ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

PHYTOCHEMICAL AND *IN VITRO* ANTIOXIDANT ASSAY OF *FUMARIA OFFICINALIS* LEAF EXTRACT

Rintu Dutta*[1], Mukesh Kumar Sharma[1], Azaz Khan[2], Megha Jha[2]

[1]*Maharaj Vinayak Global University, Jaipur, Rajasthan, India*

[2]*Pinnacle Biomedical Research Institute, Near, Bharat Scout and Guides Campus, Shanti Marg, Shyamla Hills Road, Bhopal, Madhya Pradesh, India*

**Corresponding author: rintudutta555@gmail.com*

ABSTRACT

Biologically active compounds from natural sources are of interest as possible new drugs for different diseases. Over many centuries humans have been mining the bounties of nature for discovering natural products that have been used for the treatment of all human diseases. *Fumaria officinalis* (*F. officinalis*) belongs to family papaveraceae and is traditionally used to treat hypertension, hepatitis diabetes, many inflammatory and painful-ailments. The aim of the present study was to evaluate qualitative and quantitative phytochemical analysis and *in vitro* antioxidant activities of leaf of *F. officinalis* collected from Bhopal region of Madhya Pradesh. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent method and aluminium chloride method respectively. The *In vitro* antioxidant activity of *Petroleum ether,* chloroform, ethyl acetate and methanolic extract of the leaf was assessed against DPPH, H_2O_2, and reducing power assay method using standard protocols. Phytochemical analysis revealed the presence of carbohydrates, glycosides, alkaloids, flavonoids, triterpenoids and steroids. The total phenolics content of leaves methanolic extract was (169.16mg/100mg), followed by flavonoids (202.33mg/100mg). The activities of all leaves extracts against DPPH, H_2O_2 and reducing power assay method were concentration dependent. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

Keywords: Fumaria officinalis, Phytochemical, Antioxidant, DPPH, H_2O_2, Reducing power assay.

1. INTRODUCTION

There has been intense interest recently among the public and the media in the possibility that increased intake of dietary antioxidants may protect against chronic diseases, which include cancers, cardiovascular, and cerebrovascular diseases. Antioxidants are substances that, when present at low concentrations, compared with those of an oxidizable substrate, significantly prevent or delay a pro-oxidant-initiated oxidation of the substrate [1]. A pro-oxidant is a toxic substance that can cause oxidative damage to lipids, proteins, and nucleic acids, resulting in various pathological events or diseases. Examples of pro-oxidants include reactive oxygen and nitrogen species (ROS and RNS), which are products of normal aerobic metabolic processes. ROS include superoxide ($O_2-\cdot$), hydroxyl ($OH\cdot$), and peroxyl ($ROO\cdot$) radicals, and hydrogen peroxide (H_2O_2). RNS include nitric oxide ($NO\cdot$) and nitrogen dioxide ($NO_2\cdot$) [2, 3]. There is considerable biological evidence that ROS and RNS can be damaging to cells and, thereby, they might contribute to cellular dysfunction and diseases. The existence and development of cells in an oxygen-containing environment would not be possible without the presence of a complicated antioxidant defense system that includes enzymatic and nonenzymatic components. The nonenzymatic antioxidants, most of which have low molecular weights and are able to directly and efficiently quench ROS and RNS, constitute an important aspect of the body's antioxidant system components [4]. The interaction among these antioxidants and the difficulty in measuring all of them individually prompted the development of assays for measuring total antioxidant capacity. The measurement of total antioxidant capacity of all these nonenzymatic antioxidants is necessary and important in evaluating in vivo antioxidant status in many clinical and nutritional studies. During the last decade, there was a growing demand for natural plants having diverse activities towards diseases especially chronic ones

that need long term management [5]. *F. officinalis*, family Papaveraceae (Fumariaceae), also named smoke of the earth is a tiny plant that grows in many Eastern-Mediterranean countries. It has been used in the Asian folk-medicine in many inflammatory and painful ailments like conjunctivitis and rheumatism [6-9]. Additionally, researchers had proven its efficacy as an antioxidant, antiviral and antimicrobial agent [10]. Te plant phytochemically comprises many secondary metabolites especially the isoquinoline alkaloids [11-13]. These alkaloids are determined in literature by diverse techniques mainly reversed Phase-HPLC methods [12]. The folk use of *F. officinalis* in various chronic ailments, made it of interest to explore its effect on other inflammatory and metabolic disorders and their complications. The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds and *in vitro* antioxidant activity of leaf of *F. officinalis* in Bhopal region of Madhya Pradesh.

2. MATERIAL AND METHODS

2.1. Plant material

The leaves of *F. officinalis* were collected from local area of Bhopal (M.P.) in the month of July, 2019. The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Safia College of Arts and Science, peer gate Bhopal. A voucher specimen number 203/Saif./Sci./Clg/Bpl**.** was kept in Department of Botany, Safia College of Arts and Science, peer gate Bhopal, for future reference.

2.2. Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). Clonidine (Unichem, Ltd.); Chlorpheniramine maleate (Alkem, Mumbai), All the chemicals used in this study were of analytical grade.

2.3. Extraction

In present study, plant material was extracted by using cold maceration method; the leaves of *F. officinalis* were collected, washed and rinsed properly. About 3kg of the powder was extracted with different organic solvent petroleum ether, ethyl acetate, chloroform and methanol and allow standing for 4-5days each. The extract was filtered through Whattman no.1 filter paper to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. Extract was transferred to beaker and evaporated & excessive moisture was removed and extract was collected in air tight container. Dimethyl sulfoxide (DMSO) was used to dissolve each extracts and sterilized using 0.22µm syringe filters (Axiva, Scichem Biotech) for further use [14, 15].

2.4. Qualitative phytochemical analysis of plant extract

The *F. officinalis* leaves extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate [16, 17]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

2.5. Quantification of secondary metabolites

2.5.1. Total phenolic content estimation

The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Concentration of (20-100µg/ml) of gallic acid was prepared in methanol. Concentration of 100µg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2 ml of a 10 fold dilute folin Ciocalteu reagent and 4 ml of 7.5% sodium carbonate. The tubes were covered with parafilm and it was then incubated at room temperature for 30 min with intermittent shaking and the absorbance were taken at 765 nm against using methanol as blank. Total phenolic content was calculated by the standard regression curve of gallic acid and the results were expressed as gallic acid equivalent (mg/g) [18].

2.5.2. Total flavonoid content estimation

Different concentration of rutin (20 to 100µg/ml) was prepared in methanol. Test sample of near about same polarity (100µg/ml) were prepared. An aliquot 0.5ml of diluted sample was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a 5% $NaNO_2$ solution. After 6 min, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for 5min, and then 2 ml of 4% NaOH solution was added to the mixture. The final volume was adjusted to 5ml with distilled water and allowed to stand for another 15 min. Absorbance was determined at 510 nm against water as blank. Total

flavonoid content was calculated by the Standard regression curve of Rutin/ Quercetin [19].

2.6. *In-vitro* Antioxidant activity

2.6.1. DPPH radical scavenging activity

For DPPH assay, the method of Gulçin *et al.,* 2006 [20] was adopted. A solution of 0.1mM DPPH (4mg/100ml) in methanol was prepared and 1 ml of this solution was mixed with 1 ml of different concentrations of the different extracts. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Ascorbic acid was used as reference standard while methanol was used as control. Reduction of the stable DPPH radical was used as a marker of antioxidant capacity of *F. officinalis* extracts. The change in colour was measured at 517 nm wavelength using methanolic solution as a reference solution. This was related to the absorbance of the control without the plant extracts. The percentage inhibition of free radical DPPH was calculated from the following equation:

% inhibition = [(absorbance of control – absorbance of sample)/absorbance of control] × 100%.

All the tests were carried out in triplicates. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

2.6.2. Hydrogen peroxide (H_2O_2) scavenging assay

Hydrogen peroxide scavenging activity of the plant extracts was determined using the procedure explained by *Jayaprakasha et al.,* 21]. A solution of hydrogen peroxide (20mM) was prepared in phosphate buffered saline (PBS; pH7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230nm by using the molar absorptivity of $81M^{-1}cm^{-1}$. Different concentrations of extract (20 to 100µg/ml) in ethanol were prepared.1 ml of ethanolic standard and test were added to 2 ml of hydrogen peroxide solution in PBS. After 10 min the absorbance was measured at 230 nm against a blank solution that contained hydrogen peroxide solution without the extract.

The percentage of H_2O_2 scavenging of the plant extract was calculated as follows:

$$\%\ \text{scavenged [H2O2]} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} x100$$

2.6.3. Reducing power assay

A spectrophotometric method was used for the measurement of reducing power. For this 0.5 ml of each of the extracts was mixed with 0.5ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of 1% potassium ferricyanide (10 mg/ml). The reaction mixture was incubated at 50°C for 20 min separately, and then rapidly cooled, mixed with 1.5 ml of 10% trichloroacetic acid and centrifuged at 6500 rpm for 10 min. An aliquot (0.5ml) of the supernatant was diluted with distilled water (0.5ml) and then ferric chloride (0.5ml, 0.1%) was added and allowed to stand for 10 min. the absorbance was read spectrophotometrically at 700 nm. Ascorbic acid (AA) was used as standard for construction of calibration curve [22].

$$\text{Reducing Power (\%)} = (As / Ac) \times 100$$

Here, Ac is the absorbance of control (AA) and as is the absorbance of samples (extracts) or standards.

3. RESULTS AND DISCUSSION

The crude extracts so obtained after each of the successive cold maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from the leaves of the plants using petroleum ether, ethyl acetate, chloroform and methanol as solvents are depicted in the Table 1.

Table 1: Results of percentage yield of leaves extracts

Fumaria officinalis Extract	% Yield
Pet. ether extract	1.71
Chloroform extract	6.44
Ethyl acetate extract	12.11
Methanolic extract	14.83

The results of qualitative phytochemical analysis of the crude powder of leaves of *F. officinalis* are shown in Table 2. Ethyl acetate, chloroform and methanolic extracts of leaves sample of *F. officinalis* showed the presence of carbohydrates, glycosides, alkaloids, flavonoids, triterpenoids and steroids. Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoid content (TFC). The TPC was calculated with respect to gallic acid (standard) and TFC was then calculated with respect to rutin taken as standard. The TPC and TFC in ethyl acetate and methanolic extract

were found to be 94.66,169.16mg/gm and 115.0, 202.33mg/gm respectively Table 3, 4 & Fig 1, 2. Antioxidant activity of the samples was calculated through DPPH, H_20_2 and reducing power assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition, the better is the activity. Ascorbic acid was taken as standard in all the 4 tests and the values were comparable with concentration ranging from 20µg/ml to 100µg/ml. The reduction ability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants [23].

Table 2: Phytochemical evaluation of *F. officinalis* leaves

Tests	Petroleum ether	Ethyl acetate	Chloroform	Methanol
Carbohydrates				
Molish test	+Ve	+ Ve	+ Ve	+ Ve
Benedict's test	+Ve	+ Ve	+ Ve	+ Ve
Proteins and Amino Acids				
Biuret's test	-Ve	-Ve	- Ve	-Ve
Glycosides				
Borntrager	- Ve	+Ve	- Ve	+Ve
Killar killani	-Ve	+Ve	- Ve	+Ve
Alkaloids				
Mayer	- Ve	+Ve	- Ve	+Ve
Hager	-Ve	+Ve	- Ve	+Ve
Wager	-Ve	+Ve	- Ve	+Ve
Saponins				
Froth's test	- Ve	- Ve	- Ve	- Ve
Flavonoids				
Alkaline reagent test	- Ve	+Ve	+ Ve	+Ve
Treterpenoids and Steroids				
Salkowski's test	+Ve	+Ve	+ Ve	+Ve
Libbermann burchard's test	+Ve	+ Ve	+ Ve	+ Ve

Table 3: Total phenolic content (TPC) of extract of *F. officinalis*

TPC Expressed as mg/gm Gallic Acid Equivalent				
S. No.	Pet ether extract	Chloroform extract	Ethyl Acetate extract	Methanolic extract
1	0.088	0.189	0.247	0.391
2	0.087	0.183	0.247	0.399
3	0.085	0.19	0.245	0.395
Mean Absorbance	0.087	0.187	0.246	0.395
TPC value	**14.33**	**65.167**	**94.66**	**169.16**

Table 4: Total flavonoid content (TFC) of extract of *F. officinalis*

TFC Expressed as mg/gm Gallic Acid Equivalent				
S. No.	Pet ether extract	Chloroform extract	Ethyl Acetate extract	Methanolic extract
1	0.101	0.179	0.201	0.293
2	0.099	0.178	0.209	0.293
3	0.103	0.178	0.205	0.291
Mean Absorbance	0.101	0.178	0.205	0.292
TFC value	**11.00**	**88.33**	**115.00**	**202.33**

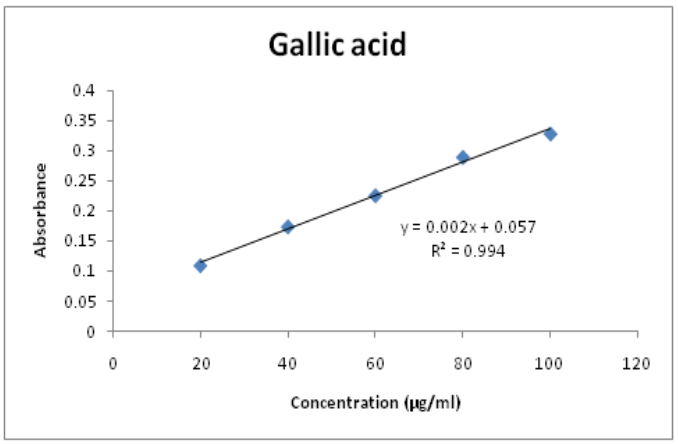

Fig. 1: Graph of estimation of Total Phenolic Content

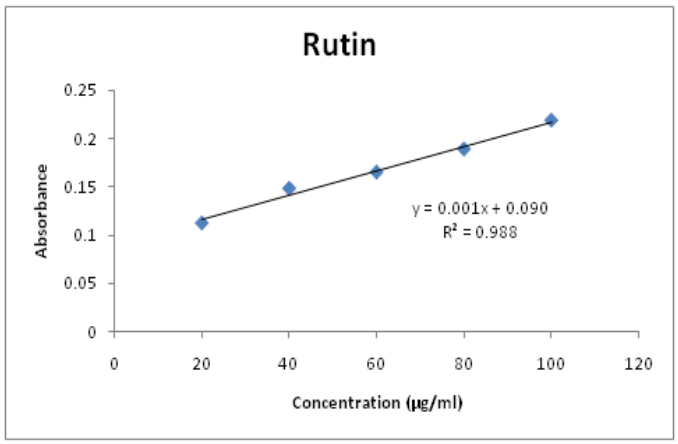

Fig. 2: Graph of estimation of Total Flavonoids Content

Table 5: DPPH assay of ascorbic acid, petroleum ether, ethyl acetate, chloroform and methanolic extract

S. No.	Conc. (µg/ml)	(% Inhibition)				
		Ascorbic acid	Pet ether Extract	Ethyl acetate Extract	Chloroform Extract	Methanolic Extract
1.	20	55.04	30.97	48.50	36.46	50.44
2.	40	59.65	35.04	51.50	40.71	55.44
3.	60	65.13	39.29	55.58	49.73	58.65
4.	80	75.75	44.78	58.41	53.81	64.88
5.	100	84.25	47.43	66.19	60.35	71.66
IC 50 Value		**11.80**	**109.38**	**31.56**	**65.98**	**20.69**

All the 4 extracts of *F. officinalis* observed a good inhibitory activity against DPPH radical. The scavenging activity of extracts and standard on the DPPH radical expressed as IC_{50} values: AA (11.80) MET (20.69), EA (31.56), PE (109.38µg/ml). Highest quenching ability was shown by methanol extract while petroleum extract showed lowest scavenging activity. The experimental data revealed that polar extracts had stronger free radical scavenging effect than the non polar ones. IC_{50} value of methanolic extract was close to ascorbic acid which is a well known antioxidant Table 5. The antioxidant activity of plant extracts is due to polyphenols present in them which show redox properties. These are important since they decompose peroxides, neutralize free radicals, and quench singlet and triplet oxygen [24]. In reducing power assay**,** conversion of the Fe^{3+}/ferricyanide complex to Fe^{2+}/ferrocyanide complex occurs due to presence of reducers. The yellow colour of the test sample changes to different shades of green and blue depending on the reducing power of each compound. This colour change was measured at 700 nm by spectrophotometer [25].

Table 6: Result of reducing power assay

S. No.	Conc. (µg/ml)	Absorbance				
		Ascorbic acid	Pet ether Extract	Ethyl acetate Extract	Chloroform Extract	Methanolic Extract
1.	20	0.127	0.031	0.102	0.097	0.11
2.	40	0.144	0.038	0.107	0.103	0.115
3.	60	0.156	0.052	0.112	0.109	0.124
4.	80	0.168	0.06	0.118	0.113	0.131
5.	100	0.187	0.069	0.121	0.123	0.143

The reducing power of extracts is shown graphically by depicting absorbance as a function of concentration. The reducing power of all the extracts increased with increase in concentration. Reducing power of methanol extract is highest which is comparable to standard compound ascorbic acid Table 6 & Fig. 3. Table 7 show the scavenging ability of all 4 extract and ascorbic acid on hydrogen peroxide at different concentrations. Extracts was capable of scavenging hydrogen peroxide in an amount dependent manner at all the tested concentrations. Hydrogen peroxide itself is a rather weak oxidant and most organic compounds (except for some sulfur containing molecules) are virtually inert to attack by it at ordinary environmental or cellular concentrations and temperatures. In the presence of reduced transition metal ions, however, hydrogen peroxide is converted to the much more reactive oxidant, hydroxyl radical in the cells by Fenton reaction. Besides this, studies have shown that other transition metals such as copper (I), cobalt (II) and nickel (II) also take part in the process [26]. Thus, the removing is very important for antioxidant defense in cell or food systems. The methanol extract showed good scavenging ability compared to all 3 extract but less than the standard compound.

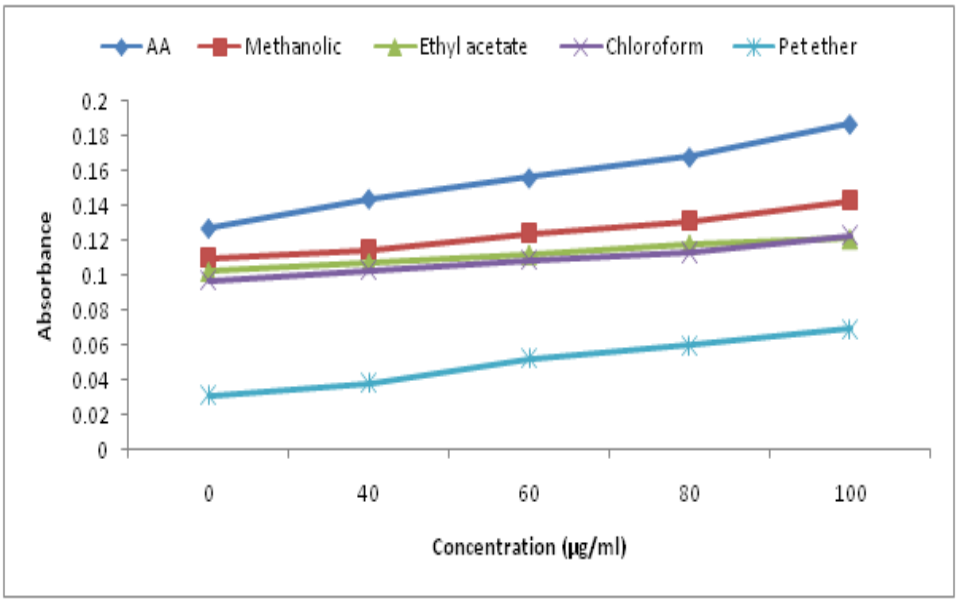

Fig. 3: Reducing power assay

Table 7: % Inhibition of ascorbic acid, petroleum ether, ethyl acetate, chloroform and methanolic extract using H_2O_2 method

S. No.	Conc. (µg/ml)	(% Inhibition)				
		Ascorbic acid	Pet ether Extract	Ethyl acetate Extract	Chloroform Extract	Methanolic Extract
1.	20	52.33831	17.21393	45.67164	29.55224	52.63682
2.	40	56.61692	22.98507	50.64677	38.40796	57.91045
3.	60	60.89552	33.03483	59.10448	41.09453	63.58209
4.	80	64.67662	40.49751	65.67164	45.87065	71.04478
5.	100	71.24378	46.56716	70.44776	48.95522	78.00995
	IC 50		**11.39**	**107.10**	**34.37**	**100.04**

4. CONCLUSION

It can be concluded from present investigation that the observed level of phytoconstituents revealed that *F. officinalis* is a rich source of antioxidant compounds proved by in vitro studies. Currently available synthetic antioxidants are suspected to cause or prompt negative health effects, hence strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. The broad range of activity of the extracts suggests that multiple mechanisms are responsible for the antioxidant activity. The multiple antioxidant activity of extract demonstrated

in this study clearly indicates the potential application value of the *F. officinalis*. However, the in vivo safety of *F. officinalis* needs to be thoroughly investigated in experimental rodent models prior to its possible application as an antioxidant ingredient, either in animal feeds or in human health foods. Further studies, on the use of above plants for their antioxidant role in various systems may provide potential natural antioxidants.

5. REFERENCES

1. Prior RL, Cao G. *Free Rad. Biol, Med.*, 1999; **27**:1173-1181.
2. Gulçin I. *Arch Toxicol.*, 2012; **86**:345-391.
3. Gulçin İ, Topal F, Çakmakçı R, Bilsel M, et al. *J. Food Sci.*, 2011; **76**:C585-C593.
4. Cao G, Prior RL. CRC Press, 2nd edition. Marcel Dekker Inc, New York, 2002; pp 1–2.
5. Raafat K. 1st ed. Beirut: Dar Al-Nahda Al-Arabia; 2016.
6. Ivanov I, Vrancheva R, Marchev A, Petkova N, et al. *Int. J. Curr. Microbiol. Appl. Sci.*, 2014; **3(2)**:296-306.
7. Rakotondramasy-Rabesiaka L, Havet J-L, Porte C, Fauduet H. *Sep Purif. Technol.*, 2007; **54(2)**:253-61.
8. Khamtache-Abderrahim S, Lequart-Pillon M, Gontier E, Gaillard I, et al. *Ind. Crops Prod.*, 2016; **94**:1001-1008.
9. Fatima S, Akhtar MF, Ashraf KM, Sharif A, et al. *Cell Mol Biol.,* 2019; **65(2)**:50-57.
10. Orhan I, Şener B, Choudhary M, Khalid A. *J Ethnopharmacol.*, 2004; **91(1)**:57-60.
11. Manske RH. *Can J. Res.,* 1938; **16(12)**:438-444.
12. Sousek J, Guedon D, Adam T, Bochorakova H, et al. *Phyto-chem. Anal.*, 1999; **10(1)**:6-11.
13. Sturm S, Strasser E-M, Stuppner H. *J. Chromatogr A*, 2006; **1112(1-2)**:331-338.
14. Sangeetha J, Vijayalakshmi K. *Int. J. Pharm. Sci. Drug Res.*, 2011; **3(2)**:116-122.
15. Jain DK, Gupta S, Jain R, Jain N. *Res. J. Pharm. Tech* 2010; **3(3)**:882-884.
16. Khandelwal KR. 23rd Ed. Nirali Prakashan; 2005.
17. Kokate CK. Practical pharmacognosy. 4th Ed. Vallabh Prakashan; 1994.
18. Ainsworth EA, Gillespie KM. *Nature Protocol,* 2007; **2(4)**:875-877.
19. Zhishen J, Mengcheng T, Jianming W. *Food Chem.* 1999; **64**:555-559.
20. Gulçin I, Elias R, Gepdiremen A, Boyer L. *Eur. Food Res. Technol.,* 2006; **223**:759-767.
21. Jayaprakasha GK, Rao LJ, Sakariah KK. *Bioorg. Med. Chem.*, 2004; **12**:5141-5146.
22. Jain R, Jain SK. *Pharmacologyonline,* 2011; **2**:755-762.
23. Rigane G, Ben Younes S, Ghazghazi H, Ben Salem R. *Int. Food Res. J.,* 2013; **20(6)**:3001-3007.
24. Saha MR, Hasan SMR, Akter R, Hossain MM. *Bangl. J. Vet. Med.*, 2008; **6(2)**:197-202.
25. Jayanthi P, Lalitha P. *J. Pharm. Res.,* 2011; **4(11)**:4003-4005.
26. Halliwell B, Aeschbach R., Lolliger J, Aruoma O I. *Food Chem. Toxicol.*, 1995; **33**:601.