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International Journal of Pharmaceutical and Phytopharmacological Research(eIJPPR)

Research Article

ISSN(Print): 2250-1029
ISSN(Online): 2249-6084
CODEN(USA): IJPPMD

Comparative Antioxidant Potential of Bark and Leaves of *Terminalia arjuna* (Roxb) Wight & Arn from Himachal Pradesh

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ABSTRACT

The present study aimed to compare antioxidant activities, total phenolic and flavonoid content present in leaves and bark of Terminalia arjuna and check whether there is any correlation between phenolic content and flavonoid content with antioxidant activities or not. Phytochemical screening of ethanolic extract of leaves and bark revealed the presence of phenols, flavonoids, tannins, carbohydrates, glycosides, saponin, phytosterols and phytosteroids. Total phenolic content was found to be higher in bark (272.71±3.18 mg/g gallic acid equivalents) as compared to that of leaves (95±3.11 mg/g gallic acid equivalents). Similarly, flavonoid content of ethanolic extract of bark was found to be higher (203.95±5.13 mg/g rutin equivalents) than that of leaves (87.625±4.28 mg/g rutin equivalents). DPPH activity of ethanolic extract of bark (IC_{50}-17.41 µg/ml) was more than that of leaves (IC_{50}-20.22µg/ml). FRAP activity of bark (IC_{50}- 4.781 µM Fe (II) equivalents) is more than that of leaves (IC_{50}-7.572 µM Fe (II) equivalents). Nitric oxide (NO) scavenging activity of bark (IC_{50}-12.87 µg/ml) was higher than that of leaves (IC_{50}-13.91 µg/ml).The present study clearly showed that there is a correlation between total phenolics, flavonoid contents and antioxidant activity of leaves and bark of T. arjuna. The phenolic compounds and flavonoids could be major contributor to antioxidant activity of T. arjuna.

Keywords: *Terminalia arjuna,* DPPH, FRAP, NO, IC_{50}, Antioxidants
DOI: 10.24896/eijppr.2016615

INTRODUCTION

From the dawn of civilization, man has been utilizing the important biological properties of various plants for the treatment of different types of the diseases. Plants-based drugs have been used for treatments of various human ailments because of their low cost, easily availability and less toxicity. Oxidative stress is an imbalance between systemic manifestation of reactive oxygen species (O^{2-} (superoxide radical), OH (hydroxyl radical) and H_2O_2 (hydrogen peroxide) and ability of biological system to detoxify reactive intermediates or its harmful effect [1]. Several degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc. are caused due to oxidative stress. In modern system of medicine, there is a need of proper balance between antioxidants and oxidants in maintaining proper human health [2-5]. Although our body possesses natural antioxidant defense mechanism to protect against oxidative stress but antioxidants from natural sources could provide enhanced protection against newly emerged diseases. Medicinal plant parts are rich source of phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have large number of biological effects including antioxidant activity and may help to protect the cells against the oxidative damage caused by free-radicals [6]. They act as free radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers.

Terminalia arjuna (T. arjuna) belonging to family combretaceae is one of the most versatile medicinal plants having a wide spectrum of biological activity. *T. arjuna* is an important cardiotonic plant described in the Ayurveda [7].

The bark, leaves and fruits of *T. arjuna* have been used in indigenous system of medicine for different ailments [8]. Bark of *T. arjuna* is rich in phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrates [9]. Its leaves have been reported to have analgesic and anti-inflammatory properties [10]. Aqueous paste of roots is applied on headache whereas leaf paste with sugar and milk is given in the treatment of spermatorrhoea [11]. Studies have also reported that *T. arjuna* is rich source of antioxidants like vitamin C, vitamin E, carotenes, polyphenols, and many other compounds help to reduce risk of degenerative diseases such as cancer, coronary heart disease and even Alzheimer's disease [12]. Therefore, present was undertaken to evaluate phenolic and flavonoid contents in ethanolic extract of bark and leaves and correlation between total phenolic content, flavonoid and antioxidant activity of bark and leaves of *T. arjuna.*

MATERIALS AND METHODS

2.1 Collection of plant material

Leaves and bark of *T. arjuna* were collected in the month of October from Kangra, Himachal Pradesh, India. The leaves and bark were washed thoroughly with tap water and then surface sterilized with 70% ethanol followed by drying in hot air oven at 40°C. The dried leaves and bark were homogenized to fine powder using electric grinder and stored in air tight bottles in dark until use.

2.2 Chemicals and Reagents

Ascorbic acid, aluminum chloride, 2,2'-diphenyl-2-picrylhydrazyl (DPPH), Sodium nitrite ($NaNO_2$), 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma Chemical Co., U.S.A. Ferric chloride, Folin-Ciocalteu reagent, Gallic acid and Rutin were procured from Loba Chemie Pvt. Ltd, Mumbai, India. All the chemicals and reagents used in this study were of analytical grade.

2.3 Preparation of leaves and bark extract: The dried powder of leaves and bark of *Terminalia arjuna* were defatted with petroleum ether (60-80°C) and then macerated in ethanol. 10 g of dried powder were taken in 100 ml of ethanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 72 h. After 72 h, the solvent was filtered through Whatmann filter paper No. 1 and then centrifuged at 4000 rpm for 5 min. Supernatant was collected and then allowed to evaporate at room temperature. The powdered dry extracts were stored at 4 °C in air tight bottles.

2.4 Qualitative screening of phytochemicals: Crude extracts of leaves and bark were examined for the presence of various secondary metabolites such as phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrate as described by Khandelwal [13] and Harbourne [14].

2.5 Determination of total phenolic contents: The total phenolic content of the leaves and bark extract was estimated according to the method described by Singleton *et al* [15]. Total phenolic content was calculated from calibration curve of gallic acid (25-200 µg) and expressed in terms of gallic acid equivalents (GAE) per gram of extract.

2.6 Determination of total flavonoid Content: The total flavonoids content in ethanolic extract of bark and leaves of *T. arjuna* were determined by using aluminium chloride ($AlCl_3$) method as described by Zhishen *et al* [16]. The flavonoid content was calculated from standard curve of rutin (25-200 µg/ml) and expressed as rutin equivalents (RE) per gram of extract.

2.7 *In-vitro* antioxidant activity:

2.7.1 DPPH radical scavenging activity: DPPH radical scavenging activity of the extract was measured by modified method described by Barros *et al.* [17].

2.7.2 Ferric Reducing Antioxidant Power (FRAP) assay: The ability to reduce ferric ions was measured using the method described by Benzie and Strain [18]. Ascorbic acid was used as positive reference standard. The antioxidant capacity based on the ability to reduce ferric ions of extract was calculated from the linear calibration curve of $FeSO_4$ (2.5-20 µM) and expressed as µmol $FeSO_4$ equivalents per gram of extract.

2.7.3 Nitric oxide (NO) scavenging assay: Nitric oxide scavenging assay was carried out using sodium nitroprusside method as described by Sreejayan and Rao [19] with ascorbic acid as positive standard.

2.8 Statistical analysis

Inhibition of concentration and total phenolic and antioxidant were determined by linear regression analysis method which was used to calculate IC_{50}. Each sample was analyzed individually in triplicates and the results are expressed

as the mean value (n = 3) ± standard deviation. The correlation coefficients between studies parameters were demonstrated by linear regression analysis.

RESULTS AND DISCUSSION

3.1 Phytochemical screening of extracts

Phytochemical analysis of ethanolic extract of leaves and bark of *T. arjuna* showed the presence of alkaloids, phenolic compounds, tannins, flavonoids, carbohydrates, glycosides, phytosterols, phytosteroids and saponins, whereas proteins and amino acids were absent (Table-1).

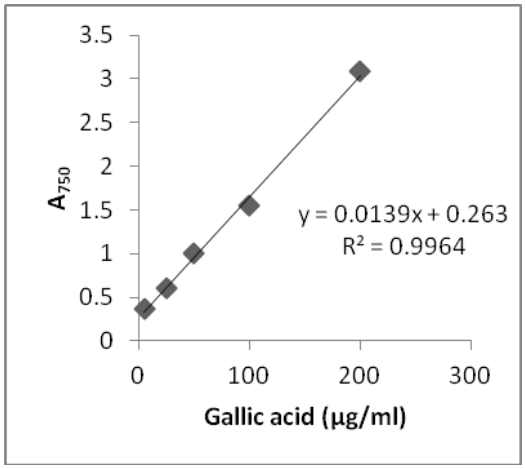
Table 1: Phytochemical constituents of ethanolic extract of leaves and bark of *T. arjuna*

Sr. No.	Phytoconstituents	Tests	Leaves	Bark
1.	Alkaloids	Dragendroff's test	+	+
		Hager test	+	+
2.	Phenolics and Tannins	Ferric chloride test	+	+
		Gelatin test	+	+
3.	Phytosteroids	Liebermann-Burchard's test	+	+
4.	Phytosterol	Salkowski reaction test	+	+
5.	Saponin	Foam test	+	+
6.	Carbohydrates	Barfoed test	+	+
		Fehling test	+	+
7.	Glycosides	Borntrager test	+	++
8.	Proteins	Millon test	-	-
		Ninhydrin test	-	-
9.	Flavonoids	Lead acetate test	+	+

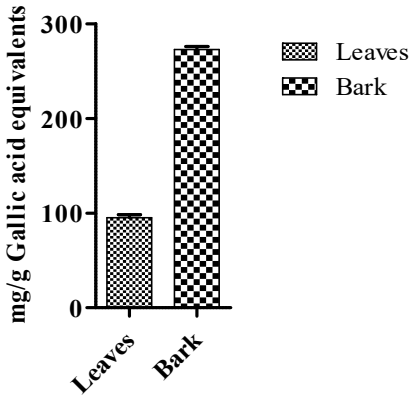
'+' indicates presence and '–' indicates absence.

3.2 Total phenolic content determination

Total phenolic content of the ethanolic extract of leaves and bark of *T. arjuna* was determined using standard curve of gallic acid (y= 0.012x + 0.313; R^2 = 0.994) (Fig-1a). Ethanolic extract of bark possess higher amount of phenolic content (272.71±3.18 mg/g gallic acid equivalents) as compared to that of leaves (95±3.11 mg/g gallic acid equivalents) (Fig-1b).



A



B

Fig 1: Total phenolic content in leaves and bark of *T. arjuna*. A) Standard curve of gallic acid. B) Phenolic content of ethanolic extract of leaves and bark represented as mg/gram gallic acid equivalents

Values are expressed as mean ± standard deviation (n = 3).

3.3 Total Flavonoid Content determination

The total flavonoid content in ethanolic extract of bark and leaves of *T. arjuna* was determined by using aluminium chloride ($AlCl_3$) method. The flavonoid content was calculated from standard curve of rutin (y = 0.0031x - 0.0104; R^2=0.9926) (Fig-2a). Ethanolic extract of bark (203.95±5.13 mg/g rutin equivalents) had higher amount of total flavonoid content as compared to that of leaves (87.625±4.28 mg/g rutin equivalents) (Fig-2b).

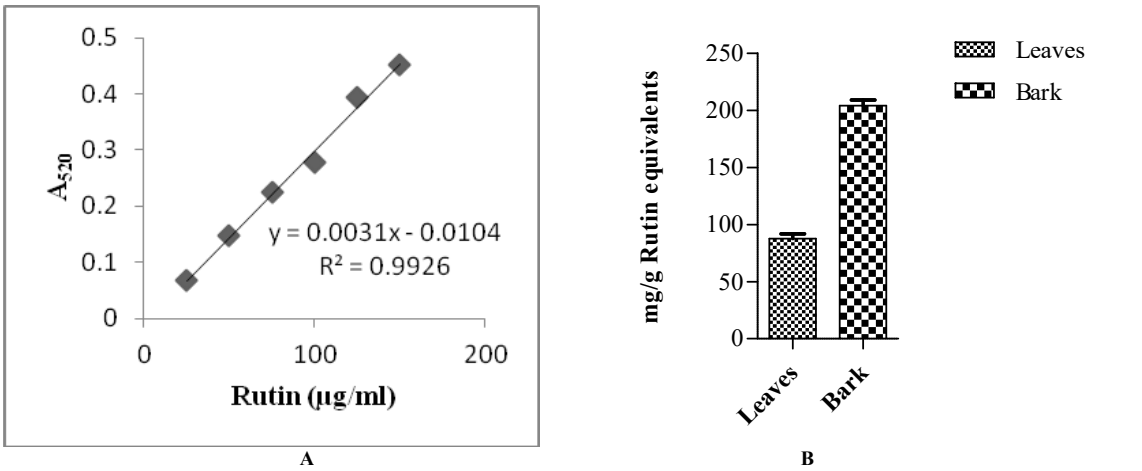


Fig-2: Total flavonoid content in leaves and bark of *T. arjuna:* A) Standard curve of rutin. B) Flavonoid content of ethanolic extract of leaves and bark

Values are expressed as mean ± standard deviation (n = 3).

3.4 In-vitro antioxidant activity

There are different methods to evaluate the antioxidant characteristics, but no single method alone can provide the proper antioxidant property of the extracts. Therefore, it is necessary to characterize the extract by different antioxidant mechanism [20-21].

3.4.1 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH test showed the ability of the test compound to act as a free radical scavenger. DPPH assay method is based on the ability of 2, 2-diphenyl-1-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants [22]. DPPH, a protonated radical, has characteristic absorbance maximal at 517 nm, which decreases with the scavenging of the proton radical. This property has been widely used to evaluate the free radical scavenging effect of natural antioxidants [23]. Both the extracts exhibited good scavenging activity comparing with the standard ascorbic acid in dose dependent manner (Fig-3). IC_{50} value of ethanolic extract of bark (17.41µg/ml) is lower than that of leaves (20.22 µg/ml) whereas ascorbic acid has IC_{50}-14.52 µg/ml.

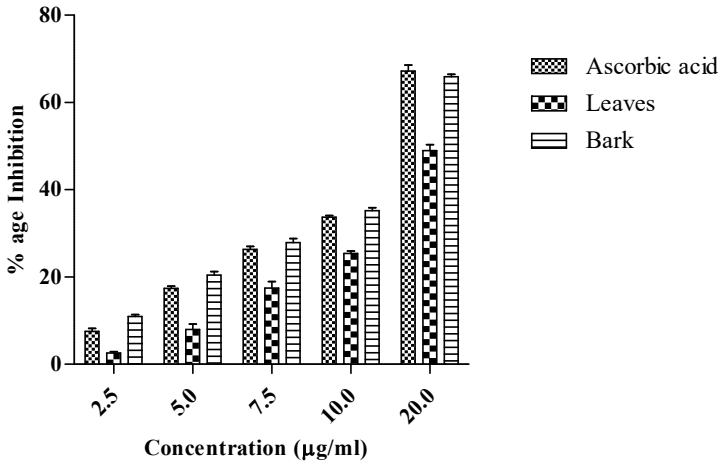


Fig 3: DPPH radical scavenging activity of ethanolic extract of leaves and bark of *T. arjuna* in different concentrations with standard ascorbic acid

Values are expressed as mean ± standard deviation (n = 3)

3.4.2 Ferric Reducing Antioxidant Power (FRAP) assay

Frap assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine [Fe^{3+}- TPTZ] complex and producing a colored ferrous tripyridyltriazine [Fe^{2+}-TPTZ] [18]. Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom. The ethanolic extract of bark and leaves showed increased ferric reducing power with the increased concentration as compared to ascorbic acid (Fig- 4). The IC_{50} values were found to be 8.056 µg/ml, 7.572 µg/ml and 4.781 µg/ml for ascorbic acid, bark and leaves extract *respectively***.**

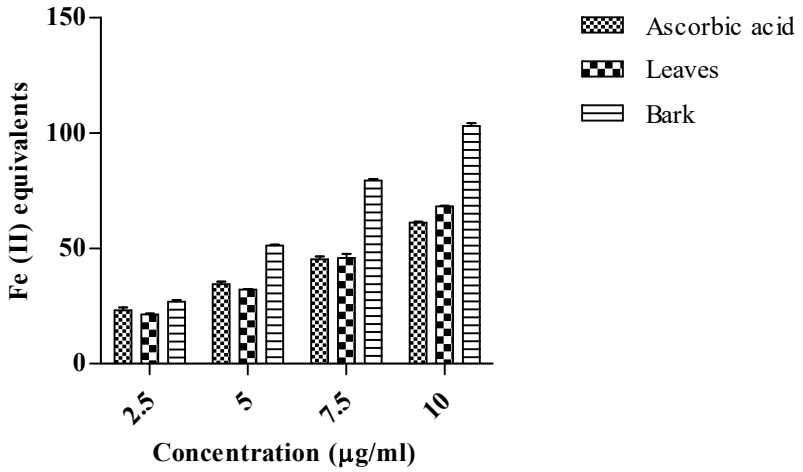


Fig 4: Ferric reducing antioxidant power of ethanolic extract of leaves and bark of *T. arjuna* in different concentrations with standard ascorbic acid

Values are expressed as mean ± standard deviation (n = 3)

3.4.3 Nitric oxide scavenging activity

Nitric oxide is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities [24]. The scavenging of NO by the extract was increased in concentration dependent manner. The ethanolic extract of bark of *T. arjuna* showed maximum activity of 68.76% at 20 µg/ml as compared to that of leaves and ascorbic acid at the same concentration exhibited 65.34% and 61.44% inhibition *respectively* (Fig-5). IC_{50} values were found to be 12.81 µg/ml, 13.91 µg/ml and 12.87 µg/ml for ascorbic acid, bark and leaves extract *respectively*.

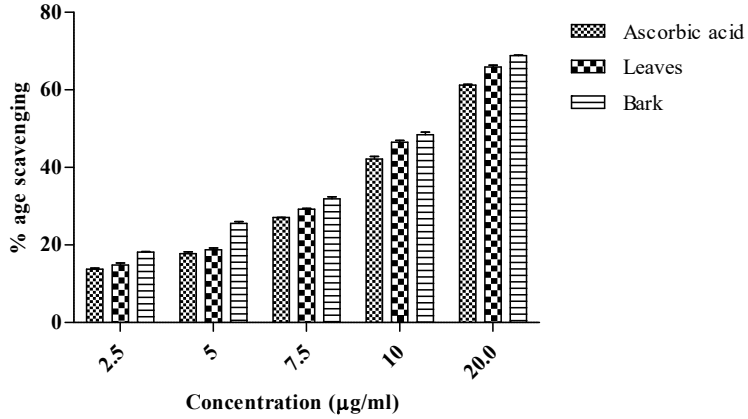


Fig 5: Nitric oxide scavenging activity of ethanolic extract of leaves and bark of *T. arjuna* in different concentrations

Values are expressed as mean ± standard deviation (n = 3)

3.5 Correlation of total phenolic content and flavonoid content with antioxidant activity:

Phenolic compounds are the secondary metabolites which act as natural antioxidants. Phenolic content of the plants are directly correlated with their antioxidant activity [25]. Several studies reported that higher antioxidant activity associated with medicinal plants can be attributed to their total phenolic compounds. Several studies have reported on the relationships between phenolic content and the antioxidant activity. Velioglu *et al* [26], Borneo *et al* [27] , Katalinic *et al* [28], Petridis *et al* [29], Rakholiya *et al* [30] found strong relationship between total phenolic content and antioxidant activity, whereas Kahkonen *et al* [31] and Singuel *et al* [32] found no correlation between phenolic content and antioxidant activity. Shariar *et al* [33] showed positive correlation between total antioxidant activity and total phenolic content of ethanol extract of bark of *T. arjuna* , whereas El Ameen *et al* [34] found that 70 % acetone extract of leaves of *T. arjuna* possess more phenolic compound and thereby having high antioxidant

activity. Our results also showed that total phenolics and flavonoids were directly correlated with antioxidant capacity of both leaves and bark of *T. arjuna* as shown in table-5.

Table 5: Correlation between phenolics and flavonoids with antioxidant activities of leaves and bark extracts of *T. arjuna*

Antioxidant Assays	Correlation coefficient (R^2)			
	Total phenolics		Total flavonoids	
	Leaves	Bark	Leaves	Bark
DPPH radical scavenging activity	0.98	0.998	0.96	0.97
Ferric Reducing Antioxidant Power (FRAP) assay	0.93	0.96	0.98	0.98
Nitric oxide scavenging activity	0.95	0.98	0.99	0.90

CONCLUSION

The present study showed that ethanolic extract of both leaves and bark of *T. arjuna* possess antioxidant activity. The total phenolics and flavonoids in bark were more as compared to that of leaves. There is a direct correlation of phenolics and flavonoids with antioxidant activities in bark and leaves which clearly indicate that phenolic compounds and flavonoids may be responsible for antioxidant activities of *T. arjuna.* The present study validates the use of bark of *T. arjuna* for treatment of various ailments.

Acknowledgements
The authors acknowledge the infrastructure and financial support provided by the Shoolini University.

REFERENCES

[1] Chandra K, Salman AS, *et al.* Protection against FCA Induced Oxidative Stress Induced DNA Damage as a Model of Arthritis and In vitro Anti-arthritic Potential of *Costus speciosus* Rhizome Extract. Int J Pharmacogn Phytochem Res, 2015; 7(2); 383-89.
[2] Davies KJA. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life, 2000; 50:279–89.
[3] Dreost IE. Trace elements, micronutrients, and free radicals. Totowa: Human Press: 1991, pp-149–199.
[4] Finkel T. Oxidants, oxidative stress and the biology of aging. Nature (London): 2000, 408: pp-239–248.
[5] Tiwari AK. Imbalance in antioxidant defense and human diseases: Multiple approach of natural antioxidant therapy. Curr Sci, 2001; 8: 1179–87.
[6] Packer L, Rimbach G, *et al.* Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (*Pinus maritima*) bark, pycnogenol. Free Radic Biol Med, 1999; 27: 704–24.
[7] Tripathi VK, Singh B, *et al. Terminalia arjuna* its present status. Orient J Chem, 1996; 1:1–16.
[8] Warrier PK, Nambiar VPK, *et al. Terminalia arjuna* In: Indian Medicinal Plants – A Compendium of 500 Species, (Eds. Warrier PK, Nambiar VPK, Ramankutty C) Vol. 5, 1st Ed. Orient Longman Limited, Madras, India, 1996, pp- 253-57.
[9] Ghani, A. Medicinal Plants of Bangladesh, 2nd edition. The Asiatic Society of Bangladesh: 2003, pp- 1-2, 55-57, 402, 500.
[10] Biswas M, Biswas K, *et al.* Evaluation of analgesic and anti-inflammatory activities of *Terminalia arjuna* leaf. J Phytol, 2011; 3(1):33–38.
[11] Sikarwar RLS, Pathak B, *et al.* Some unique ethnomedicinal perceptions of tribal communities of Chitrakoot, Madhya Pradesh. *Indian J Trad Knowledge,* 2008; 7(4): 613-17.
[12] Ames BN, Shigenaga MK, *et al.* Oxidants, antioxidants and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA, 1993; 90(17): 7915-22.
[13] Khandelwal KR. Practical pharmacognosy techniques and experiments. 17th Ed. India, 2007.
[14] Harbourne JB. Phytochemical methods: A guide to modern techniques of plant analysis. London: Chapman and Hall, 1984.
[15] Singleton V, Orthoper R, *et al.* Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteu reagent. Methods Enzymol, 1999; 299:152-78.
[16] Zhishen J, Mengcheng T, *et al.* The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. Food Chem*,* 1999; 64(4): 555-59.
[17] Barros L, Baptista P, *et al.* Effect of Lactariuspiperatus fruiting body maturity stage on antioxidant activity measured by several biochemical assays. Food Chem Toxicol, 2007; 45:1731-37.
[18] Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as measurement of ‘‘antioxidant power’’: The Frap assay. Anal Biochem, 1996; 239: 70–76.
[19] Sreejayan, Rao MN. Nitric oxide scavenging by curcuminoids. J Pharmacol Pharmacother, 1997; 49: 105–7.

[20] Chanda S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. Afr J Microbiol Res, 2009; 3: 981- 96.
[21] Chanda S, *et al*. Evaluation of antioxidant properties of some Indian vegetable and fruit peels by decoction extraction method. Am J Food Tech, 2013; 8: 173-182.
[22] Kumarasamy Y, Byres M, *et al.* Screening seeds of some Scottish plants for free-radical scavenging activity. Phytother Res, 2007; 21: 615-21.
[23] Jao CH, Ko WC. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging by protein hydrolyzates from tuna cooking juice. Fish Sci, 2002; 68(2): 430–35.
[24] Hagerman AE, Riedl KM, *et al*. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J Agric Food Chem*,* 1998; 46: 1887-92.
[25] Orak HH, Yagar H, *et al.* Comparison of Antioxidant Activities of Juice, Peel and Seed of Pomegranate (*Punica granatum* L.) and Inter-relationships with Total Phenolic, Tannin, Anthocyanin, and Flavonoid Contents. Food Sci Biotech, 2012; 21: 373-87.
[26] Velioglu YS, Mazza G, *et al.* Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agric Food Chem, 1998; 46: 4113-17.
[27] Borneo R, Leon EA, *et al.* Antioxidant capacity of medicinal plants from the province of Cordoba (Argentina) and their *in vitro* testing in model food system. Food Chem, 2008; 112: 664-70.
[28] Katalinic V, Milos M, *et al.* Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem, 2004; 94: 550-57.
[29] Petridis A *et al.* Salinity-induced changes in phenolic compounds in leaves and roots of four olive cultivars (*Olea europaea* L.) and their relationship to antioxidant activity. Env Exp Bot, 2012; 79: 37-43.
[30] Rakholiya K *et al.* Comparative study of hydroalcoholic extracts of *Momordica charantia* L. against foodborne pathogens. Ind J Pharm Sci, 2014; 76: 148-56.
[31] Kähkönen MP, Hopia AI, *et al.* Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem, 1999; 47: 3954–62.
[32] Sengul M, Yildiz H, *et al*. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pak J Pharm Sci, 2009; 22(1): 102-6.
[33] Shahriar M, Akhter S, *et al.* Evaluation of *in-vitro* antioxidant activity of bark extracts of *Terminalia arjuna*. J Med Plants Res, 2012; 6(39): 5286-98.
[34] El-Ameen SM, Refahy LA, *et al*. Chemical Investigation and Antioxidant Activity of Phenolic Acids from the Leaves of *Terminalia arjuna*. Global J Pharmacol, 2013; 7(4): 448-56.