

**PHYTOCHEMICAL INVESTIGATION AND IN-VITRO BIOLOGICAL ACTIVITY
OF *SONCHUS OLERACEUS* AND *LACTUCA SERRIOLA***

NAYEEM N* AND IMRAN M

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Northern Border University
Saudi Arabia

*Corresponding Author: Email: naira_64@yahoo.co.in

Received 21st March 2018; Revised 15th April 2018; Accepted 17th April 2018; Available online 1st May 2018

DOI: <https://doi.org/10.31032/IJBPAS/2018/7.5.4422>

ABSTRACT

In the present study the methanolic extracts of *Lactuca serriola* and *Sonchus oleraceus* collected from Rafha, Northern Border Province were evaluated for the the total phenolic content, flavonoid content and invitro antioxidant activities. The total flavonoid and phenolic contents were investigated by the Folin ciocalteu method and Aluminum chloride colorimetric method respectively. While the antioxidant activity was evaluated by DPPH Radical Scavenging Activity Assay, Hydrogen Peroxide Scavenging Activity and Reducing Power Assay. The amount of phenolic compounds and flavonoids was found to be more in the methanolic extract of *Lactuca serriola* (LS(Me)) when compared to the extract of *Sonchus oleraceus* (SO(Me)). *Lactuca serriola* extract showed better antioxidant activity in both DPPH Radical Scavenging Activity Assay and Hydrogen Peroxide Scavenging Activity, when compared to the methanolic extract of *Sonchus oleraceus*, and this was attributed to the difference in amount of phenolic acids and flavonoids. The methanolic extracts of *Lactuca serriola* extract contained 400 µgm of phenolic acid while the other extract contained 320 µgm. However there was no significant variation in the antioxidant activity of the two extracts in Reducing Power Assay.

Keywords: *Lactuca serriola*, *Sonchus oleraceus*, antioxidant, flavonoid content, phenolic content

INTRODUCTION

In recent past there has been an increased interest to bring about new developments in the prevention and treatment of diseases. The phyto constituents of plants have been reported to possess various medicinal properties. Phenolics and Flavonoids are among the most important bioactive principles in plants and serve as sources of natural antioxidants in human diet [1]. These antioxidants are capable of scavenging free superoxide radicals and protecting the human biological system against the harmful effects of oxidative processes. It is believed that flavonoids and phenolics contribute to a major extent towards improving the total antioxidant defense system of the human body [2]. These compounds have a capacity ascribed to their antioxidant abilities, capacity to transfer electrons, quenching of free radicals and chelating abilities, activate antioxidant enzymes, reduce α tocopherol, radicals and inhibit oxidases. Diverse biological activities have been reported for the phenolics some of them being antiulcer, anti-inflammatory, antidiabetic, antioxidant, cytotoxic and antitumor as reported in the literature [3]. An imbalance between antioxidants and free radicals results in oxidative stress, which will lead to cellular damage [4]. Oxidative stress occurs when there is an excess of ROS and decrease in antioxidant levels, this may cause tissue

damage resulting in different diseases [5]. Researchers have reported that the antioxidants of plant origin could be of great importance as therapeutic agents in the disorders that are caused due to oxidative stress [6]. Phenolic antioxidants have also been shown to delay the development of chronic diseases such as cardiovascular diseases and Alzheimer's disease [7]. The health-promoting effects of these plant constituents may be due to interactions with key enzymes, signaling cascades involving cytokines and transcription factors [8]. Knowing the importance of antioxidants it is undeniable to have interest when it comes to finding natural antioxidants from plant materials. *Lactuca serriola* and *Sonchus oleraceus* are plants belonging to the Asteraceae family and are wildy grown in the Northern Border Province, Saudi Arabia [9]. The study is designed with an aim to evaluate the in-vitro antioxidant activity along with evaluation of the total phenolic acid and total flavonoids which are considered to be the class of phytoconstituents that are responsible for anti-oxidant activity.

Lactuca serriola (Arabic name: Fajtaloon) is also referred to as prickly lettuce. It is biennial herb. Literature review reveals that the plant contains lactucopicrin, lactucin, lactucone, vitamins A, B1, B2, and beta

carotene and iron. The phytochemical investigations of seeds revealed the presence of alkaloids, bitter substance lettuce, oxalic acid, triterpenes and sesquiterpene esters [10, 11]. It has been reported to be used in whooping cough, rheumatic pain, antipyretic, ailments of the urinary tract, Anti-cancer, spasmogenic, spasmolytic, bronchodilator, vasorelaxant activities, diuretic and laxative [12].

***Sonchus oleraceus*:** (Arabic name: Adheed). This plant is annual herb. The reported chemical constituents are lupeol, α and β -amyrin, ursonic acid, oleanolic acid, betulinic acid, Loliolide, 15-O- β -glucopyranosyl-11 β ,13-dihydrourospermal A 2 and β -sitosterol [13]. Traditionally it has been used for the treatment of a wide variety of infections like headache, diarrhea, menstrual problems, fever, hepatitis, liver infections, inflammation and rheumatism. The other activities reported are antioxidant, antidiabetic, anxiolytic, cytotoxic, antinociceptive, Anti-inflammatory and antipyretic, Anti HIV Antibacterial and Anticonvulsant [14].

MATERIAL AND METHODS

1.Collection and extraction: The plants under investigation ie *Lactuca serriola* and *Sonchus oleraceus* were collected from Rafha, Northern Border Province, Saudi Arabia. They were dried in the research lab of Faculty of Pharmacy, pulverized and stored until further use. Plant material were

extracted with methanol and water and vacuum dried to get constant weight followed by calculation of percentage yield.

2. Phytochemical analysis

The various extracts of the plant were subjected to qualitative analysis for the phytoconstituents like alkaloids, carbohydrates, glycosides, steroids, tannins, proteins, amino acids and flavonoids as per the standard procedures [15]. The percentage of total phenolic content was estimated by the Folin ciocalteu method for the aqueous and methanolic extracts of both the plants. The absorbance was measured at 750 nm in the UV spectrometer. A standard graph was obtained for gallic acid was using different dilutions by plotting absorbance versus the concentration. The total phenolic content was expressed as gallic acid equivalent [16].

The flavonoid content of the plant extracts was evaluated by the Aluminum chloride colorimetric method using standard Quercetin. The absorbance of this mixture was measured at 415 nm. Quercetin was used as a stand. Different dilution of quercetin were prepared and a standard graph was obtained by plotting absorbance versus the concentration. The total flavonoid contents were calculated as quercetin equivalent from the calibration curve [16].

3: Antioxidant activity: [16]

DPPH Radical Scavenging Activity

Assay

Anti-oxidant activity of the extracts was evaluated by 1, 1-Diphenyl-2-picrylhydrazyl (DPPH). The stock solution of the extract (10 mg/ml) was in methanol. The working solutions of the extracts were prepared from the stock solution using suitable dilutions. The anti-oxidant activity of the plant extract and the isolated compounds were determined based on the radical scavenging effect of the stable DPPH. DPPH was prepared as 0.002 % solution in methanol and mixed with the extracts. The prepared solutions were kept in the dark for ½ an hour and the absorbance was measured at 517 nm. The % absorbance was calculated using the formula:

$$\% \text{ inhibition} = \frac{A - B}{A} \times 100$$

Where A is the absorbance of the blank and B is the absorbance of the sample.

Hydrogen Peroxide Scavenging Activity

The ability of the extracts to scavenge hydrogen peroxide was estimated as follows. Different concentrations of the samples were taken in test tubes. To this 0.6ml hydrogen peroxide solution was added and the volume was made up to 1ml by addition of phosphate buffer. The absorbance of hydrogen peroxide was determined by absorption at 230 nm using a

spectrophotometer. Phosphate buffer without hydrogen peroxide was taken as blank. Hydrogen peroxide scavenging activity was expressed as the percentage inhibition. The abilities of the extracts to scavenge the hydrogen peroxide was calculated using the following equation:

$$\% \text{ inhibition} = \frac{A - B}{A} \times 100$$

Reducing Power Assay

Various concentrations (20-100 µgms) of the two plant extracts were prepared in methanol. To this 2.5ml each of phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide were added. This mixture was then placed in a water bath for about 20 minutes. This was then centrifuged after the addition of 5ml of 10% trichloro acetic acid. The upper layer was collected and an equal volume of water was added to it. This was followed by the addition of 0.5ml of freshly prepared FeCl₃ solution. The Prussian blue color complex that was formed was measured at 700nm.

RESULTS AND DISCUSSION

Phenolic compounds, flavonoids, Sterols, glycosides, alkaloids, carbohydrates and proteins were detected in the preliminary phytochemical investigation. Phenolic compounds and flavonoids play an important role in many of the activities like analgesic, anti-inflammatory, anti-oxidant and anti-microbial activity as revealed in the literature survey [17]. Hence the total

phenolic acids and flavonoid content was evaluated for the extracts using the standard methods i.e. Folin Ciocalteu method and Aluminum chloride colorimetric methods respectively. The phenolic acids were estimated by the Folin Ciocalteu method as mentioned in the experimental section. The calibration curve was obtained for standard gallic acid in the concentrations 100 to 700 $\mu\text{g/ml}$. The concentration of the phenolic acids present in the methanolic extracts was extrapolated from the standard graph. The total phenolic acid content was calculated as gallic acid equivalent. The content of the phenolic acid is as depicted in the fig 1. The amount of phenolic acid was found to be maximum (400 μg) at absorbance value of 0.0660 in LS(Me) when compared to the methanolic extract of SO(Me) (320 μg) whose absorbance was 0.0562. The flavonoid content was evaluated by the Aluminum chloride colorimetric method. A calibration curve for the standard quercetin was obtained by taking different concentrations of quercetin. The total flavonoid contents were calculated as quercetin equivalent from the calibration curve by plotting the absorbance versus concentration. From the calibration curve the amount of flavonoids was found to be maximum in LS(Me) i.e. 490 μg followed by 340 μg , 230 μg and 190

μg for SO(Me), LS(Aq) and SO(Aq) respectively as shown

in figure 2.

From the results it was evident that the amount of phenolic acids and flavonoids was more in the methanolic extract of *Lactuca serriola*. In our study the methanolic extracts of both the extracts had illustrated the high total phenolic content when compared to the aqueous extracts, which is in concurrence of reports of other research groups [16,18]. This may be attributed to the formation of complexes by certain fractions of phenolic compounds with carbohydrates and proteins, which may be more easy to extract in methanol when compared to other solvents.

Oxidative stress is one of the important risk factor in the pathogenesis of a number of diseases. Antioxidants play an important role in protecting the body from damage caused by free radical induced oxidative stress. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals and inhibit the oxidative mechanisms that lead to degenerative diseases. Polyphenols present in plant act as reducing agents and antioxidants because of the hydrogen-donating property of hydroxyl groups present in them [19]. The phenolics present in the plant may exert their activity by scavenging some reactive species, suppressing lipid peroxidation recycling,

may bind pro-oxidant metals, and some may increase the activity of antioxidant enzymes. Antioxidant activity can be evaluated using a number of models with different mechanisms, including hydrogen atom transfer single electron transfer, reducing power, and metal chelation, among others. The anti-oxidant activity was evaluated by the DPPH method, Hydrogen peroxide scavenging assay and Reducing power assay methods. The DPPH method is widely used method to investigate the scavenging activity of several natural phytochemicals as it is an easy, rapid and sensitive method. DPPH is stable free radical which has a maximum absorbance at 517 nm in methanolic solution. This radical contains an odd electron, which is responsible for the absorbance at 515 nm and for the appearance of deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance [20]. The results of the anti-oxidant activity revealed that LS (Me) exhibited better activity when compared to the SO(Me).

Hydrogen peroxide scavenging assay was the second method used for the evaluation of the antioxidant activity.

Hydrogen peroxide is not very reactive by itself, but it may give rise to hydroxyl

radicals. As it is a weak oxidizing agent and has an ability to inactivate a few enzymes directly by oxidation of essential thiol groups, it can also react probably with Fe^{2+} and Cu^{2+} ions once inside the cell to form hydroxyl radical and which may lead to its toxic effects and the extracts containing phenolic compounds are reported to scavenge the H_2O_2 by donating electrons and thus neutralizing it to water [21]. The percentage of H_2O_2 scavenging activity was found to be 84.49% and 78.29% at 100 $\mu\text{g}/\text{ml}$ for LS (Me) and SO (Me) respectively.

Reducing power assay is based on the principle that the substances having reduction potential will react with potassium ferricyanide forming potassium ferrocyanide. On addition of ferric chloride the potassium ferrocyanide forms a ferric ferrous complex which has an absorption maximum at 700nm. Increased absorbance of the reaction mixture indicates an increase in reducing power [22]. The reducing power of the methanolic extracts of the two plants under investigation is as shown in table 3 and figure 5. The reducing power of all the extracts was proportional to the increase in concentration. However there was no significant difference in the reducing power of the two extracts.

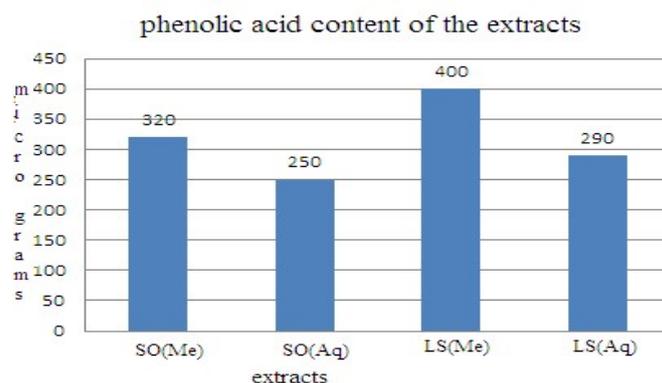


Figure 1: Phenolic content of the extracts

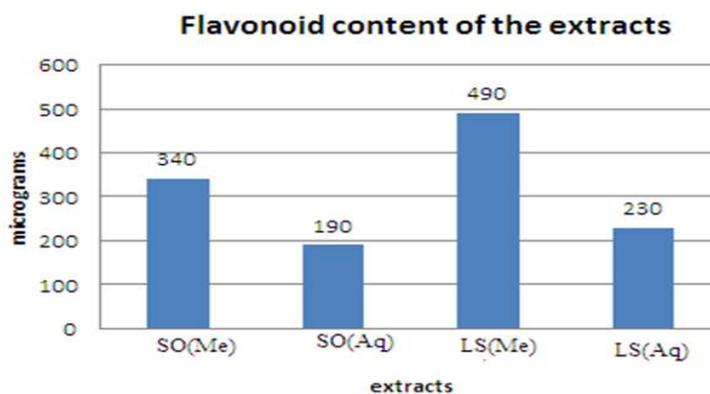


Figure 2: Flavonoid content of the extracts

Table 1: Anti oxidant activity of the methanolic extracts by DPPH method:

µg/m	<i>Lactucaserriola</i>		<i>Sonchusoleraceus</i>	
	Abs	% Inh	Abs	%Inh
10	0.033	89.93	0.050	84.75
20	0.030	90.85	0.047	85.67
40	0.028	91.46	0.044	86.58
80	0.025	92.37	0.040	87.80
100	0.020	93.90	0.037	88.71
120	0.018	94.51	0.035	89.32
140	0.017	94.81	0.030	90.85
180	0.016	95.12	0.029	91.15
200	0.016	95.12	0.027	91.76

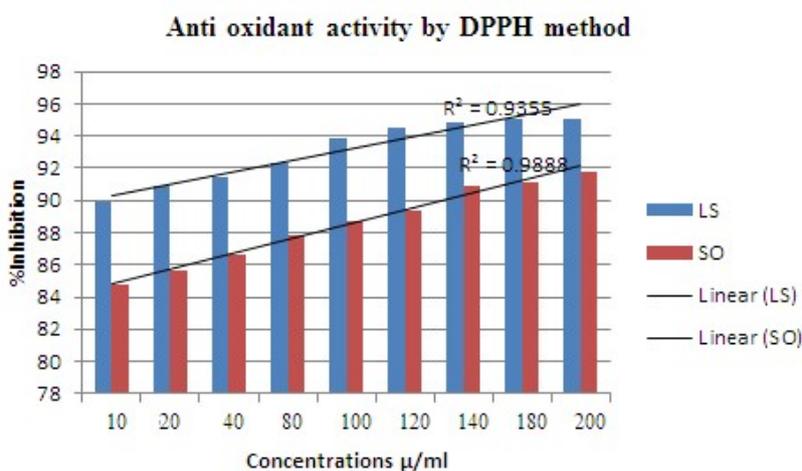


Fig 3: Anti oxidant activity of the methanolic extracts by DPPH method:

Table 2: Antioxidant activity by hydrogen peroxide method

µg/m	<i>Lactucaserriola</i>		<i>Sonchusoleraceus</i>	
	Abs	% Inh	Abs	%Inh
20	0.070±0.002	45.73	0.084±0.001	34.88
40	0.055±0.002	57.36	0.074±0.003	42.63
60	0.037±0.001	71.31	0.062±0.001	51.93
80	0.024±0.002	81.39	0.053±0.001	60.94
100	0.020±0.002	84.49	0.028±0.004	78.29

Antioxidant activity by hydrogen peroxide method

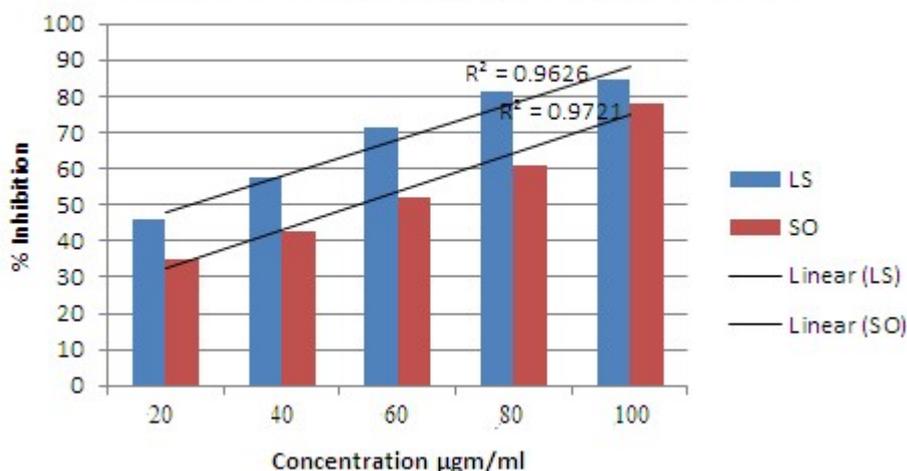


Fig 4:Antioxidant activity by hydrogen peroxide method

Table 3:Antioxidant activity by reducing power method

Concenµgms/ml	Absorbance	
	LS	SO
20	0.056	0.045
40	0.089	0.08
60	0.158	0.149
80	0.22	0.214
100	0.29	0.281

Reducing power assay

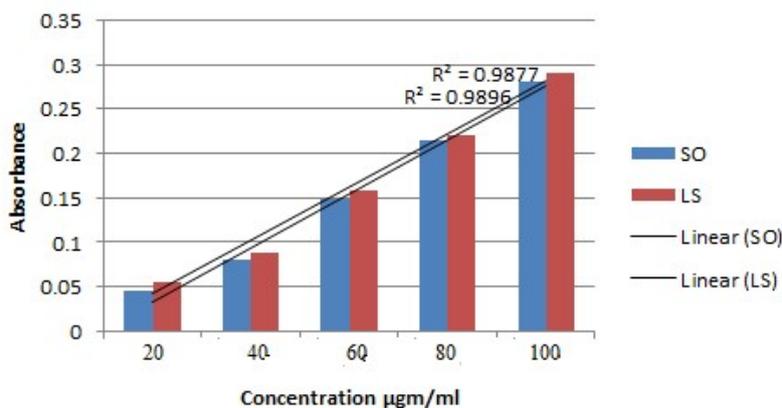


Figure 5: Antioxidant activity by reducing power method

CONCLUSION

In the present study, the two plants were evaluated with respect to their total phenolic content, flavonoid content and antioxidant capacity. The presence of phenolic compounds and flavonoids were confirmed by the standard methods. The methanolic extracts of LS exhibited good antioxidant activity which was measured by the DPPH and H₂O₂. This difference in activity was attributed to the difference in the phenolic acid and flavonoid content. However there was no significant difference in the anti oxidant activity by reducing power methods. The results of this study indicate that the plants under investigation contain certain amounts of phenolic compounds and flavonoids, and these plants could be used as natural sources of antioxidants.

ACKNOWLEDGEMENT

The authors are grateful to the Deanship of Scientific Research, Northern Border University, Saudi Arabia for approving, providing necessary facilities and financial support by; grant 6991-PHM-2017-1-7-F.

REFERENCES

- [1] Kim D, Jeond S, Lee C, Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry*. 2003; 81: 321-326.
- [2] Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal

ions in human disease: An overview. *Methods Enzymol* 1990; 186: 80-85.

- [3] Kumar S, Pandey AK. Chemistry and Biological Activities of Flavonoids: An Overview. *Sci World J*. 2013 ; 16 pages.
- [4] Kukic J, Petrovic S, Niketic M, Antioxidant activity of four endemic *Stachys* taxa. *BiolPharmaceut Bull*. 2006; 29:725-729.
- [5] Tian Y, Jiang B, An L, Bao Y, Neuroprotective effect of catalpol against MPP⁺-induced oxidative stress in mesencephalic neurons. *Eur J Pharmacol*. 2007; 568:142-148,.
- [6] Ramchoun M, Harnafi H, Alem C, Benlys M, Elrhaffari L, Amrani S, Study on antioxidant and hypolipidemic effects of polyphenol rich extract from *Thymus vulgaris* and *Lavendula multifida*. *Pharmacol Res*. 2009; 1: 106-112.
- [7] Halliwell B, Antioxidants and human diseases: a general introduction. *Nutrition Reviews*. 1997; 55: 44-52.
- [8] Polya GM, Biochemical Targets of Plant Bioactive Compounds: A Pharmacological Reference Guide to Sites of Action and Biological

- Effects, Taylor & Francis New York. 26, 124-135, 2003.
- [9] Ahmed K Osman, Faraj Al-Ghamdi, Abdulhakim Bawadekjia, Floristic diversity and vegetation analysis of Wadi Arar: A typical desert Wadi of the Northern Border region of Saudi Arabia. Saudi J Biol Sci. 2014; 21(6): 554–565.
- [10] Baquar S R. Medicinal and Poisonous Plants of Pakistan, Printas, Karachi, Pakistan, 1989.
- [11] www.naturalmedicinalherbs.net/herbs/l/lactuca-serriola. retrieved on 20/3/2017.
- [12] Medicinal herbs: Prickly Lettuce – *Lactuca serriola*, www.naturalmedicinalherbs.net/herbs/l/lactuca-serriola=prickly-lettuce.php retrieved on 20/3/2017
- [13] Yan XU, Jing-Yu .Chemical constituents of *Sonchusoleraceus*, J China Pharma Uni, 2005; 36(5): 411-413.
- [14] Medicinal uses of Common Sowthistle (*Sonchusoleraceus*) natureconservation.in › Flora › Shrubs. retrieved on 20/3/2017.
- [15] Kokate CK, Purohit AP, Gokhale SB. 17th Edn. Pune: NiraliPrakashan; 2001.
- [16] Esmacili AK, Taha RM, MohajerS, Banisalam B. Antioxidant Activity and Total Phenolic and Flavonoid Content of Various Solvent Extracts from In Vivo and In Vitro Grown *Trifoliumpratense* L. (Red Clover). Biomed Res Int. 2015; 2015: 643285.
- [17] Bent HH. The biochemistry and medical significance of flavonoids. Pharmacol Thera. 2002; 96: 80-112.
- [18] Sahreen S, Khan MR, Khan RA. Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. Food Chemistry. 2010; 122(4): 1205–1211.
- [19] Aberoumand A, Deokule SS. Comparison of phenolic compounds of some edible plants of Iran and India. Pak J Nut. 2008; 7: 582–585.
- [20] Sahaa MR, Hasana S M R, Aktera R, Hossaina MM, Alamb MS, Alam MA, Mazumderc M. EH. In vitro free radical scavenging activity of methanol extract of the leaves of *mimusopselengilinn*. Bangla J Vet Med. 2008; 6 (2): 197–202.
- [21] Gulcin I, Huyut Z, Elmastas M, Aboul-Enein HY. Radical scavenging and antioxidant activity of tannic acid. Arab J Chem 2010;3(1): 43-53.
- [22] Chanda S, Dave R. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. Afri J MicroRes. 2009; 3(13): 981-996.