were mixed with 3 mL of FRAP reagent and samples were incubated at 37◦C for 10 min. After incubation, the absorbance of samples was readed at 593 nm. The standard curve of ferric sulphate ($FeSO_4$.$7H_2O$) (0–1 mM) was made.The results of samples were expressed in mmol Fe2+/g of dried extract [20].

3. RESULTS AND DISCUSSION

3.1. Extraction yields

The yield of extraction was depending on temperature of extraction, the kind of solvents and time. In the present study the obtained extraction yields for the different solvents (Table 1) stratified in the following order: methanol>ethyl acetate > dichloromethane extracts.

The presence of saponins, flavonoids, and tannins in the methanol extract of *H. calycinus* was investigated and the presence of flavonoid and absence of saponins and tannins were recorded.

3.2. Total phenol and flavonoid contents

The phenolic compounds act as reducing agents and antioxidants because they intercept chain oxidation reactions by give of a hydrogen atom or chelating metals [21]. Total phenolic content of extracts was done with Folin-Ciocalteu test. Standard curve for total phenolic contents was drawn and the regression equation was: $y = 0.003 x + 0.061$(R^2= 0.985). The phenolic content of extracts is shown in Table 1. Phenolic content in the extracts was in the range of 114 to 140 mg gallic acid equivalent (GAE)/g of extract and total flavonoid contents were obtained from the equation: $y = 0.004 x + 0.006$ (R^2= 0.996), the amount of flavonoid ranged from 42.25 to 76.75 mg/g of extract and the results are given in Table 1. As shown in table 1, the extraction solvents affected the amount of phenolic and flavonoid compounds and methanol solvent was found to be more efficient in the extraction of flavonoid and phenolic compounds. Our results are in agreement with other results that reported methanol solvent was more efficient for extracting total phenolic [22, 23].

3.3. Antioxidant activity of extracts

The antioxidant activities of five concentrations (0.5, 1, 2, 4, 8 mg/mL) of the extracts were measured with DPPH test. This method is a valid and easy method to evaluate the antioxidant activity. The radical scavenging effects of the extracts were depended on concentration (Fig 1).

In accordance with the DPPH scavenging ability of the extracts, IC50 values increased in the following order: ascorbic acid <BHT < methanol extract< ethylacetate extract < dichloromethane extract (Table 1). Results of the measurement of the total antioxidant activity of the extracts with the FRAP method are presented in Table 1.. The reducing power of the extracts in FRAP method demonstrates their ability for donating electron. The ferric reducing antioxidant ability increased in the following order of ascorbic acid < methanol extract < ethyl acetate extract < dichloromethane extract < BHT (Table 1). Methanol extract has the highest amount of phenol and flavonoids and this extract showed the strongest ferric reducing antioxidant ability. As shown in figure 2 and 3, a good correlation was found between the antioxidant activity and total phenolic content in DPPH and FRAP assays (R^2 = 0.915, 0.912), also there is a good correlation between the antioxidant and total flavonoid content R^2 = 0.950, 0.948 (DPPH and FRAP method).

Table 1. Extraction yield, total phenolic, total flavonoid content and antioxidant properties of Dichloromethane, Ethyl acetate and Methanol extracts from *H. calycinus (Boiss.) Benth*

Extracts	Extraction yield (%)	Total phenolic (Gallic acid equivalents mg/g of dry extract)	Total flavonoid (quercetin equivalents mg/g of dry extract)	IC_{50} (mg/mL)	FRAP value (µmolFe^{2+}/g dry extract
Dichloromethane	0.9%	114±0.029	42.25±0.005	4.36	696±0.035
Ethyl acetate	1.49	131±0.017	67±0.013	1.31	1750±0.059
Methanol	12.425%	140±0.035	76.75±0.057	1.12	1810±0.066
BHT				0.59	550±0.026
Ascorbic acid				0.17	2100±0.0107

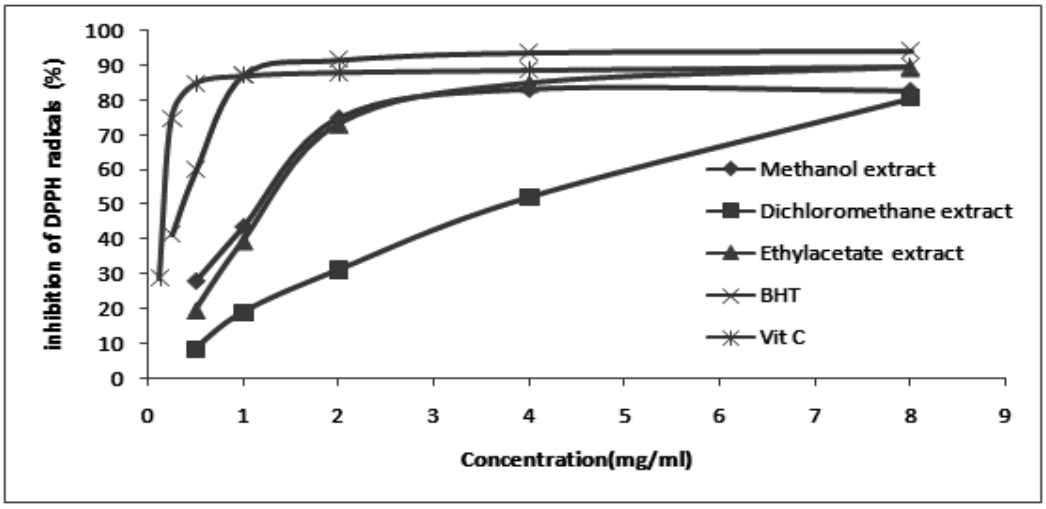

Fig. 1 – Scavenging activity against DPPH radicals

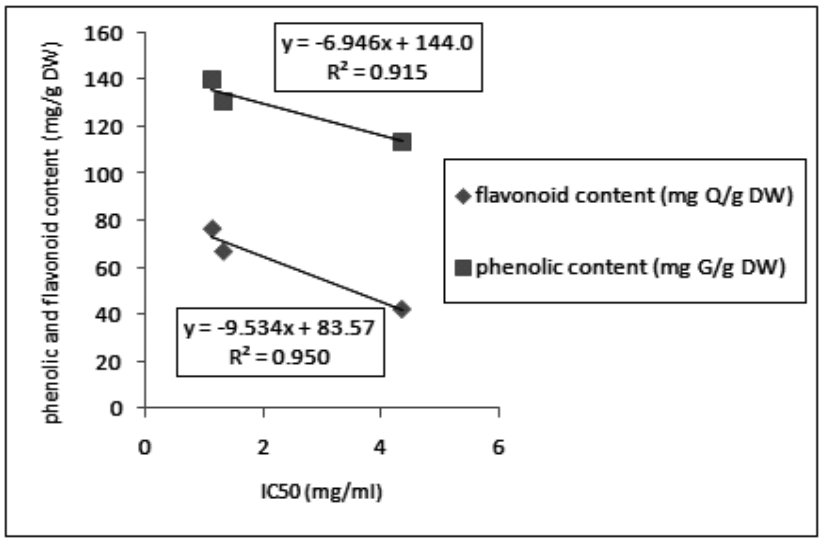

Fig .2- The effect of phenolic and flavonoid content on the free radical scavenging assay

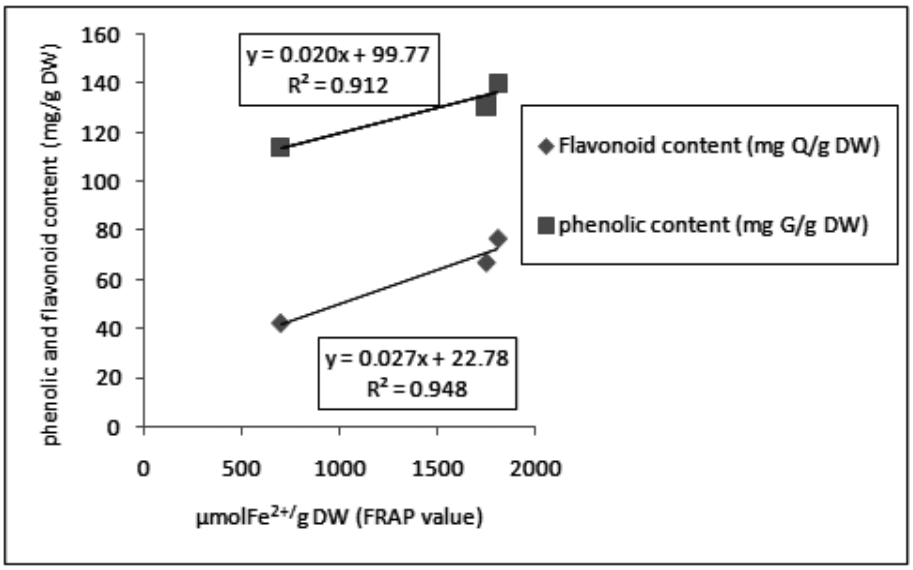

Figure .3- The effect of phenolic and flavonoid content on the FRAP assay

4. Conclusion

This work showed the extracts of aerial parts of *H. calycinus* (*Boiss.*) can use as a source of antioxidants. This work showed that the extraction yield, total phenolic content and antioxidant ability were depending on the type of solvent.

5. REFERENCES

1. Türkoğlua, A., E.M. Durub, N. Mercanc, 2007, Antioxidant and Antimicrobial Activity of Russula delica Fr: An Edidle Wild Mushroom. Eurasian J Anal Chem; 2(1): 54-67.
2. Lai, L. S., S. Chou, W. Chao, 2001, Studies on antioxidative activities of hsain-tsao (*Mesona procumbent* Shemls) leaf gum. J. Agric. Food. Chem., (49): 963-968
3. John, J. A., F. Shahidi, 2010. Phenolic compounds and antioxidant activity of Brazil nuts (Bertholletia excelsa). Journal of Functional Foods, 2: 196–209.
4. Jafari, A., F. Jafarzadeh, 2008, Anatomical and pollen ornamentation study on Hymenocrater species in North East of Iran. Pakistan J Biol Sci 11(17): 2149–2153.
5. Rechinger, K.H. 1982. Flora Iranica (Labiatae). Vol. 150., Subtitle: Labiatae. Akademische Druck-u. Verlagsanstalt, Graz. Austria, ISBN: 3-201-00728-5, pp: 1-250 (In Latin).
6. Naghibi, F., M. Mosaddegh, S.M. Motamed, A. Ghorbani, 2005, Labiatae family in folk medicine in Iran, from ethnobotany to pharmacology. Iran. J.Pharmaceutical Res, 2:63-79.
7. Akramian, M., S.N. Ebrahimi, M.R. Joharchi, 2008, Essential oil composition of *Hymenocrater latystegius Rech.f.* from Iran .I. Essential oil 11 (2): 199-202.
8. Zadidi, M.A., S.A. Crow, 2005, Biologically active traditional medicinal herbs from balochestan. Pakistan Ethnol. Pharmacol. 96: 331-334.
9. Gohari A.R., S. Saeidnia, A.R. Shahverdi, N. Yassa, M. Malmir, K. Mollazade, A.R. Naghinejad, 2009, Phytochemistry and antimicrobial compounds of *Hymenocrater calycinus*. EurAsian Journal of BioSciences . EurAsia J BioSci 3: 64-68
10. Taherpour, A., H. Maroofi, M. Changizi, R. Vafaei Shoushtari, K. Larijani, A. Kazempour, 2011, Chemical compositions of the essential oil and calculation the biophysicochemical coefficients of the components of *Hymenocrater longiflorus Benth.* of Iran. Natural Science Vol.3, No.2: 104-108.
11. Prachayasittikul, S., P. Buraparuangsang, A. Worachartcheewan, C. Isarankura-Na-Ayudhya, S. Ruchirawat, V. Prachayasittikul, 2008 , Antimicrobial and antioxidative activities of bioactive constituents from Hydnophytum formicarum Jack. Molecules 13: 904–21.
12. Markham, R.K .1982, Techniques of Flavonoid Identification, Academic Press, London pp 15–45.
13. Kardong, D., S. Upadhyaya, L.R. Saikia, 2013, Screening of phytochemicals, antioxidant and antibacterial activity of crude extract of Pteridium aquilinum Kuhn. journal of pha rmacy research 6: 179 -182.
14. Hostettmann, K., A, Martson, 1995, Saponin, Cambridge, Univ Press, p 233.
15. Evans, E, 2009, Trease and Evans Pharmacognosy, 16th edn, Saunders, Edinburgh.
16. Golmakani, E., A. Mohammadi, T. Ahmadzadeh Sani, H. Kamali, 2014, Phenolic and flavonoid content and antioxidants capacity ofpressurized liquid extraction and perculation method from roots of Scutellaria pinnatifida A. Hamilt. subsp alpina (Bornm) Rech. f. J. of Supercritical Fluids 95: 318–324.
17. Chang C.C., M.H, Yang, H.M, Wen, J.C. Chern, 2002, Estimation of total flavonoid content in propolis by two complementary colorimetric methods, J. Food and DrugAnalysis 10: 178–182.
18. Fazly Bazzaz, B.S., M. Hassanzadeh Khayat, S.A. Emami, J. Asili, A. Sahebkar, E. Javadi Neishabory, 2011, Antioxidant and antimicrobial activity of methanol, dichloromethane, and ethyl acetate extracts of Scutellaria litwinowii, ScienceAsia 37: 327–334.
19. Chen Z., R. Bertin, G, Froldi, 2013, EC50 estimation of antioxidant activity inDPPH assay using several statistical programs, Food Chemistry 138: 414–420.
20. Xu, X., H. Xie, Y. Wang, X .Wei, 2010, A-type proanthocyanidins from Lychee seeds andtheir antioxidant and antiviral activities, J. Agricultural and Food Chemistry 58: 11667–11672.
21. Bursal, E., E. Koksal, 2011, Evaluation of reducing power and radical scavenging activities of water and ethanol extracts from sumac (Rhus coriaria L.). Food Research International 44, 7: 2217–2221.
22. Yu, J., M. Ahmedna, I. Goktepe, A. Proctor, 2005, Effects of processing methods and extraction solvents on concentration and antioxidants activity of peanut skin phenolics. Food Chemistry,90/1-2 :199-206
23. Wang, T., R. Jonsdottir, G. oLafsdottir, 2009, Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds Food Chem. 116: 240– 248.