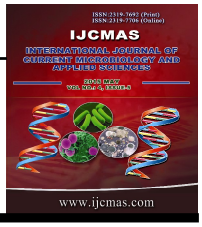
Int.J.Curr.Microbiol.App.Sci (2015) 4(5): 728-736

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 5 (2015) pp. 728-736**
http://www.ijcmas.com



Original Research Article

Antibacterial and antioxidant efficacy analysis of leaves extracts of *Prunus amygdalus* (Badam) in different solvents

Udita Tiwari[1], Nishi Dubey[1] and Shuchi Kaushik[2]*

[1]Department of Biochemistry; School of Life Sciences, Dr.B.R.Ambedkar University, Agra -282002, India
[2]Amity Institute of Biotechnology, Amity University, Madhya Pradesh, Gwalior-474005 (MP), India
**Corresponding author*

Keywords

Antioxidant, Antimicrobial, Natural Medicine, Secondary Metabolites

A B S T R A C T

Antioxidants play important role in body defense system against reactive oxygen species (ROS) as they combine with reactive oxygen species and nullify their toxic effects. A variety of free radical scavenging antioxidants exist within the body, many of them are derived from dietary sources. Medicinal plants have great importance in formulation of medicines due to enriched quality of phytochemicals produced by them as the byproduct of secondary metabolism. Keeping these points in view, the present investigation was designed to investigate the phenolic contents and evaluate the *in vitro* antibacterial as well as antioxidant activities of Badam fresh and dry leaves in different solvents. Owing to the antioxidant and antibacterial activities exhibited by the leaf extracts investigated in this study, this plant could be considered natural antioxidant and antimicrobial agents source that can be used in food and pharmaceutical industries. However, further studies are needed to obtain purified compounds that may be responsible for the activities observed from the tested leaves.

Introduction

In healthy individuals, the production of free radicals is balanced by the antioxidative defense system; however, oxidative stress is generated when equilibrium favors free radical generation and depletion of antioxidant levels (Young and Woodside, 2001). Reactive Oxygen Species (ROS) are major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro* and create oxidative stress which results in numerous diseases and disorders (Halliwell, 2006; Fernande *et al*., 2013). Reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide and other exogenous factors are generally the cause of several fatal diseases such as coronary heart disease, stroke, rheumatoid arthritis, diabetes, cancer, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, atherosclerosis and ageing (Hyun *et al*., 2006; Upston *et al*., 2003; Sian, 2003).

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress (Tirzitis and Bartosz, 2010). A variety of free radical scavenging antioxidants exists within the body, many of them are derived from dietary sources like fruits, vegetables and teas (Diaz *et al*., 1997*)*. It is also used to preserve food quality mainly because they arrest oxidative deterioration of lipids.

Plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies (Nair *et al*., 2005). They are important source of potentially useful structures for the development of new chemotherapeutic agents. The use of plant extracts and phytochemicals with known antimicrobial, antioxidants properties can be of great significance in therapeutic treatments (Ifesan *et al*., 2013).

Medicinal plants have great importance in formulation of medicines due to enriched quality of phytochemicals produced by them as the byproduct of Secondary metabolism. In Southeast Asia, leaves and barks of Indian almond tree are widely used in human as a folk medicine to treat dermatosis, hepatitis, thrush and other oral infections, and intestinal ailments in children.

Indian almond leaves contain various tannins, including punicalagins, as well as several different flavanoids, such as kaempferol and quercetin, isovitexin, vitexin, isoorientin, rutin and triterpenoids (Ahmed *et al*., 2005).

Punicalagins caught the attention of cancer researchers after a Taiwanese study showed that punicalagin alpha (1-alpha-O-galloylpunicalagin) inhibited certain types of intracellular signalling molecules (extracellular signal-regulated kinases, c-Jun N-terminal kinases and protein kinases to be exact) that play roles in cancer growth (Chen *et al*., 2008).

Methanolic leaf extract of *C. viminalis* showed good antioxidant free radicals scavenging activity due to their hydroxyl groups of phenolic content. The use of such plant based medicines in treatment of infectious diseases where access to commercial antibiotics is restricted (Tiwari *et al*., 2014)

Keeping all these points in view the present investigation was carried out to see the effect of different solvents (such as distilled water, DMSO, ethanol, and methanol) in phytochemical screening of (dry and fresh) leaf extracts of Badam (*Prunus amygdalus*), evaluation of antibacterial activity against the selected bacterial species as well as estimation of total phenolic content (TPC), and total antioxidant activity by phosphomolybdenum method.

Materials and Methods

Collection of plant materials

Plant Materials Fresh and dry leaves of Badam, *Prunus amygdalus* were collected from different locations of Gwalior city. The leaves were, identified and authenticated from Botanist, Department of Botany, Maharaja Mansingh College, Gwalior.

Preparation of fresh and dry leaves extract in different solvents

The leaves were washed thoroughly with sterile distilled water and dried in shade until further use. Dried and fresh leaves of Badam (*Prunus amygdalus*) were powdered separately and 500 mg of each powder was extracted in 10 ml of different solvents using

blender and kept for thirty minutes and centrifuged at 10000 rpm for fifteen minutes.

Preliminary phytochemical studies

The supernatant of extracts were subjected to various phytochemical tests to determine the activity of constituents present in the crude extracts in different solvents (Okerulu *et al*., 2001)

Evaluation of antioxidant capacity by Phosphomolybdenum method

The total antioxidant capacity of dry and fresh leaf extracts in different solvents was evaluated by the method of Prieto *et al*., (1999). 200 µl of dry and fresh leaf extracts of Badam (*Prunus amygdalus)* in different solvents were combined with 2 ml of reagent solution (0.6 M, H_2SO_4, 28 mM Sodium hypophosphate and 4 mM of Ammonium Molybedate). The tubes were capped and incubated in a boiling water bath at 95^o C for 90 min. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm against blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as rest of the sample. Ascorbic acid 10mg/ml was used as the standard control. It was expressed in terms of ascorbic acid equivalent (mg/g). All the tests were performed in triplicate.

Total Phenolic Content Estimation

Total phenol content was estimated using Folin-Ciocelteau reagent based assay as previously described with little modification by Singleton and Rossi (1965). One ml of (dry and fresh) leaf extracts in different solvent extracts, 5ml of Folin -Ciocalteau reagent (diluted tenfold) and 4 ml (75 g/l) of Na_2CO_3 were added. The mixture was allowed to stand at 20^{o}C for 30 min and the absorbance of the developed colour was recorded at 765 nm using UV-VIS spectrophotometer. 1 ml aliquots of 20, 40, 60, 80, 100µg/ml different solvent gallic acid solutions were used as standard. Total phenolic content was expressed in (g GAE/100g) dry weight of sample. All determination was performed in triplicates and values were expressed in mean±SD.

Antibacterial Susceptibility Testing (AST)

1) Test organisms

Two pathogenic bacteria namely *E. coli* and *S. aureus* were included in the study and were procured from Institute of Microbial Technology (IMTECH), Chandigarh. The obtained cultures were received in the aseptic conditions of the laboratory and subcultured on nutrient agar medium. After 24 hours of incubation at 37°C the cultures were preserved aseptically in refrigerator until further use.

2) Agar well Diffusion Method

Agar well diffusion method described by Schillenger and Luke (1989) was used for the study. An overnight culture of *E. coli* and *S. aureus* was standardized to contain approx. 10 CFU/ml was inoculated into 100 ml of Muller Hinton agar. The culture medium was allowed to set. Thereafter, a sterile cork borer of 6.0 mm diameter was used to punch wells in the Muller Hinton agar. Five wells were made in the petri plate containing media (One in centre and four at the border); the agar plugs were removed with a flamed and cooled wire loop. The

plates were swabbed with different cultures and the cut wells were then filled with 20 µL of each plant extracts prepared in different solvents after 24hrs, the plates were observed for the zone scale and the result was recorded in millimeters. All the experiments were done in triplicates.

3) Statistical analysis

All measurements were carried out in triplicates. The results are expressed as mean values ±standard deviation (SD).

Results and Discussion

Preliminary phytochemical analysis of (fresh & dry) leaf extracts of test plant Badam (*Prunus amygdalus)* in different solvents revealed that the plant leaf extracts possessed following phytoconstituents as given in the Table 1. We observed that bioactive substances demonstrated varying degree of solubility in different solvents. The therapeutic value and bioactivity of plant extracts is attributed to phytochemical constituents. Flavonoids are a major group of phenolic compounds reported for their antiviral (Elumalai *et al*., 2011), antimicrobial and spasmolytic properties. Alkaloids are the most efficient therapeutically significant plant substance. They are isolated from plants commonly found to have antimicrobial properties (Jose *et al*., 2005). Saponin has relationship with sex hormones like oxytocin. Oxytocin is a sex hormone involved in controlling the onset of labour in women and the subsequent release of milk (Okigbo *et al*., 2009). Another important action of saponins is their expectorant action through the stimulation of a reflex of the upper digestive tract (Ayoola and Adeyeye, 2010) the cardiac glycosides therapeutically have the ability to increase the force and power of the heart-beat without increasing the amount of oxygen needed by the heart muscle. They can thus increase the efficiency of the heart and at the same time steady excess heart beats without strain to the organ (Stray, 1998).

Total phenolic content and total antioxidant activity

Total phenolic content of plant leaf extracts (both dry & fresh) in different solvents are presented in Table 2 &graph 1, 2. Maximum phenolic content in dry Badam leaf extract was observed in Methanol solvent i.e. 602.53±60.2 mg/ml while Maximum phenolic content in fresh Badam leaf extract was observed in DMSO solvent i.e. 526± 52.67mg/ml. Plant polyphenols, a diverse group of phenolic compounds (flavanols, flavonoids, anthocyanins, phenolic acids, etc.) possess an ideal structural chemistry for free radical scavenging activity. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidative properties of polyphenol arise from their ability of derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction) (Chanda and Dave, 2009).

Table -2 also illustrates Antioxidant activity was used to evaluate the antioxidant potential of different solvent extracts of Badam (dry and fresh) leaf along with reference antioxidant compound ascorbic acid. Maximum Antioxidant activity in dry Badam leaf extract was obtained from DMSO solvent i.e. 1423±149.0 mg/ml while maximum Antioxidant activity in fresh Badam leaf extract was observed in ethanol solvent i.e. 1109±109.2mg/ml. The antioxidant capacity is based on the ability to reduce Mo (VI) to Mo (V). It was expressed in terms of ascorbic acid

equivalent (mg/g). When comparing the total Antioxidant activity in both dry & fresh leaf extracts, the antioxidant potential of dry Badam leaf extracts were in the increasing order of ethanol<distilled water < methanol<DMSO and in case of fresh leaves Antioxidant potential were as their increasing order DMSO<Distilled water< methanol<ethanol. The antioxidant activity arises from the donation of hydrogen electrons or ions (H+) originating from the hydroxyl, which reduces oxidant free radicals (Hamid *et al*., 2010). The medicinal effects of plants are often attributed to the antioxidant activity of the phytochemical constituents, mostly the phenolics and due to their redox property which allows them to act as reducing agents, metal chelators and free radical quenchers (Ademiluyi and Oboh 2008). Natural phenolics exert beneficial effects mainly through their antioxidant activity.

These compounds are capable of decreasing oxygen concentration, intercepting singlet oxygen, preventing first chain initiation by scavenging initial radical, such as hydroxyl radical, chelating metal ion catalyst, decomposing primarily product of oxidation to non radical species and breaking chains to prevent continued hydrogen abstraction from substance (Shahidi, 1997). In addition, polyphenolic compounds are primarily responsible for the antioxidant activity of natural extract due to their redox properties and chemical structures (Cuppett *et al*., 2001) several researches have demonstrated that there can be a correlation between phenolic content and antioxidant capacity of plant extracts or their essential oils (Davidson and Naidu, 2000 & Yang *et al*., 2002).

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay *et al*., 2003). It can be stated that phenolic content of the plant may be a good indicator of its antioxidant capacity.

Antibacterial activity

The Badam fresh leaf extract was found to show maximum activity against *S. aureus* in all the tested solvents, than *E. coli* and approximate similar pattern was found in dry leaves extracts in different solvents. The therapeutic value and bioactivity of plant extracts is attributed to phytochemical constituents. For instance, plant rich in tannins have antibacterial potential due to their characters that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane (Ayo, 2010).

The antimicrobial activity has been reported by many researchers. The results obtained from this work revealed that the plants contained bioactive agents which are connected with antimicrobial properties in plants. The zone of inhibition varied suggesting the varying degree of efficacy and different phytoconstituents of herb on the target organism. The antimicrobial activity of the plants may be due to the presence of various active principles in their leaves. In general, the Gram-negative bacteria show less sensitivity to plant extract may be due to their extra lipopolysaccharide and protein cell wall that provides a permeability barrier to the antibacterial agent (Adwan and Abu- Hasan, 1998). Furthermore, the Gram-positive bacteria are more sensitive to the extract because of the single layer of their cell wall, whereas the double membrane of Gram-negative bacteria make them less sensitive (Kaur and Arora, 2009).

Table: **1** Phytoconstituents present in dry and fresh leaves extracts of Badam in different solvents

S l	**Phytoconstituents**	**Dry leaves extract**				**Fresh leaves Extract**			
		Distilled Water	DMSO	Ethanol	Methanol	Distilled Water	DMSO	Ethanol	Methanol
1	Resins	+	+	+	-	+	-	+	+
2	Tannin	-	+	-	+	+	-	-	+
3	Anthraquinone	+	-	-	-	-	-	+	+
4	Phytosterol	-	+	+	+	+	+	-	+
5	Saponins	-	+	-	-	-	-	-	-
6	Cardiac Glycosides	+	+	+	+	+	+	+	+
7	Flavonoids	-	-	+	-	+	+	+	+
8	Alkaloids	-	+	+	-	-	-	-	+

(+) Present and (-) Absents

Table.2 Showing Total phenol contents and total antioxidant activity of the different extracts of (and Fresh leaves) in Badam(Prunus amygdalus)

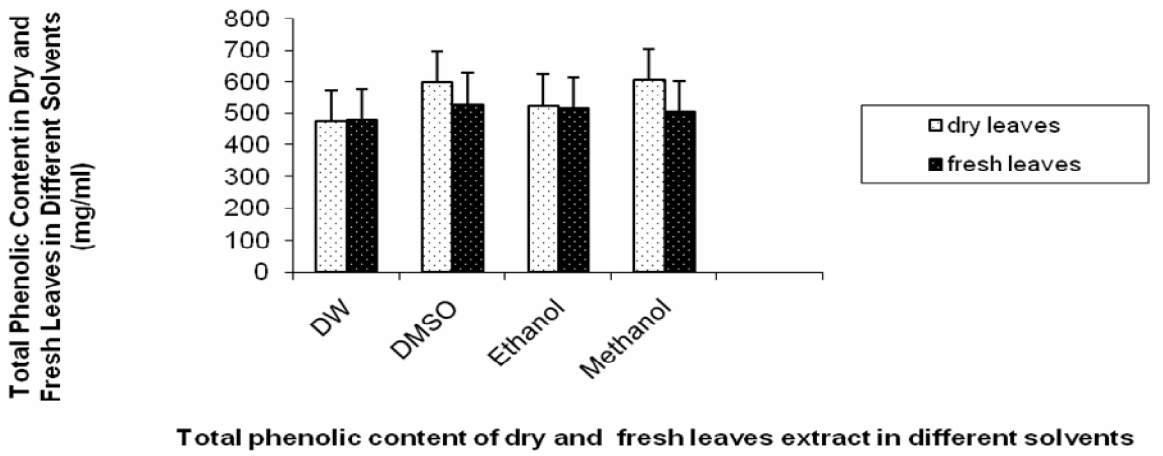
	Total Phenol Contents (mg/l)			
			Total Antioxidant Activity (mg/ml)	
Solvent	Dry leaves	Fresh leaves	Dry leaves	Fresh leaves
Distilled Water	472.7±49.45	476.66±47.66	1225.66±121.0	1166.66±116.6
DMSO	597.43±58.99	526±52.67	1423±149.0	1109±110.0
Ethanol	522.46±52.78	512.26±51.11	643±65.4	1595±158.22
Methanol	602.53±60.2	502.7±49.67	1377.33±137.0	162±17.2

All determination was performed in triplicates and values were expressed in mean ± SD

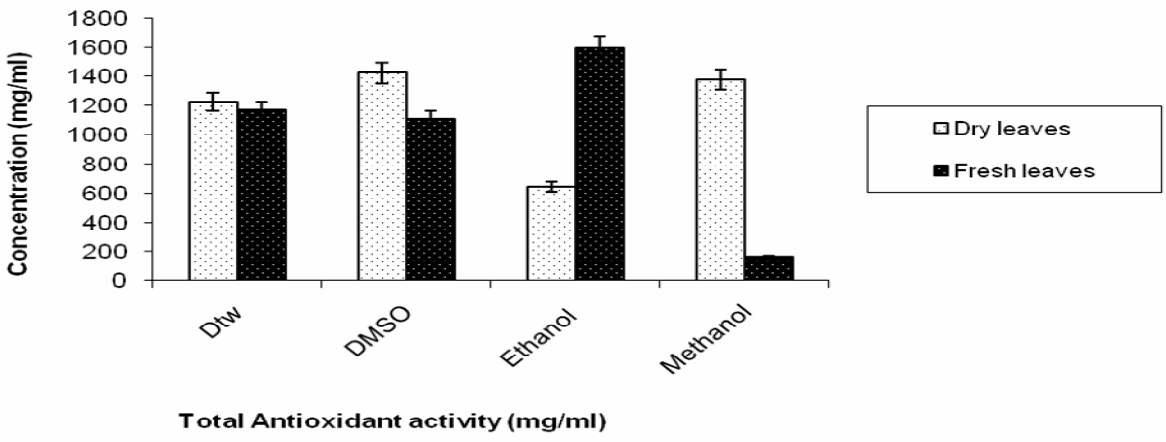
Table.3 Showing Antibiogram patterns for Badam (Prunus amygdalus) dry and fresh leaf extracts in different solvents

S. No	Micro-organism/Solvent	Diameter of zone of inhibition (in mm) for in dry leaves extracts				Diameter of zone of inhibition (in mm) in fresh leaves extracts			
		Distilled water	DMSO	Ethanol	Methanol	Distilled water	DMSO	Ethanol	Methanol
1	*E. coli*	R	S 13±1	R	R	R	S 14±1	S 11±1	R
2	*S. aureus*	R	R	S 15±1	R	S 16±1	S 16±1	S 17±1	R

Graph.1 Showing total phenolic content of dry and fresh leaves extract in different solvents



Graph.2 Showing total antioxidant activity of dry and fresh leaves extract in different solvents



Today, antioxidative properties of extracts from plants have become a great interest due to their possible uses as natural additives to replace synthetic ones. This study was designed to investigate the phenolic contents and evaluate the *in vitro* antioxidant activities of Badam fresh and dry leaves in different solvents.

Owing to the antioxidant and antibacterial activities exhibited by the leaf extracts investigated in this study, extracts of Badam plant leaves in different solvents could be considered a natural antioxidant and antimicrobial agent's source that can be used in food and pharmaceutical industries. However, further studies are needed to obtain purified compounds that may be responsible for the activities observed from the tested leaves.

Acknowledgement

We are thankful to Director, CHRI, Gwalior, for providing all the facilities required to carry out the experiments. We are also thankful to Dr. Archana Shrivastav,

Professor, College of Life Sciences for her unstinted support and guidance.

References

Ademiluyi A.O and Oboh G. (2008) Antioxidant properties of methanolic extracts of mistletoes (Viscum album) from cocoa and cashew trees in Nigeria. African Journal of Biotechnology Vol.7 (17), pp3138-3142.

Adwan K, Abu-Hasan N (1998). Gentamicin resistance in clinical strains of Enterobacteriaceae associated with reduced gentamicin uptake, Folia Microbiologica, 43, 438.

Ahmed, SM., Swamy, V., Dhanapal, PGR., Chandrashekara, VM.(2005). Anti-Diabetic Activity of Terminalia catappa 'Linn. Leaf Extracts in Alloxan-lnduced Diabetic Rats. Iranian J Pharmacol & Therapeutics. , 4(1): 36-39.

Ayo, R.G. (2010). Phytochemical constituents and bioactivities of the extracts of Cassia nigricans Vahl: A review. J Medicinal Plants Research Vol. 4(14), pp. 1339-1348.

Ayoola, P.B. & Adeyeye, A. (2010). Phytochemical and nutrient evaluation of carica papaya (pawpaw) leaves. IJRRAS 5 (3).

Chanda and Dave R. (2009) *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. African Journal of Microbiology Research Vol. 3(13) pp. 981-996.

Chen LG, Liu YC, Hsieh CW, Liao BC, Wung BS (2008). Tannin 1-alpha-O-galloylpunicalagin induces the calcium-dependent activation of endothelial nitric-oxide synthase via the phosphatidylinositol 3-kinase/Akt pathway in endothelial cells.Molecular Nutrition and food research.52 (10):1162-71. doi: 10.1002/mnfr.200700335

Cuppett, SL. (2001). The use of natural antioxidants in food products of animal origin. In: Pokorny J, Yanishkieva N, Gordon M, editors. Antioxidants in food-practical applications. Woodhead Publishing Ltd., Cambridge. , 285–310

Davidson, PM., Naidu, AS. (2000). Phyto-phenols. In: Naidu AS editor. Natural Food Antimicrobial Systems. Boca Raton, FL: CRC Press. , 265-294.

Diaz, MN., Frei, B., and Keaney, JF. (1997). "Antioxidants and atherosclerotic heart disease", *Engl. J. Med.*, 337,408- 416.

Elumalai, EK., Ramachandran, M., Thirumalai, T.,Vinothkumar, P. (2011). Antibacterial activity of various leaf extracts of *Merremia emarginata*. Asian Pacific J Tropical Biomedicine. 406-408.

Fernande Nyangono, Biyegue Christine, Marthe Tsague, Laure Ngondi Judith and Enyong Oben Julius (2013). *In vitro* antioxidant activity of *Guibourtia tessmannii* Harms, J. Leonard (Cesalpinoidae.J. Med. Plants Res. Vol. 7(42), pp. 3081-3088

Halliwell B (2006) Reactive species and antioxidants. Redox biology is a fundamental theme for aerobic life. Plant Physiol 141:312–322

Hyun DH, Hernandez JO, Mattson MP, de Cabo R (2006). The plasma membrane redox system in aging. Aging Res. Rev. 5: 209-220.

Hamid A.A. Aiyelaagbe,O.O. Usman,L.A. Ameen,O.M. and Lawal A(2010) Review:Antioxidants:Its medicinal and pharmacological

applications.African. Journal of Pure And Applied Chemistry.Vol 4(8) 142-151.

Ifesan, B.O.T., Fashakin, J.F., Ebosele, F. and Oyerinde, A.S. (2013). Antioxidant and Antimicrobial Properties of Selected Plant Leaves. European Journal of Medicinal Plants 3(3): 465-473.

Jose, MA., Ibrahim, Janardhanan, S. (2005). Modulatory effect of Plectranthus amboinicussusceptibility testing by standardized single disk method. Amer.J. Clin.Pathol.45:493-6,

Kaur GJ, Arora DS, (2009).Antibacterial and phytochemical screening of Anethum graveolens, Foeniculum vulgare and Trachysprmum ammi. BMC Complement, Alternative Medicine 9, 30.

Nair, R., Kalariya, T., Chanda, S. (2005). Antibacterial activity of some selected Indian medicinal flora. Turk J Biol. 29: 41-47.

Oktay, M., Gulcin, I. and Kufrevioglu, O.I. (2003) Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT Food Science and Technology Volume 36, Issue 2, Pages 263-271

Okerulu, IO, Ani, CJ. (2001). The Phytochemical Analysis and Antibacterial screening of extracts of *Tetracarpidium conophorum*. J. Chem. Soc. Nig., 26(1): 223-228.

Okigbo, R.N., Anuagasi, C.L. and Amadi, J.E. (2009). Advances in selected medicinal and aromatic plants indigenous to Africa. J Medicinal Plants Research Vol. 3(2), pp. 086-095

Prieto, P., Pineda, M., and Aguliar, M. (1999).Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex; specific application to the determination of Vitamin E, Anal. Biochem. 269, 337-341.

Shahidi, F. (1997). Natural antioxidants. An overview: Natural antioxidants, chemistry, health effects and applications. Champaign, IL. AOCS Press: pp. 1–11.

Sian, B.A (2003). Dietary antioxidants-past, present and future? Trends in Food Sci.Technol.14:93-98.

Singleton VL and Rossi JA. (1965) Colorimetory of total phenolics with phosphomolybdic –phosphotungstic acid and reagent Am. J. Enol. Vitic.; 16;144-158.

Stray, F. (1998). The natural guide to medicinal herbs and plants. Tiger Books International, London 1998, pp: 12-16.

Tirzitis Gunars and Bartosz Grzegorz (2010) Determination of antiradical and antioxidant activity: basic principles and new insights. Acta Biochimica Polonica.Vol 57(1)139-142.

Tiwari U., Jadon, M and Nigam, D (2014). Evaluation of antioxidant and antibacterial activities of methanolic leaf extract of *Callistemon viminalis*. International Journal of Pharmaceutical Sciences and Business Management (IJPSBM) Vol.2 Issue. 12, pg1-12

Upston JM, Kritharides L, Stocker R (2003). The role of vitamin E in atherosclerosis. Prog. Lipid Res. 42: 405-422.

Yang, JH., Lin, HC., Mau, JL. (2002). Antioxidant properties of several commercial mushrooms. Food Chem., 77:229-235.

Young IS and Woodside, JV. (2001). "Antioxidants in health and disease", *J. Clin. Pathol.* 54, 176-186.