ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2019; 5(3): 256-260
www.allresearchjournal.com
Received: 16-01-2019
Accepted: 20-02-2019

Manik Sharma
P.G. Department of Zoology, Career College, Bhopal, Madhya Pradesh, India

Jagrati Tripathi
P.G. Department of Botany, Govt. L.B.S. College Sironj Vidisha, Madhya Pradesh, India

Mukesh Kanesh
P.G. Department of Chemistry, Govt. Geetanjali Girls P.G. College Bhopal, Madhya Pradesh, India

Correspondence
Manik Sharma
P.G. Department of Zoology, Career College, Bhopal, Madhya Pradesh, India

Antioxidant activity, total phenolic and total flavonoid contents of rhizome extracts of herb *Rheum emodi* wall. ex Meissn

Manik Sharma, Jagrati Tripathi and Mukesh Kanesh

Abstract
In this study, the antioxidant properties and total phenolic and flavonoid contents of *Rheum emodi* rhizome extracts were examined. The radical scavenging capacity of Petroleum ether, chloroform, ethyl acetate and 90% ethanol extracts was estimated by 2, 2-diphenyl-1-picrylhydrazil (DPPH) assay. Phenols and flavonoids were present only in the ethyl acetate and 90% ethanol extracts, their total phenols and flavonoids contents were determined. Total phenolic content in the 90% ethanol and ethyl acetate extracts were 51.57±0.09, 69.75±1.13 mg gallic acid equivalent (GAE)/g and total flavonoid content 11±0.14, 29.5±0.01 mg Quercetin equivalent (QE)/g of extract respectively. The highest DPPH antioxidant activity was shown by 90% ethanol extract IC_{50} 52.04 followed by ethyl acetate extract IC_{50} 59.68 and least by petroleum ether extracts IC_{50} 156.62 while standard ascorbic acid was IC_{50} 28.26. Our results of antioxidant assays were justified and partially supported the popular usage of the tested plants. The high antioxidant activity found in the plant can be a good source of natural antioxidants which might have benefits for health.

Keywords: extracts; antioxidant activity; phenols; flavonoids

Introduction
The use of oxygen as part of the process for generating metabolic energy produces reactive oxygen species (ROS), as a product of normal cell metabolism [1] These include hydrogen peroxide (H_2O_2) and free radicals such as superoxide anion (O_2–) and hydroxyl radical (HO•) [2]. Free radicals can cause oxidative damage to cellular components including DNA, RNA, globular protein [3], Proteases and unsaturated fatty acids [4]. Oxidative damage has been indicated as a primary cause of several diseases such as Alzheimer's disease [5], hepatitis, cirrhosis and liver cancer [6], the innate immune system [7]. Antioxidants are compounds that can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction. Synthetic antioxidants are widely used, but their use is being restricted nowadays because of their toxic and carcinogenic side effects [8]. For this reason, currently there is a growing interest toward natural antioxidants from herbal resources, able to protect organisms from damage induced by oxidative stress. The useful pharmacological effects of medicinal plants are attributed to the presence of phytoconstituents which are primarily secondary plant metabolites [9]. Phytoconstituent Flavonoids are known as "catchers" of superoxide anions and hydroxyl radicals [10] inhibit the oxidation of lipids, inhibit some of the enzyme systems, have an influence on the formation and transformation of peroxyl radicals, etc. [11]. In a worldwide review on herbal medicines by World Health Organization, it has been reported that approximately three-fourth of the populations in developing countries use traditional and folkloric herbal medicines for their common ailments [12]. *Rheum emodi* commonly known as Rhubarb is a perennial herb belongs to family Polygonaceae. It is a food plant with medicinal value restricted to the temperate, subalpine, and alpine zones of the Himalayas in altitudes ranging from 2,800 to 3,800 m [13]. Traditionally plant is widely used as laxative, tonic, diuretic and to treat ulcers, diarrhea, fever, cough and indigestion. *R. emodi* possess a number of phytoconstituents [14-17] and showed Antiulcer [18], Hepatoprotective [19], Antidiabetic [20], Bidirectional effect on Intestinal function [21], Antiplatelet and Anticoagulant [22], Immuno-enhancing effect [23], Nephroprotective [24] and Antifungal [25] biological activities. The present study aims to investigate the total phenolic and flavonoid

contents in the *R. emodi* rhizome extracts and antioxidant potential by DPPH radical scavenging assay.

Material and Methods

Collection of plant material and preparation of Extract

The rhizomes of *Rheum emodi* was collected from Pabbar valley of Himachal Pradesh. These were washed throughly and shade dried at room temperature. The dried plant material was grinded to a coarse powdered form, and extracted successively with the soxhlet apparatus in increasing order of polarity of solvents viz Petroleum ether, chloroform, ethyl acetate and 90% ethanol. The percentage yield 0.052, 0.11, 0.15 and 3.2% obtained respectively. The extracts were concentrated in a vaccum rotary evaporator. In phytochemical screening there was presence of Anthraquinones, Terpinoids, Steroids, Flavonoids, Carbohydrates, glycosides, Saponins and phenols in the extracts. The presence of phenol and flavonoid occurs only in ethyl acetate and 90% ethanol extract.

Quantitative phytochemical analysis

Determination of total phenolic content (TPC)

The amount of total phenolics in the plant extract was determined with the Folin-Ciocalteu reagent **[26]**. The extracts stock solution of 1mg/ml concentration was prepared in methanol. To 0.5ml of extracts solution add 2.5ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5ml 7.5% $NaHCO_3$ and blank containing 0.5ml methanol, 2.5ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5ml of 7.5% of $NaHCO_3$. A dilution series of 10, 20, 30, 40 and 50µg/ml concentration of standard gallic acid was prepared in methanol and same amount of reagent was added in each concentration as described in extract and blank. The samples were then incubated at room temperature in dark for 45min. The absorbance was determined by a spectrophotometer at 765nm. The concentration of phenolics was determined from the calibration line, the content of phenolics in the extracts were expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

Determination of total flavonoids content (TFC)

The determination of the total flavonoid content was carried out as described by [27]. All extracts and standard Quercetin stock solution was made at conc. of 1mg/ml in methanol. A dilution series of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml was prepared from the stock of Quercetin. To 1 ml of extracts solution add 1ml of 2% $AlCl_3$ solution dissolved in methanol. The same procedure was repeated for the dilution series of quercetin. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at 415nm. The calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in the extracts was expressed in terms of rutin equivalent (mg of QE/g of extract).

In-vitro antioxidant activity assay

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antiradical activity of the extract was estimated according to the procedure described by [28] 0.135mM DPPH solution was prepared in methanol and 1.0ml of this solution was mixed with 1.0ml of extract prepared in methanol containing 10, 20, 60, 80, 100 µg/ml and standard drugs separately (Ascorbic acid). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30min. The absorbance of the mixture was measured spectrophotometrically at 517nm. The ability of plant extract to scavenge DPPH radical was calculated by the equation

$$\text{DPPH radical scavenging activity} = \frac{\text{Ab(Control)} - \text{Ab(Sample)}}{\text{Ab(Control)}} \text{ x } 100$$

Statistical analysis

Tests were carried out in triplicate experiments and mean values were calculated with EXCEL program from MS office package. IC_{50} were graphically estimated using a linear regression algorithm.

Results and Discussion

Total Phenolics

The total phenolic contents in the examined extracts using the Folin Ciocalteu's reagent the standard curve equation is : y = 0.004x – 0.362, R2 = 0.961 (Graph 1).The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract (Table 2). The total phenolic contents in the 90% ethanol and Ethyl acetate extract is 51.57±0.09 and 69.75±1.13 mg of GA/g of extract respectively. The total phenolic content in plant extracts depends on the type of extract, i.e. the polarity of solvent used in extraction. The high solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction [29, 30]. Phenolic compounds protect plants from oxidative damage and perform the same function for humans. Several types of polyphenols (phenolic acids, hydrolysable tannins, and flavonoids) show anticarcinogenic and antimutagenic effects.

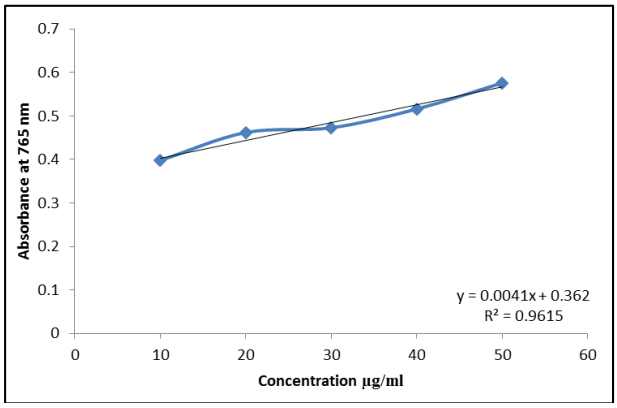
Total Flavonoids

The concentration of flavonoids in plant extract of *Rheum emodi* was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of quercetin equivalent. The standard curve equation: y = 0.002x + 0.289, R2 = 0.983 (Graph 2), mg of QE/g of extract (Table 4). The total Flavonoid contents in the 90% ethanol and Ethyl acetate extract is 11±0.14 and 29.5±0.01 mg of QE/g of extract respectively. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation **[34]**. Flavonoids are the most ubiquitous groups of plant secondary metabolites [35]. Flavonols consumption has been associated with a variety of beneficial effects including increased activity of erythrocyte superoxide dismutase, a decrease in lymphocyte DNA damage, a decrease in urinary 8-hydroxy-2-deoxyguanosine (a marker of oxidative damage) and an increase in plasma antioxidant capacity [36]. Flavonoids have been widely used in cancer treatments, coronary heart diseases, gastrointestinal ulcers and rheumatic diseases [37].

DPPH radical scavenging assay

DPPH is a free radical compound and has been widely used to test the free radical scavenging ability of various samples [38]. It is a stable free radical with a characteristic absorption at 517nm that was used to study the radical-scavenging

effects of extracts. As antioxidants donate protons to this radical, the absorption decreases. Antioxidants, on interaction with DPPH, either transfer an electron or hydrogen atom to DPPH, thus neutralizing its free radical character [39]. The colour changed from purple to yellow and the absorbance at wavelength 517 nm decreased. The extracts had varying degrees of antioxidant activity in the DPPH assay. The Table 5 have shown % of inhibition correspond to their conc. The IC_{50} value was determined from the plotted graph of Concentration vs % of inhibition. The DPPH scavenging of standard ascorbic acid and extracts was found to be in the order of Ascorbic acid > 90% Ethanol > Ethyl Acetate > Chloroform > Petroleum ether and the IC_{50} values 28.26, 52.04, 59.68, 72.29, 156.62 µg/ml respectively. The IC_{50} value of a compound is inversely related to its antioxidant capacity. A lower IC_{50} value indicates a stronger antioxidant activity of the extract or compound [40]. The DPPH scavenging activity is lowest in pet ether followed by chloroform and higher in 90% ethanol and Ethyl acetate that may be due to presence of high phenolic and flavonoid constituents.



Graph 1: Calibration curve of Standard gallic acid

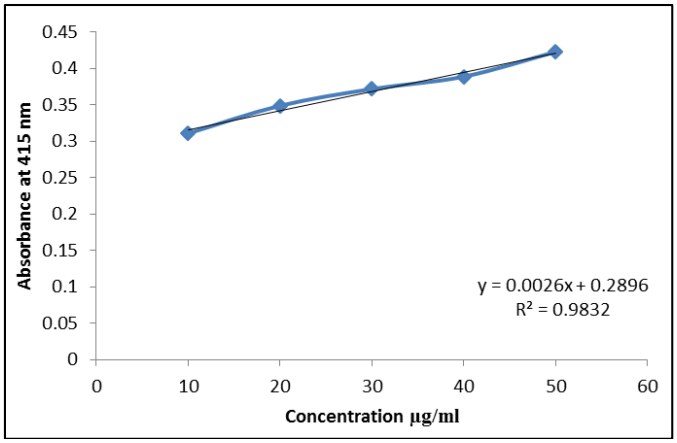
Table 1: Concentration Vs Absorbance curve of Gallic acid

S. No.	Conc. µg/mL	Absorbance (765 nm)
1.	10	0.398
2.	20	0.462
3.	30	0.473
4.	40	0.516
5.	50	0.576

Table 2: Total phenolic content in fractions expressed in mg/g equivalent to gallic acid

Extracts	Absorbance (765 nm)	Conc. of Extract	TPC mg/g GAE
90% Ethanol	0.569 ± 0.0001	1mg/mL	51.57 ± 0.09
Ethyl acetate	0.641 ± 0.0006	1mg/mL	69.75 ± 1.13

All values are in Mean ± Standard deviation



Graph 2: calibration curve of Standard Quercetin

Table 3: Concentration Vs Absorbance curve of Quercetin.

S. No.	Conc. µg/mL	Absorbance at 415 nm
1.	10	0.311
2.	20	0.349
3.	30	0.372
4.	40	0.389
5.	50	0.423

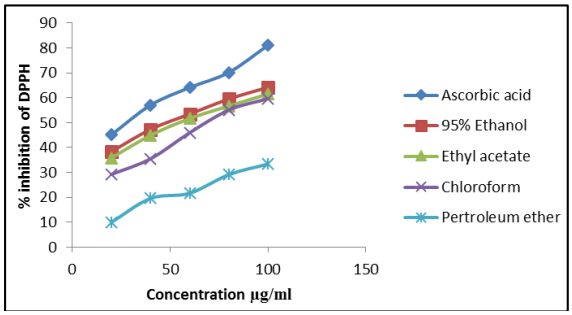
Table 4: Total Flavonoid content in Fractions expressed in mg/g equivalent to Quercetin.

Extracts	Absorbance	Conc. of extracts	mg/g equivalent to Quercetin
90% Ethanol	0.311 ± 0.0021	1mg/mL	11 ± 0.14
Ethyl acetate	0.348± 0.0001	1mg/mL	29.5 ± 0.01

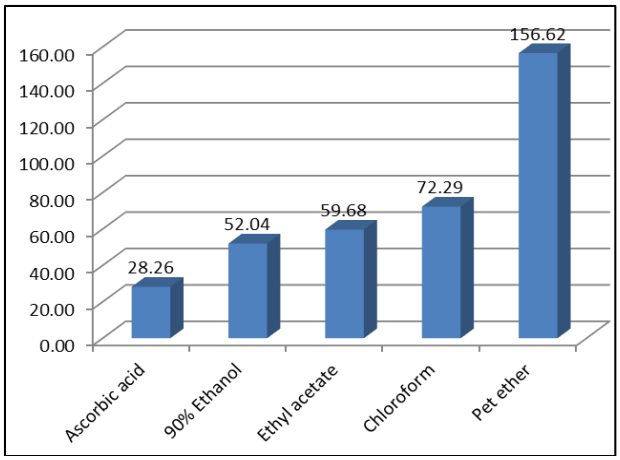
All values are in Mean ± Standard deviation

Table 5: Percentage of inhibition of DPPH by extracts

Conc. µg/ml	Ascorbic acid	90% Ethanol	Ethyl acetate	Chloroform	Petroleum ether
20	45.13	38.37	35.87	29.21	9.98
40	57.08	47.32	44.92	35.51	19.57
60	64.13	53.47	51.68	45.98	21.67
80	70.2	59.52	56.72	55.11	29.15
100	81.14	64.23	61.55	59.66	33.39
IC_{50}	28.26	52.04	59.68	72.29	156.62



Graph 3: Percentage of Inhibition of DPPH by extracts



Graph 4: IC_{50} values of extracts

Conclusion

The replacement of synthetic with natural antioxidants (because of implications for human health) is advantageous. In the present study, analysis of free radical scavenging activity and total phenolic and flavonoid contents showed that *Rheum emodi* rhizome extracts can be the potent source of natural antioxidants. However, further detailed investigation, especially *in-vivo* antioxidant and toxicity studies are needed to justify its use as a natural source of antioxidants to prevent the progression of many diseases.

References

1. Raha S, Robinson BH. Mitochondria, oxygen free radicals, disease and ageing. Trends Biochem Sci; 2000; 25(10):502-508.
2. Sharma B, Kalikotay S. Screening of antioxidant activity of lichens *Parmotrema reticulatum* and *Usnea* sp. from Darjeeling hills, India. IOSR J Pharm; 2012; 2(6):54-60.
3. Chamani J, Moosavi-Movahedi AA, Rajabi O, Gharanfoli M, Momen-Heravi M, Hakimelahi GH. Cooperative α-helix formation of β-lactoglobulin induced by sodium n-alkyl sulfates. Journal of colloid and interface science; 2006; 293(1):52-60.
4. Sivanandham V. Free radicals in health and diseases-a mini review. Pharmacologyonline. 2011; 1:1062-1077.
5. Wojtunik-Kulesza KA, Oniszczuk A, Oniszczuk T, Waksmundzka-Hajnos M. The influence of common free radicals and antioxidants on development of Alzheimer's Disease. Biomedicine & Pharmacotherapy. 2016; 78:39-49.
6. Hefnawy HTM, Ramadan MF. Protective effects of *Lactuca sative* ethanolic extract on carbon tetrachloride induced oxidative damage in rats. Asian Pacific Journal of Tropical Disease. 2013; 3(4):277-285.
7. Asoodeh A, Zardini HZ, Chamani J. Identification and characterization of two novel antimicrobial peptides, temporin-Ra and temporin-Rb, from skin secretions of the marsh frog (Rana ridibunda). Journal of Peptide Science. 2012; 18(1):10-16.
8. Verma N, Behera BC, Makhija U. Antioxidant and hepatoprotective activity of lichen *Usnea ghattensis* in vitro. Appl Biochem Biotechnol. 2008; 151(2, 3):167-181.
9. Negi PS, Jayaprakasha GK, Jena BS. Antioxidant and antimutagenic activities of pomegranate peel extracts. *Food Che*. 2003; 80:393-7.
10. Halliwell B. Arch Biochem. Biophys. 2008; 476:107-112
11. Cetkovic G. The chemistry of natural products, Faculty of Technology, University of Novi Sad, (in Serbian), 2008.
12. World Health Organization. Regulatory situation of herbal medicines a worldwide review. Geneva: World Health Organization, 1998.
13. Nautiyal BP, Prakash V, Maithani UC, Chauhan RS, Purohit H, Nautiyal MC. "Germinability, productivity and economic viability of *Rheum emodi* Wall. ex Meissn. Cultivated at lower altitude," 2003; 84(2):143-148.
14. Sudhir SS, Satish CP, Rajesh S, Santosh KA. 1,8 Dihydroxyanthraquinone derivatives from rhizomes of *Rheum emodi* Wall. Indian J Chem. 2005; 43B:1494-1496.
15. Krenn L, Pradhan R, Presser A, Reznicek G, Kopp B. Anthrone C-glucosides from *Rheum emodi*, Chem Pharm Bull (Tokyo); 2004; 52(4):391-3.
16. Babu KS, Srinivas PV, Praveen B, Kishore KH, Murthy US, Rao JM. Antimicrobial constituents from the rhizomes of *Rheum emodi*. Phytochem. 2003; 62:S203-207.
17. Summerah N, Manik S, Manjushah S, Mir A. *Rheum emodi:* phytochemistry, bioactive compounds and their biological activity. Int J Phyto pharm. 2013; 4(4):272-276.
18. Amandeep K, Sunil K, Ramica S. Assessment of Anti-Ulcer Activity of *Rheum emodi* Rhizomes Extract. IGJPS; 2012; 2(3):333-341.
19. Akhtar MS, Amin MA, Maqsood, Alamgeer A, Akseer-e-Jigar. Hepatoprotective Effectof *Rheum emodi* Roots (Revand chini) against Paracetamol-induced Hepatotoxicity in Rats. Ethnobotanical Leaflets. 2009; (2):310-315.
20. Radhika R, Krishna K, Sudarsanam D. Antidiabetic activity of *Rheum emodi* in Alloxan induced diabetic rats. IJPSR; 2010; 1(8):296-300.
21. Qin Y, Wang J, Kong W, Zhao Y, Yang H, Dai C. The diarrhoeogenic and antidiarrhoeal bidirectional effects of rhubarb and its potential mechanism. J Ethnopharmacol. 2011; 133:1096-1102.
22. Seo EJ, Ngoc TM, Lee SM, Kim YS, Jung YS. Chrysophanol-8-O-glucoside, an Anthraquinone Derivative in Rhubarb, Has Antiplatelet and Anticoagulant Activities. J Pharmacol Sci. 2012; 118:245-254.
23. Fozia K, Zargar MA. *Rheum emodi* Induces Nitric Oxide Synthase Activity in Murine Macrophages. Am J Biomed Sci. 2010; 2(2):155-163.
24. Alam MM, Javed K, Jafri MA. Effect of *Rheum emodi* (Revand Hindi) on renal functions in rats. J Ethnopharmacol. 2005; 96(1, 2):121-5.
25. Agarwal SK, Singh SS, Verma S, Kumar S. Antifungal activity of anthraquinone derivatives from *Rheum emodi*. J Ethnopharmacol. 2000; 72(1, 2):43-6.
26. Singleton VL, Orthofer R, Lamuelaraventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999; 299:152-178.
27. Quettier DC, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx MC *et al*. Bailleul F & Trotin F. Phenolic compounds and antioxidant activities of buckwheat

(*Fagopyrum esculentum* Moench) hulls and flour. J Ethnopharmacol. 2000; 72:35-42.
28. Liyana-Pathiranan CM, Shahidi F. Antioxidant activity of commercial soft and hard wheat as *Triticum aestivum* L. affected by gastric pH conditions. Journal of Agriculture and Food Chemistry. 2005; 53:2433-2440
29. Mohsen MS, Ammar SMA. Total phenolic contents and antioxidant activity of corn tassel extracts. Food Chem. 2008; 112:595-598.
30. Zhou K, YU L. Effects of extraction solvent on wheat bran antioxidant activity estimation. *LWT*; 2004; 37:717-721.
31. Duthie SJ, Collins AR, Duthie GG, Dobson VL. Quercetin and myricetin protect against hydrogen peroxide-induced DNA damage (Strand breaks and oxidised pyrimidines) in human lymphocytes. Mutat Res. 1997; 393:223-331.
32. Skaper SD, Fabris M, Ferrari V, DalleCarbonare M, Leon A. Quercetin protects cutaneous tissueassociated cell types including sensory neurons from oxidative stress induced by glutathione depletion: cooperative effects of ascorbic acid. Free Radic. Biol. Med. 1997; 22:669-678.
33. Urquiaga I, Leighton F. Plant polyphenol antioxidants and oxidative stress. Biol. Res. 2000; 33(2):55-64
34. Min G, Chun-Zhao L. Comparison of techniques for the extraction of flavonoids from cultured cells of *Saussurea medusa* Maxim. World J Microb. Biot. 2005; 21:1461-1463.
35. Prasain JK, Wang CC, Barnes S. Flavonoids and isoflavones (Photoestrogens): absorption, metabolism, and bioactivity. Free Radical Bio Med. 2004; 37:1324-1350.
36. Williamson G, Manach C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. Am J Clin Nutr. 2005; 81:243S-255S.
37. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Therapeut. 2002; 96:67-202.
38. Sakanaka S, Tachibana Y, Okada Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (Kakinoha-cha). J Food Chem. 2005; 89:569-575.
39. Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohoni PP, Biyani MK. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. J. Phytochem. 2003; 63:97-104.
40. Liu S, Lin J, Wang C, Chen H, Yang D. Antioxidant properties of various solvent extracts from lychee (*Litchi chinenesis* Sonn.) flowers. Food Chem. 2009; 114:577-81.