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Evaluation of Antioxidant Activity of Stem-Bark Extracts of the Selected Plants

Rahul Kumar Jonwal[1*] | Rashmi Kundra[2]

[1]Assistant Professor (Botany), S.P.N.K.S. Government P. G. College, Dausa, Rajasthan, India.
[2]Assistant Professor (ABST), S.P.N.K.S. Government P. G. College, Dausa, Rajasthan, India.

*Corresponding Author: rameshjony89@gmail.com

ABSTRACT

Nature has been a source of various medicines from multiple years. Traditional drugs for about over all urbanizations of the globe bounded in herbal treatments. Due to the presence of many biochemicals, plant extracts have antioxidant property. In this investigation, methanolic extracts of stem-bark of the selected trees (Holoptelia integrifolia, Pscidium guajava, Pongamia pinnata, Syzygium cumini and Bombax cieba) were estimated for their antioxidant [DPPH (1,1-diphenyl 2-picryl-hydrazil) radical scavenging activity assay and FRAP (Ferric Reducing ability of Plasma) assay] at different concentrations (10 mg/L to 100 mg/L). Results exposed the occurrence of good antioxidant property of all the extracts. The maximum free radical scavenging potential was observed in stem bark of Psidium guajava with lowest IC50 value (43.76 mg/L) and the minimum free radical scavenging potential was observed in stem bark of Holoptelea integrifolia with the highest IC50 value (400 mg/L) by DPPH method. Among the selected plants, methanolic extracts of Syzygium cumini was observed to show maximum FRAP activity with the lowest IC50 value (71.08 mg/L) while the lowest FRAP activity was shown by Psidium guajava with the highest IC50 value (112.98 mg/L). Results of this study resolved that the stem-bark of selected trees have active biochemicals which are cause for their antioxidant property. Further investigation, and isolation of those active biochemicals may pave path for pharmaceutical industries to formulate new antioxidant drugs.

***Keywords**: Antioxidant, FRAP, Phytochemicals, Stem Bark, DPPH.*

Introduction

Nature has been a source of various medicines from multiple years. Traditional drugs for about over all urbanizations of the globe bounded in herbal treatments (Kala *et al.,* 2004). Due to the presence of many biochemicals, plant extracts have antioxidant property. Antioxidants are substances which lowers oxidation of certain compounds in body even found in low concentrations (Halliwell, 2007). Some antioxidants are formed in body like SOD, GSH etc. while others are taken as dietary supplements (Sies, 1997). Plants are considered to be rich sources of dietary antioxidants. It is considered that almost all medicinal plants have antioxidant compounds within different parts. Ascorbic acid was the first natural antioxidant which was derived from plants. After that finding, many researches were done to find out new natural antioxidant compounds in plants (Szent-Giörgyi, 1963). Against oxidative stress, antioxidant compounds become promising agents (Kasote *et al.,* 2013).

Nowadays, there are approx. nineteen *in vitro* and ten *in vivo* approach for evaluation of antioxidant nature; can be used to measure the antioxidant potential of plant isolates (Alam *et al.,* 2013). In maximum of these *in vitro* methods plant extracts indicated good antioxidant potential. Their natural ability to produce non-enzymatic antioxidants like glutathione and ascorbic acid may be the reason of this and at the same time secondary metabolites like phenolic compounds.

Plants also form and store different PSMs which have prevented ROS production and avoid excessive oxidation of biomolecules. These molecules are also significant for adapting the plants to environmental changes (Baier and Dietz, 2005). PSMs give passive as well as active resistance. The

continuous availability of metabolites in the presence of stressors, is known as passive resistance whereas the production of metabolites in response to specific stressors is known as active resistance (Korkina, 2007).

Up to date, multiple kinds of PSMs has been find out in plants (Korkina, 2007). These may have nitrogen in their structure or may be without nitrogen (Patra *et al.,* 2013). Many plants have alkaloids, terpenoids etc. In laboratory assay of free radical scavenging activity, activity of alkaloids is considered negligible. In terpenoid family, about 40,000 different products are found which are PSMs (Aharoni *et al.,* 2005). Different kinds of terpenes (like monoterpenes, diterpenes, sesquiterpenes) have good antioxidant potential (Baratta *et al.,* 1998).

Phenolic acid, flavonoids, lignans, tannins are compounds which are related to a single group named as plant phenolics (Duthie *et al.,* 2000; Myburgh, 2014; Blokhina *et al.,* 2003). Aromatic rings are present in phenolic compounds in which single or many -OH groups are present. Free radical scavenging capacity of phenolics is positively reliant on number of free hydroxyl groups (Morgan *et al.,* 1997). Flavonoids are considered to have very good antioxidant potential which is enhanced when this work together with other antioxidant molecules (Croft, 1998).

Bark is a significant part of plant find generally from tree species. The word barks represent all outside tissues of axis's cambium, both in primary as well as secondary growth phase (Srivastav, 1964). The word bark "The outer integument of the wood and exterior to it i.e., all tissues outside the cambium" (Esau, 1960). In young stems of woody plants, the bark is made up of following tissues arranged from the outer surface to the inner surface: corks (phellem), cork cambiums (phellogen), secondary cortex (phelloderm), cortex (the primary tissue of the stems and roots) and the phloem (Kuribara, 2000).

Bark tissues are necessary for plants in defending them from pathogens, herbivorous, sun irradiation, desiccation, wind, fire, hail, flooding and snow by either their bioactive compounds or by a thick cork layer. The roles of the outer bark are the prevention of water loss from roots and stems, pathogen entry, injury to underlying tissues (Biggs, 1986) and the inner bark have functions of transport and storage of photosynthates (Berryman, 1972).

In this present resesrch, methanolic extracts of stem-bark of selected trees (*Holoptelia integrifolia, Pscidium guajava, Pongamia pinnata, Syzygium cumini and Bombax cieba*) were estimated for their antioxidant [DPPH (1,1-diphenyl 2-picryl-hydrazil) radical scavenging activity assay and FRAP (Ferric Reducing ability of Plasma) assay] at variable concentrations (10 mg/L to 100 mg/L).

Materials and Methods

Collection of Plants and Extract Preparation

From the University of Rajasthan campus in Jaipur, Rajasthan, the stem bark of the chosen trees - *Holoptelia integrifolia, Pscidium guajava, Pongamia pinnata, Syzygium cumini, and Bombax cieba* - were collected. The stem bark was washed in the laboratory and dried in air at room temperature. All of the obtained samples were placed in a flat-bottom dish in an air oven set to 105°C for 1, 3, and 5 hours and after cooling to room temperature in a desiccator, the samples were weighed in order to estimate the moisture content. The process was repeated until successive weighing agrees to as constant weighing. The plant material's weight loss was taken as a gauge of its moisture content.

The air-dried and coarsely powered, about 30 g of plant material were places in Soxhlet extractor with 150 ml methanol for 24 h. Then, these were filtered by Whatman filter paper No. 1. The extracts were then concentrated to dryness by reduced pressure and controlled temperature by a rotary evaporator. The resulting extracts were used for additional analysis after being dried over anhydrous $CaCl_2$.

Antioxidant Assay

- **DPPH Radical Scavenging Activity Assay**

For estimation of DPPH radical scavenging potential of extracted samples 1,1-diphenyl 2-picryl-hydrazil (DPPH) procedure given by Alothman *et al*., (2009) was used. The mixing of 100 µl aliquot form peel extract was done in 3.9 ml taken from 0.1 mM DPPH (methanolic) solution. Then blend was exposed to vortex and left for incubation in the dark for 30 min. Its OD was calculated at 515 nm while methanol was used as blank.

$$\text{The radical scavenging activity was determined by the ratio} = \frac{Ab_{control} - Ab_{sample}}{Ab_{control}} \times 100$$

Where $Ab_{control}$ is the absorbance of DPPH solution and absorbance of the DPPH solution with sample is denoted by Ab_{sample}.

Linear plot of concentration versus % inhibition was plotted and by this IC50 values were estimated. The antioxidant property of each extract was showed in form of IC50 (stated as the quantity of concentration necessary to prevent DPPH radical development by 50%), find out with the help of inhibition curve.

- **FRAP Assay (Reducing Ability Assay)**

FRAP test also known as Ferric Reducing ability of Plasma assay by Benzie and Strain, 1996 is slightly changed for estimation of the overall antioxidant property in the plant extract. The stock solutions contained 300 mM of acetate buffer (0.3 M acetic acid and sodium acetate pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine in 40 mM HCl) and 20 mM FeCl3.6H2 solution. By adding 25 ml acetate buffer into 2.5 ml FeCl3.6H2O and TPTZ solution, fresh working solution was prepared. The solution temperature was increased to 37 °C just before use. Plant extracts prepared in 100 µl of methanol, hexane and ethyl acetate were used to do reaction in the dark condition, with 290 µl of the FRAP solution for 30 min. Absorbance of the coloured complex of ferrous tripyridyl triazine was noted at 593 nm. The standard curve was drawn and it is found linear between the concentration 100 and 1000 µM ferrous sulphate. Results are shown in µgTE/gdw (Trolox equivalent antioxidant capacity). The catalase enzyme concentration was found out using following equation-

$$\text{Catalase enzyme concentration} = \frac{(A_0 - A_{60}) \times Vt}{\varepsilon_{240} \times d \times V_8 \times C_t \times 0.001}$$

A0 = Initial absorbances of the assay mixture,

A60 = Absorbances of assay mixture after 1 minute,

Vt = Total volumes of the assay mixture in ml,

ε240 = Molar extinction coefficients - H2O2 at OD240 (34.9 mol - 1 cm - 1),

d = Optical lengths of cuvette (1 cm),

Vs = Volume of sample in ml.

Results

- **DPPH Radical Scavenging Activity Assay**

Results revealed that methanolic extracts of all the bark of selected plants exhibit free radical scavenging potential of diverse level. It was also concluded that increasing concentration of extracts influence free radical scavenging property positively.

Free radical scavenging potential by different extracts of all the carefully chosen plant parts by DPPH assay and their IC50 values are shown in Table 1. Percent inhibition in plants is graphically represented in Figure 1 to 5.

Extract of *Holoptelea integrifolia* (at concentration fluctuating from 10 mg/L to 100 mg/ L) showed 0.4±0.06 % to 16.3±1.24 % free radical scavenging potential. IC50 values of the samples as calculated were 400 mg/ L.

Extract of *Syzygium cumini* (at concentration fluctuating from 10 mg/L to 100 mg/L) showed 4.7±0.76 % to 73.4±1.69 % free radical scavenging potential. IC50 values of the samples as calculated were 74.51 mg/L.

Extract of *Bombax ceiba* (at concentration fluctuating from 10 mg/L to 100 mg/L) showed 3.0±1.22 % to 85.4±1.38 % free radical scavenging potential. IC50 values of the samples as calculated were 55.30 mg/L.

Extract of *Psidium guajava* (at concentration fluctuating from 10 mg/L to 100 mg/L) showed 9.8±1.22 % to 88.6±1.43 % free radical scavenging potential. IC50 values of the samples as calculated were 43.76 mg/L.

Extract of *Pongamia pinnata* (at concentration fluctuating from 10 mg/L to 100 mg/L) showed 8.4±2.91 % to 18.6±1.78 % free radical scavenging potential. IC50 values of the samples as calculated were 227.27 mg/L.

Statistical analysis showed that the minimum IC50 value for the antioxidant potential by DPPH assay was found in *Psidium guajava* (43.76 mg/L) while maximun in *Holoptelea integrifolia* (400 mg/L).

- **FRAP Assay (Reducing Ability Assay)**

The enhancement in the potential of ferrous reducing power of the sample was showed by the rise in the amount of the extract and therefore the rise in optical density. Ferrous reducing power by diverse extracts of all the chosen plant parts by FRAP assay and their IC50 values are revealed in Table 2. Percent inhibition in plants is graphically represented in Figure 6 to 10.

It was observed that methanolic extracts of *Holoptelea integrifolia* exhibited FRAP activity as Trolox equivalent antioxidant capacity (TEAC) from 7.16±2.11 µgTE/gdw to 44.32±3.11 µgTE/gdw at the concentration from 10 mg/L to 100 mg/L with IC50 value of 110.64 mg/L.

FRAP activity shown by methanolic extract of stem bark of *Syzygium cumini* was 1.35±0.22 µgTE/gdw to 51.64±0.72 µgTE/gdw from the concentration of 10 mg/L to 100 mg/L with an IC50 value of 71.08 mg/L.

Methanolic extract of stem bark of *Bombax ceiba* showed FRAP activity ranging from 2.34±0.12 µgTE/gdw to 41.73±2.93 µgTE/gdw at the concentration of 10 mg/L to 100 mg/L with an IC50 value of 100.58 mg/L.

Methanolic extract of *Psidium guajava* showed FRAP activity from 4.17±0.73 µgTE/gdw to 38.61±1.62 µgTE/gdw from the concentration of 10 mg/L to 100 mg/L with an IC50 value of 112.98 mg/L.

Methanolic extracts of *Pongamia pinnata* showed FRAP activity ranging from 3.51±0.22 µgTE/gdw to 41.47±2.88 µgTE/gdw at the concentration of 10 mg/L to 100 mg/L with an IC50 value of 103.68 mg/L.

Statistical analysis showed that the minimum IC50 value for the antioxidant potential by FRAP assay was found in *Syzygium cumini* (71.08 mg/L) while maximun in *Psidium guajava* (112.98 mg/L).

Table 1: Antioxidant Potential of Methanolic Extracts of Stem Bark of the Selected Trees by DPPH Assay

S. N.	Name of plant	Concentration (mg/L)	% Free radical scavenging activity	Regression equation	IC_{50} value (mg/L)
1.	*Syzygium cumini* (L.)	10	4.7±0.76	y= 0.7671x-6.6713	74.51
		20	7.5±2.55		
		30	13.40±1.58		
		40	20.31±3.24		
		50	36.3±3.64		
		60	39.3±2.15		
		70	45.8±3.66		
		80	57±2.84		
		90	57.5±4.26		
		100	73.4±1.69		
2.	*Bombax ceiba* (L.)	10	3±1.22	y = 0.9136x-0.6067	55.30
		20	19.9±2.10		
		30	26±4.34		
		40	38.9±2.28		
		50	46.6±3.75		
		60	53.7±2.33		
		70	65.1±1.86		
		80	77.6±2.28		
		90	80.2±3.17		
		100	85.4±1.38		
3.	*Psidium guajava* (L.)	10	9.8±1.22		
		20	23.2±2.14		
		30	51.4±2.71		

		40	69.9±1.65	y = 0.8528x+ 20.387	**43.76**
		50	79.7±1.98		
		60	86.3±2.78		
		70	87.9±1.20		
		80	88.0±1.56		
		90	88.1±2.67		
		100	88.6±1.43		
4.	*Pongamia pinnata* (L.) Pierre.	10	8.4±2.91	y= 0.118x+7.2	227.27
		20	9.02±1.32		
		30	10.1±1.10		
		40	12.3±1.36		
		50	13.8±1.21		
		60	14.6±2.78		
		70	16.2±1.18		
		80	16.6±1.21		
		90	17.3±1.11		
		100	18.6±1.78		
5.	*Holoptelea integrifolia* (Roxb.) Planch.	10	0.4±0.06	y= 0.1588x- 2.34	400
		20	1.82±0.81		
		30	2.3±0.22		
		40	3.5±0.68		
		50	4.3±±0.15		
		60	6.3±0.37		
		70	7.5±0.71		
		80	10.7±1.18		
		90	10.8±0.58		
		100	16.3±1.24		
Note*: All values are denoted as Mean ± Standard deviation.					

Table 2: Antioxidant Potential of Methanolic Extracts of Stem Bark of the Selected Trees by FRAP Method

S. N.	Name of plant	Concentration (mg/L)	FRAP activity (µgTE/gdw)	Regression equation	IC_{50} value (mg/L)
1.	***Syzygium cumini* (L.)**	10	1.35±0.22	Y= 0.5613x- 10.098	**71.08**
		20	3.22±0.16		
		30	6.81±0.11		
		40	10.75±1.62		
		50	12.54±1.01		
		60	18.32±2.87		
		70	22.72±1.76		
		80	37.14±1.00		
		90	43.25±3.11		
		100	51.64±0.72		
2.	***Bombax ceiba* (L.)**	10	2.34±0.12	Y = 0.4371x- 6.0327	100.58
		20	3.12±0.21		
		30	6.76±0.44		
		40	9.83±0.18		
		50	12.62±0.23		
		60	18.52±1.17		
		70	23.11±0.82		
		80	28.87±3.27		
		90	33.18±1.82		
		100	41.73±2.93		
3.	***Psidium guajava* (L.)**	10	4.17±0.73		
		20	5.92±0.52		

		30	8.33±1.31	Y= 0.4153x-3.0753	112.98
		40	9.51±4.27		
		50	16.27±2.72		
		60	21.82±1.88		
		70	26.65±0.36		
		80	31.61±3.71		
		90	34.76±2.80		
		100	38.61±1.62		
4.	***Pongamia pinnata* (L.) Pierre.**	10	3.51±0.22	Y = 0.4451x-3.8507	103.68
		20	6.58±0.43		
		30	8.82±1.26		
		40	10.26±3.62		
		50	16.86±4.11		
		60	21.92±2.93		
		70	26.51±1.88		
		80	31.77±0.71		
		90	38.62±3.12		
		100	41.47±2.88		
5.	***Holoptelea integrifolia* (Roxb.) Planch.**	10	7.16±2.11	Y = 0.4422x-1.072	110.64
		20	9.62±0.77		
		30	13.44±1.62		
		40	18.81±2.87		
		50	21.13±3.11		
		60	27.64±3.56		
		70	31.48±1.88		
		80	38.55±0.74		
		90	41.78±2.64		
		100	44.32±3.11		

Note*: All values are denoted as Mean ± Standard deviation.

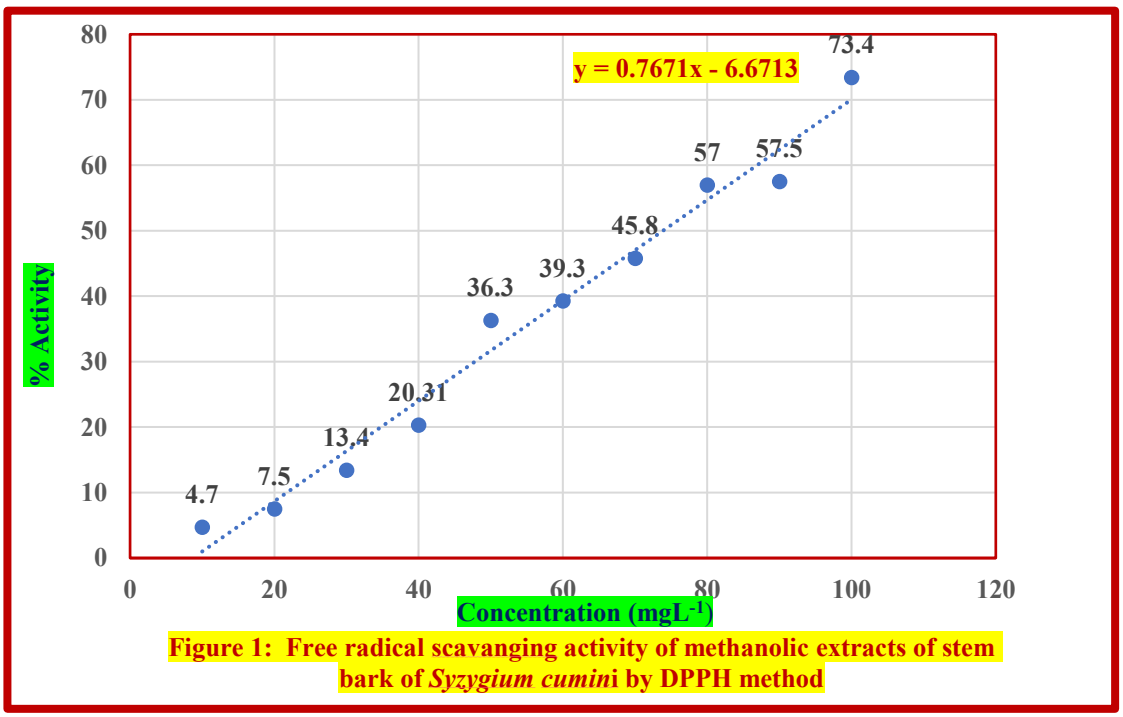

Figure 1: Free radical scavanging activity of methanolic extracts of stem bark of *Syzygium cumini* by DPPH method

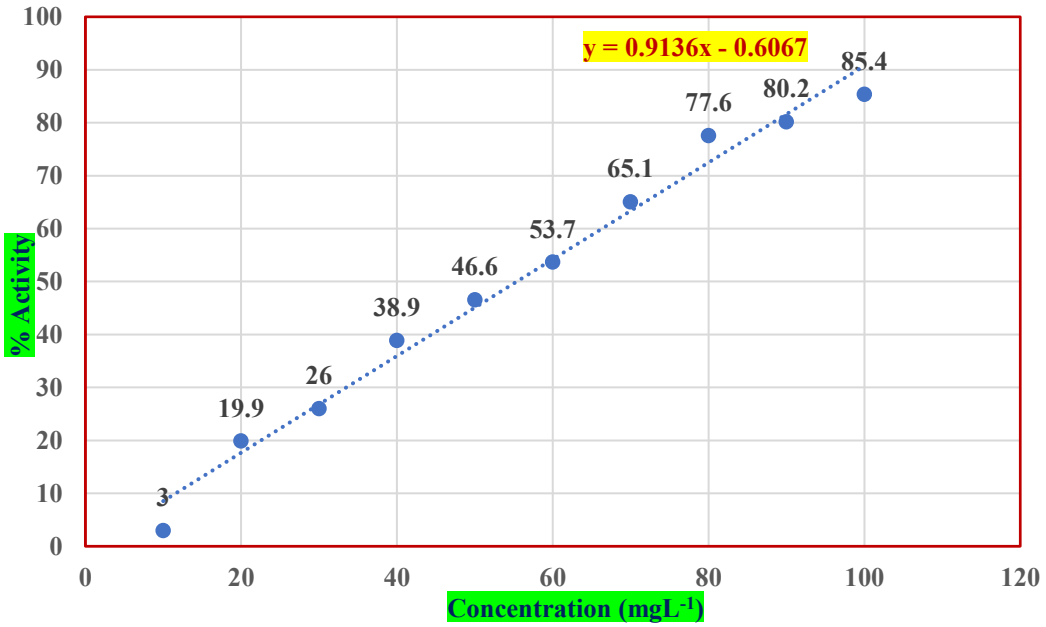

Figure 2: Free radical scavanging activity of methanolic extracts of stem bark of *Bombax ceiba* by DPPH method

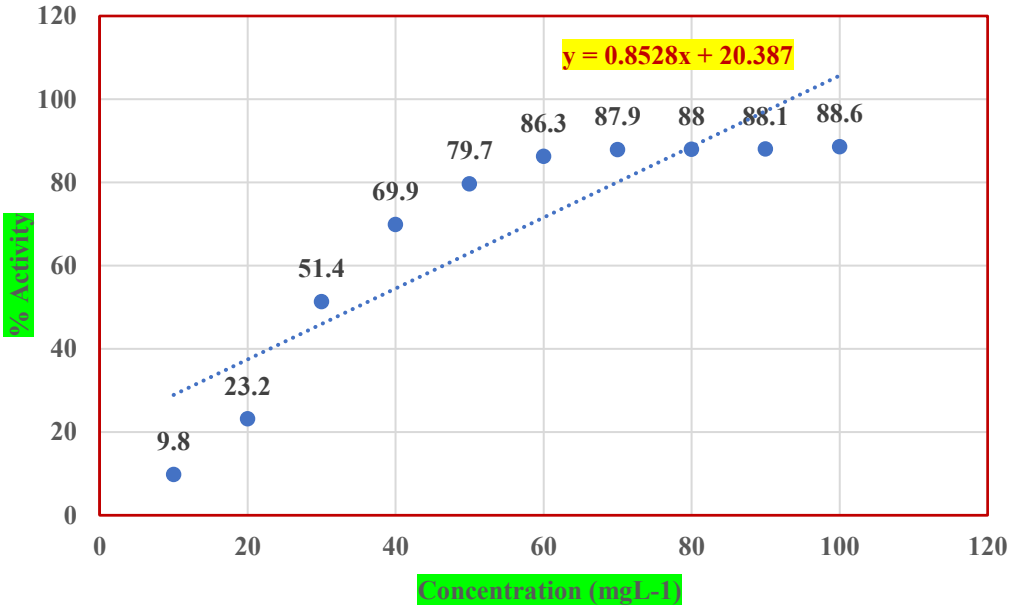

Figure 3: Free radical scavanging activity of methanolic extracts of stem bark of *Psidium guajava* by DPPH method

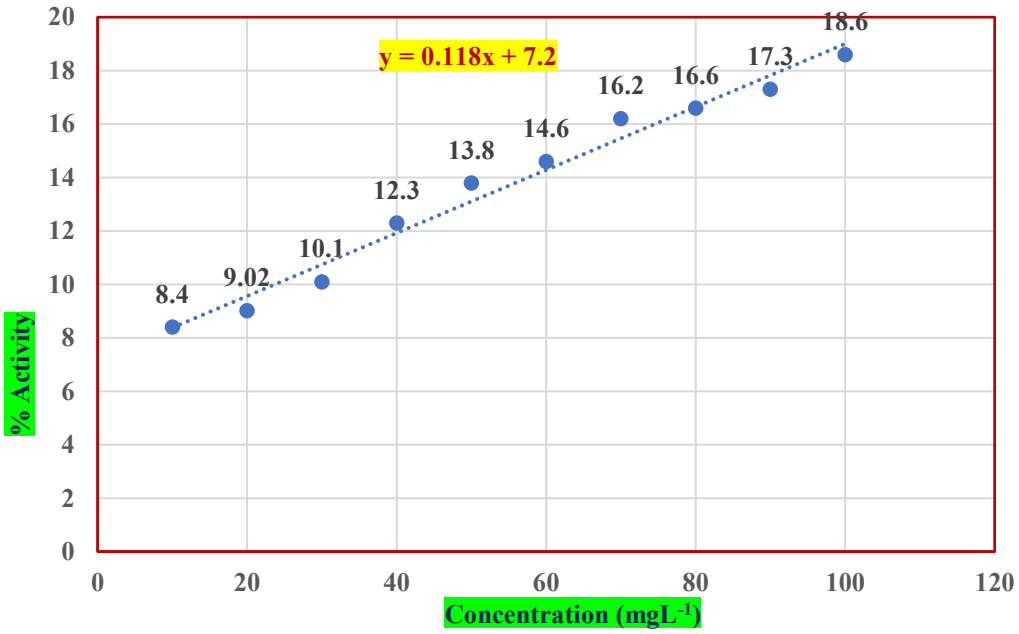

Figure 4: Free radical scavanging activity of methanolic extracts of stem bark of *Pongamia pinnata* by DPPH method

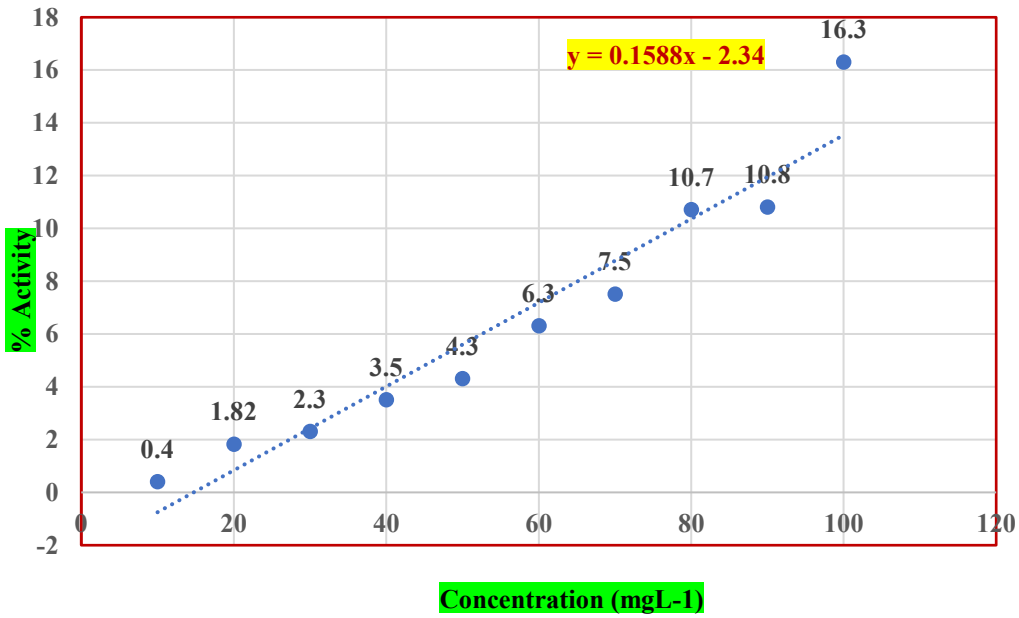

Figure 5: Free radical scavanging activity of methanolic extracts of stem bark of *Holoptelea integrifolia* by DPPH method

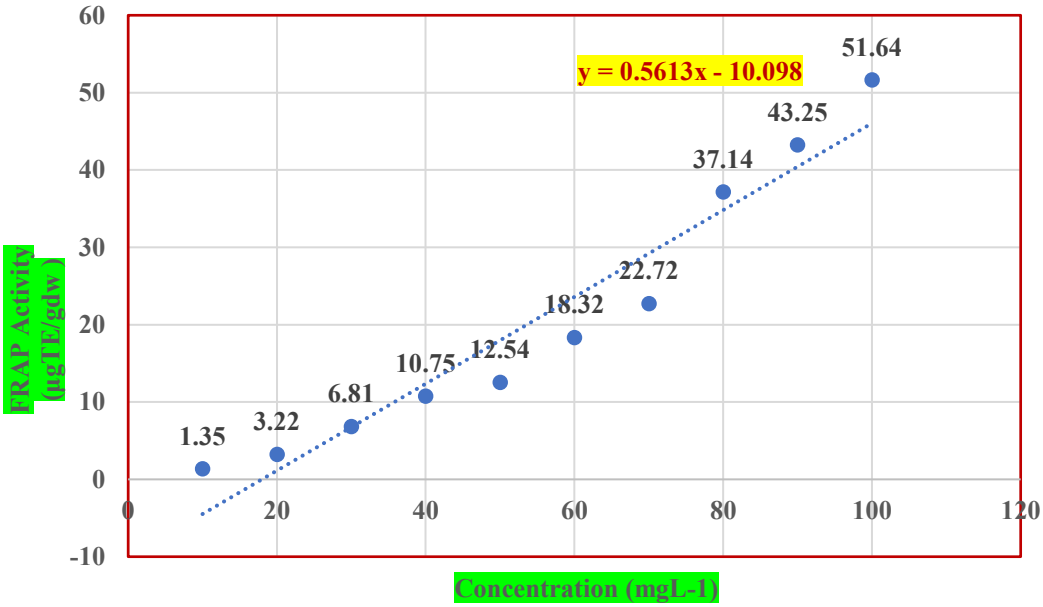

Figure 6: Estimation of antioxidant potential of methanolic extracts of stem bark of *Syzygium cumini* by FRAP assay

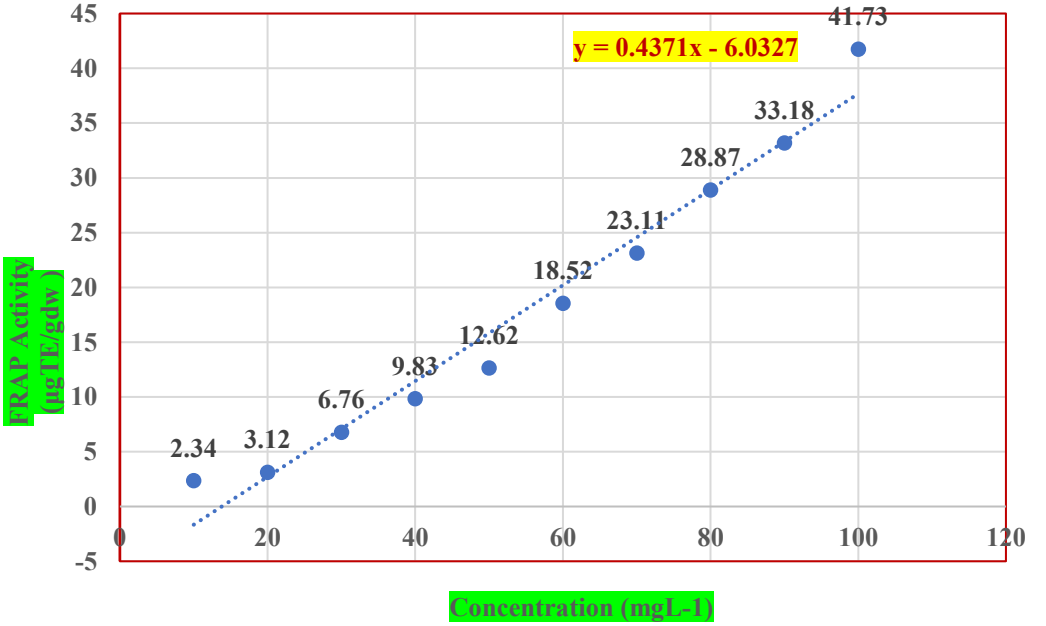

Figure 7: Estimation of antioxidant potential of methanolic extracts of stem bark of *Bombax ceiba* by FRAP assay

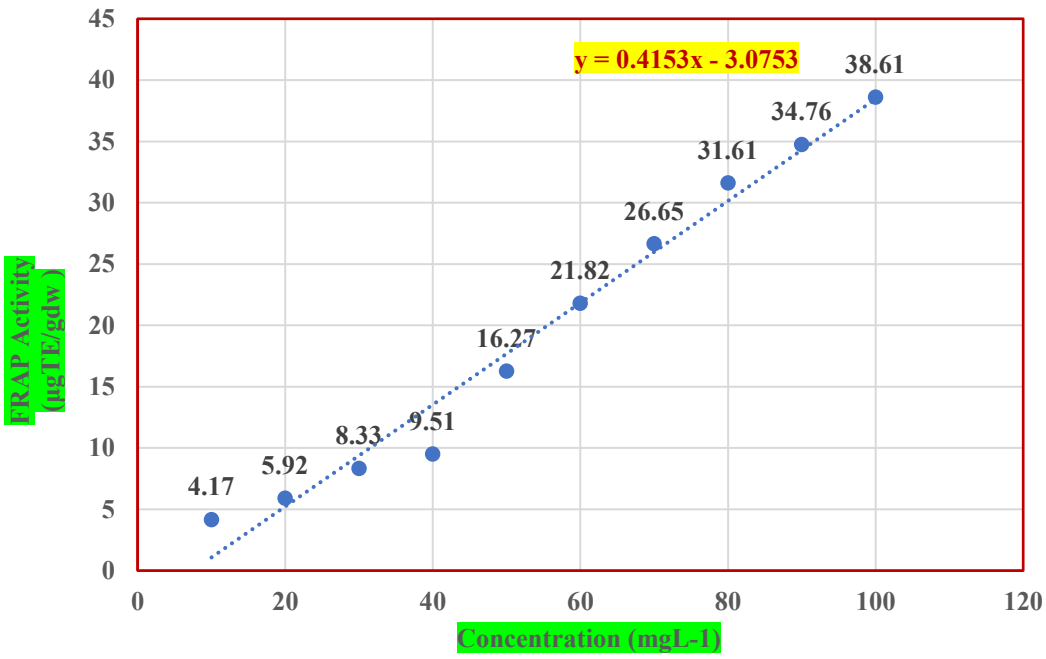

Figure 8: Estimation of antioxidant potential of methanolic extracts of stem bark of *Psidium guajava* by FRAP assay

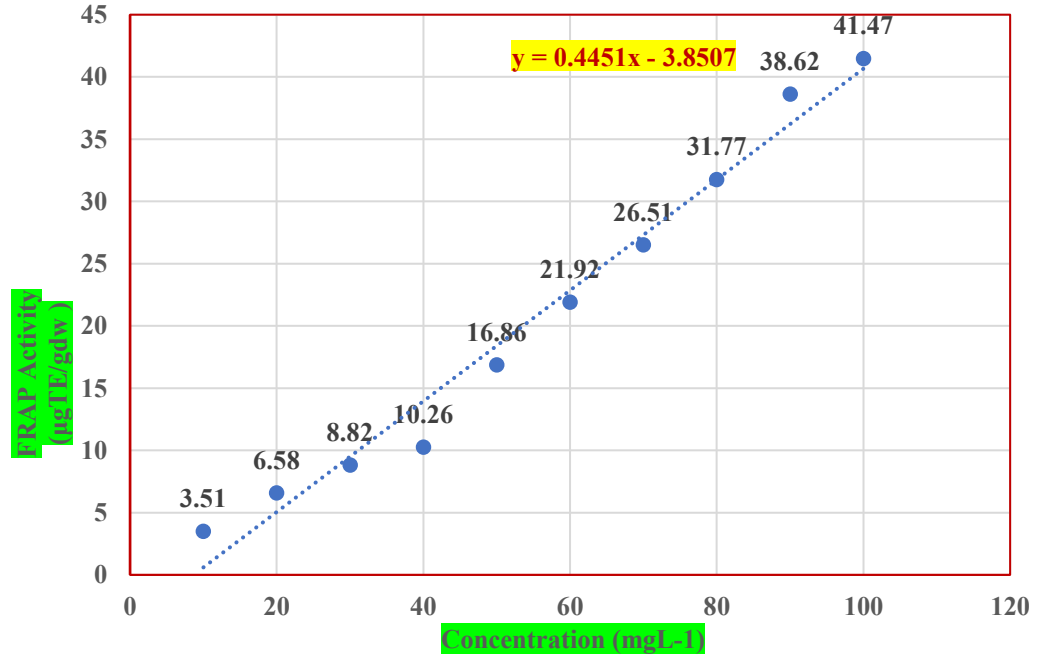

Figure 9: Estimation of antioxidant potential of methanolic extracts of stem bark of *Pongamia pinnata* by FRAP assay

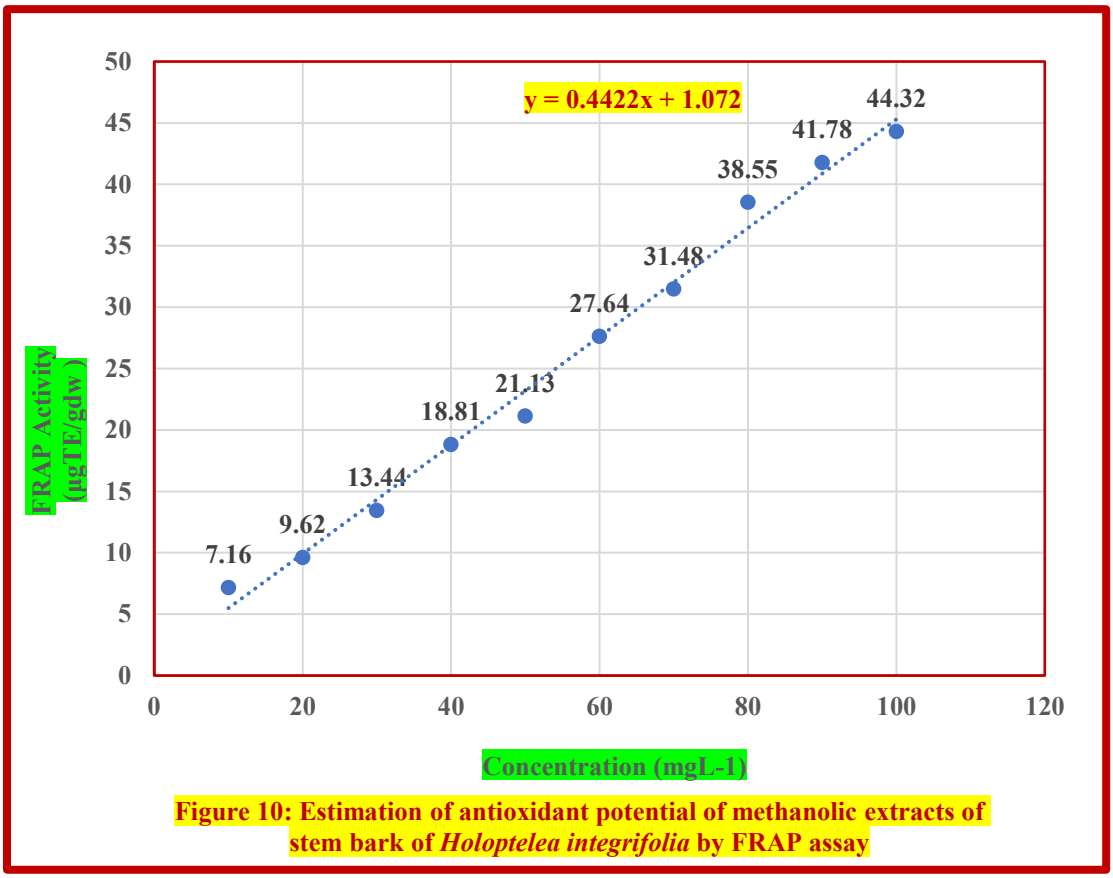

Figure 10: Estimation of antioxidant potential of methanolic extracts of stem bark of *Holoptelea integrifolia* by FRAP assay

Discussion and Conclusions

It is a well-recognized fact that plant phenolic substances are the supreme naturally present antioxidants, and a very amazing relationship between their concentrations and the antioxidant aptitudes has also been witnessed (Ozcan *et al., 2009;* Nahak and Sahu*, 2010).*

Free radicals show significant role in hundreds of diseases in humankind like atherosclerosis, arthritis, ischemia and reperfusion injury of multiple tissues, injuries of CNS, cancer, AIDS and gastritis (Cook and Samman, 1996; Kumpulainen and Salonen, 1999). Because of environmental impurities, chemicals, toxins, radiations, deep fried, spicy foods and physical stress; resulting free radicals lead to decrease of antioxidants of immune system, modification in gene expression and induction of irregular proteins.

Oxidative mechanism is one of the furthermost significant roots for production of free radicals in drugs, food, and even living beings. Hydroperoxides and catalase enzymes changes hydrogen peroxides and hydroperoxides to a non-radical form and works as natural antioxidants in multiple maladies, utilizing antioxidants as free radical scavengers may be essential (Kumpulainen and Salonen, 1999).

New era synthetic antioxidants such as butylated hydroxy anisole (BHA), tertiary butylated hydroquinone, butylated hydroxy toluene (BHT) and gallic acid esters, are seriously doubted by customers that they might have negative health impacts. For that reason, their continuous use is questioned regularly and poses as a demand to find a natural replacement of them. Moreover, these artificial antioxidants have low solubility and show modest antioxidant activity only (Branen, 1975; Barlow, 1990). At the present time, the therapeutic capabilities of medicinal plants as antioxidants have gained immense attention as they can reduce production of free radicals and tissue injuries due to them. There are a number of evidences that tea, fruits, wine, vegetables and spices have been used since centuries. A few of conventionally used such as rosemary and sage have been tested on commercial level for analysis of their performance (Schuler, 1990). Though, intensive research for finding natural antioxidants

is going on since decades (Oke and Hamburger, 2002), yet it fails to fulfill their demand in market. The antioxidant potential of plant species has been attributed to the presence of phenolics and other metabolites in them (Cook and Samman, 1996). In this concern, the flavonoids belonging to class of polyphenolic substances has been recognized to comprise free radical scavenging activity, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory effects. These properties also depend on their antioxidant nature as reported in a number of studies (Gryglewski *et al., 1987).*

In this present study, methanolic extracts of all the bark of selected plants at their various concentrations (10 mg/L to 100 mg/L of each extract) were subjected for their antioxidant capability using DPPH and FRAP methods.

In DPPH assay, it was identified that all the extracts showed free radical scavenging capacity. With rise in concentration of extracts, free radical scavenging potential was also enhanced. Among the bark of selected plants, the maximum free radical scavenging potential was observed in stem bark of *Psidium guajava* with lowest IC50 value (43.76 mg/L). The minimum free radical scavenging potential was observed in stem bark of *Holoptelea integrifolia* with the highest IC50 value (400 mg/L).

In the previous research, there was also found a strong linear correlation between the total phenol and total flavonoid contents in the stem bark extracts of *Psidium guajava* with the antioxidant property. This proved that *P. guajava* could be more operative if used separately in handling oxidative stress condition (Ibe *et al*., 2014). The biochemical investigation of acetone, ethanol and water extracts of bark of *Psidium guajava* also showed the presence of tannins, phenols, saponins and cardiac glycosides but tannins were responsible for higher scavenging property (Aziz *et al*., 2014).

By FRAP method, in was found that increasing concentrations of extracts influenced FRAP activity positively. Among the selected plants, methanolic extracts of *Syzygium cumini* was observed to show maximum FRAP activity with the lowest IC50 value (71.08 mg/L) while the lowest FRAP activity was shown by *Psidium guajava* with the highest IC50 value (112.98 mg/L).

Refrences

1. Aharoni A., Jongsma M.A., Bouwmeester H.J. Volatile science: Metabolic engineering of terpenoids in plants. *Trends Plant Sci.* 2005. 10: 594-602.
2. Alam M.N., Bristi N.J., Rafiquzzaman M. Review on *in vivo* and *in vitro* methods of evaluation of antioxidant activity. *Saudi Pharm J*. 2013. 21: 143-152.
3. Alothman M., Bhat R. and Karim A.A. UV radiation-induced changes of antioxidant capacity of fresh-cut tropical fruits. *Innovative Food Science & Emerging Technologies*. 2009. 10(4): 512-516.
4. Aziz N.A., Rafiqkhan M.O., Menon D.B. Phytochemical screening of *Psidium guajava* bark and in vitro antioxidant activity of *Psidium guajava* bark tannins. *Asian J Pharm Clin Res*. 2014. 7(3): 191-194.
5. Baier M. and Dietz K.J. Chloroplasts as source and target of cellular redox regulation: a discussion on chloroplast redox signals in the context of plant physiology. *J Exp Bot*. 2005. 56: 1449-1462.
6. Baratta M.T., Dorman H.J.D., Deans S.G. Chemical composition, antimicrobial and antioxidative activity of laure, sage, rosemary, oregano and coriander essential oils. *J Essent Oil Res*. 1998. 10: 618–627.
7. Barlow S.M. Toxicological aspects of antioxidants used as food additives. *In Food Antioxidants, Springer, Dordrecht.* 1990. 253-307.
8. Benzie I.F. and Strain J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*. 1996. 239(1): 70-76.
9. Berryman A. Resistance of conifers to invasion by bark beetle-fungus associations. *Journal of Bioscience.* 1972. 22: 598-602.
10. Biggs A.R. Prediction of lignin and suberin deposition in boundary zone tissue of wounded tree bark using accumulated degree-days. *Journal of American Society for Horticultural Science.* 1986. 111: 757-760.
11. Blokhina O., Virolainen E., Fagerstedt K.V. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann Bot.* 2003. 91: 179-194.

12. Branen A.L. Toxicology and biochemistry of butylated hydroxy anisole and butylated hydroxytoluene. *Journal of 'The American Oil Chemists' Society*. 1975. 52(2): 59-63.
13. Cook N.C. and Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources. *The Journal of Nutritional Biochemistry*. 1996. 7(2): 66-76.
14. Cook N.C. and Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources. *The Journal of Nutritional Biochemistry*. 1996. 7(2): 66-76.
15. Croft K.D. The chemistry and biological effects of flavonoids and phenolic acids. *Ann N Y Acad Sci.* 1998. 854: 435-442.
16. Duthie G.G., Duthie S.J., Kyle J.A. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutr Res Rev.* 2000. 13: 79-106.
17. Gryglewski R.J., Korbut R., Robak J. and Święs J. On the mechanism of antithrombotic action of flavonoids. *Biochemical pharmacology*. 1987. 36(3): 317-322.
18. Halliwell B. Biochemistry of oxidative stress. *Biochem Soc Trans.* 2007. 35: 1147-1150.
19. Ibe C., Jacobs C.C., Imo C., Osuocha K.U., Okoronkwo M.U. evaluation of the antioxidant activities of *Psidium guajava* and *Aloe vera*. *British Journal of Pharmaceutical Research*. 2014. 4(3): 397.
20. Kala C.P., Farooquee. N.A. and Dhar U. "Prioritization of medicinal plants on the basis of available knowledge, existing practices and use value status in Uttaranchal, India". *Biodiversity and Conservation*. 2004. 13: 453-469.
21. Kasote D.M., Hegde M.V., Katyare S.S. Mitochondrial dysfunction in psychiatric and neurological diseases: cause(s), consequence(s) and implications of antioxidant therapy. *Biofactors.* 2013. 39: 392-406.
22. Korkina L.G. Phenylpropanoids as naturally occurring antioxidants: from plant defence to human health. *Cell Mol Biol*. 2007. 53: 15-25.
23. Kumpulainen J.T. and Salonen J.T. *Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease*. 1999. 240.
24. Kuribara H. The anxiolytic effect of two oriental herbal drugs in Japan distributed to Hanokiol from *Magnolia* bark. *Journal of Pharmacy and Pharmacology.* 2000. 52 (11): 1425-1429.
25. Morgan J.F., Klucas R.V., Grayer R.J., Abian J., Becana M. Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: prooxidant and antioxidant properties. *Free Radic Biol Med.* 1997. 22: 861–870.
26. Myburgh K.H. Polyphenol supplementation: benefits for exercise performance or oxidative stress. *Sports Med.* 2014. 1: 57-70.
27. Nahak G. and Sahu R.K. Antioxidant activity in bark and roots of neem (*Azadirachta indica*) and mahaneem (*Melia azedarach*). *Continental Journal of Pharmaceutical Sciences*. 2010. 4: 28-34.
28. Oke J.M. and Hamburger M.O. Screening of some Nigerian medicinal plants for antioxidant activity using 2,2 diphenyl-picryl-hydrazyl radical. *African Journal of Biomedical Research*. 2002. 5: 1-2.
29. Ozcan M.M., Erel O. and Herken E.E. Antioxidant activity, phenolic content, and peroxide value of essential oil and extracts of some medicinal and aromatic plants used as condiments and herbal teas in Turkey. *Journal of Medicinal Food*. 2009. 12(1): 198-202.
30. Patra B., Schluttenhofer C., Wu Y., Pattanaik S., Yuan L. Transcriptional regulation of secondary metabolite biosynthesis in plants. *Biochim Biophys Acta.* 2013. 18(29): 1236-1247.
31. Schuler P. Natural antioxidants exploited commercially. *In Food Antioxidants, Springer, Dordrech.* 1990. 99-170.
32. Sies H. Oxidative stress: oxidants, antioxidants. *Exp Physiol.* 1997. 82: 291-295.
33. Srivastav L.M. Anatomy, chemistry and physiology of bark. *Int. Rev. for Res*. 1964. 1: 204-277.
34. Szent-Giörgyi A. Lost in the twentieth century. *Annu Rev Biochem*. 1963. 36: 1–15.

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