➔DOI : 10.15740/HAS/AJBS/12.2/254-258
e ISSN-0976-8343 | ■ Visit us : *www.researchjournal.co.in*
ASIAN JOURNAL OF BIO SCIENCE
Volume **12** | Issue 2 | Oct., 2017 | 254-258

RESEARCH **P**APER

In vitro total antioxidant activity and nitric oxide scavenging of *Anogeissus latifolia*

AAKANKSHA, **RAMESHWAR GROACH AND** NARENDER SINGH

Department of Botany, Kurukshetra University, KURUKSHETRA (HARYANA) INDIA

Email : rameshwar.groach@kuk.ac.in

Article Info : Received : 10.06.2017; Revised : 25.08.2017; Accepted : 25.09.2017

Anogeissus latifolia (Roxb.Ex DC.) Wall.exGuill. &Perr. (Combretaceae) commonly known as bakli, dhau, dhawa or axle wood is a moderate sized tree characteristic of dry deciduous forests flourishing mainly in India. It is used in traditional systems of medicine to enhance the immune system and in the treatment of diabetes mellitus, diarrhoea, dysuria, cough, colic, snakebite, digestive ailments, skin and cardiovascular diseases. In this study, methanol and water extracts were prepared from powdered bark and leaf of *A. latifolia*. The extracts were studied for nitric oxide scavenging and total antioxidant activities determined by spectrophotometric technique. The results of this study show that the methanol and water extracts of *A. latifolia* can be used as an easily accessible source of natural antioxidants.

Key words : *A. latifolia*, Nitric oxide scavenging, Total antioxidant

How to cite this paper : Aakanksha, Groach, Rameshwar and Singh, Narender (2017). *In vitro* total antioxidant activity and nitric oxide scavenging of *Anogeissus latifolia*. *Asian J. Bio. Sci.*, **12** (2) : 254-258.**DOI : 10.15740/HAS/AJBS/12.2/254-258.**

INTRODUCTION

Free radicals are reactive nitrogen species (RNS) or reactive oxygen species (ROS) that are generated by environmental conditions or during metabolic activities in the body. Some of the reactive species, including singlet oxygen, hydrogen peroxidesuperoxide and hydroxyl radicals, have positive roles in energy production *in vivo* systems, intercellular signal transfer, phagocytosis, regulation of cell growth and the synthesis of important biomolecules (Shoji *et al*., 2008). Overproduction of free radicals is harmful because they initiate the oxidation of biomolecules which causes cell death and creates oxidative stress. Oxidative stress is responsible for involuntary activation of enzymes that lead to damage the cellular machinery by disturbing the nucleic acid, proteins, membrane lipids (Wiseman and Halliwell, 1996 and Jung *et al*., 2009). Free radicals are responsible for variety of biological phenomena such as ageing, inflammation, ischemia-reperfusion injury, atherosclerosis, diabetes mellitus, neurodegenerative disorders, mutation and carcinogenesis (Yoshikawa *et al*., 2000). Antioxidants have an ability to overcome the oxidative stress by scavenging free radicals and protecting antioxidant defences so they play an important role in the prevention of diseases (Banerjee and Dasgupta, 2005). Plants produce numbers of antioxidants to control the oxidative stress caused by free radicals and solar radiation, Thus, they can act as a new source of compounds with antioxidant activity.

Anogeissus latifolia belongs to the family Combretaceae, commonly known as bakli, dhau, dhawa, dhawra or dhaora is deciduous forest plant which has many economic importance. It is a native to India and distributed throughout India, except Jammu and Kashmir, Sikkim, Arunachal Pradesh, Assam, Nagaland,

Meghalaya, Manipur, Tripura, Mizoram. It is used as used as fodder and for making a variety of items of domestic and agricultural use that demand strength. It is included in the list of silk-producing non-mulberry plants (Srivastav *et al.*, 1990). Gum ghatti an important commercial exudate (Whistler, 1982) is obtained from it, used as binding agent in pharmaceuticals, in preparing sweets and cold drink. Number of phytochemical compounds have been isolated from various parts of the plant, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids and tannins. Due to the presence of these compounds the plant is useful in urinary tract infections, skin diseases, liver complaints, fever, epileptic fits etc. Stem bark is useful in diarrhoea, dysuria, cough, colic, liver complaints, snakebite and skin disease. It possesses antiulcer, antidiabetic, anticancer, antihyperlipidaemic, anti-inflammatory and antimicrobial properties. The present study was, aimed to determine the nitric oxide scavenging and total antioxidant activity of methanol and water extracts of *Anogeissus latifolia.*

RESEARCH METHODOLOGY

Collection of plant material :

Anogeissus latifolia bark and leaves were collected from forest complex, Pinjore, Haryana, (India) in the month of October, 2016. The collected bark and leaves were washed with running tap water and then dried under shade at room temperature for one week. Then, they were crushed to form fine powder and was stored in airtight glass bottles at room temperature.

Preparation of the extract :

The plant extracts were prepared by macerating 30 g of the dried plant part powder separately in water (300 ml/L) for 48 h. The extracts were first filtered, then the resultant filtrates were evaporated to dryness in a rotary evaporator under reduced pressure and were stored at 4°C for further use. The methanol extract was obtained by the similar method of water extract preparation but replacing water with methanol in the method already described.

Total antioxidant activity :

Total antioxidant capacity was determined by the spectrophotometric method of Preito *et al.*(1999). Different concentration of (100 to 600 µg/ml) bark and leaf extracts were prepared by dissolving in water. 0.1 ml of each concentration of extracts was combined with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in an eppendorf tube. The tubes containing the reaction solution were incubated at 95°C for 90 min. After cooling to room temperature, the absorbance of the solution of each extract was measured at 695 nm against a blank. Water (0.1 ml) was used as the blank in the place of extract. Ascorbic acid was used as the standard. The same procedure was carried out with extracts prepared in methanol.The per cent scavenging activity was calculated using the formula:

% scavenging activity = [(Ac-At)/Ac] x 100

where Ac is the absorbance of the control reaction and At is the absorbance in the presence of samples with the extracts.

Nitric oxide scavenging :

Nitric oxide (NO) scavenging activity was determined by spectrophotometric method of Garrat (1964). For this, test solution was prepared by dissolving 2 ml of 5 mM sodium nitroprusside solution in 0.5 ml phosphate buffer saline (pH 7.4) and then mixed with different concentration (100 to 600 µg/ml) of extract dissolved in methanol. The resulting mixture was incubated at 25°C for 120 minutes. Took 0.5ml of the incubated solution and 2 ml of Griess reagent (1% Sulfanilamide, 2% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride) was used to dilute it. The absorbance was measured at 546 nm by pouring the test solution into cuvette. The absorbance of the chromophore formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylene diamine. The per cent scavenging activity was calculated using the formula:

% scavenging activity = [(Ac-At)/Ac] x 100

where Ac is the absorbance of the control reaction and at is the absorbance in the presence of samples with the extracts.

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads Table 1 and Fig. 1 and 2.

Nitric oxide scavenging activity :

Nitric oxide is an important chemical intermediator

involved in regulation of various physiological processes. In the biological process, it is generated by specific nitric oxide *synthases* (NOSs), which metabolizes arginine to citrulline during which NO is formed via a five-electron oxidative reaction (Ross, 1993). These compounds are responsible for changing the structural and functional behaviour of many cellular components. Nitric oxide has the ability to exert multiple cytotoxic effects and its excess concentration cause number of diseases. In nitric oxide scavenging activity assay, nitrites produced when sodium nitroprusside is incubated in PBS for 2 h at 25°C which is reduced by the tested extracts of the plant. NO scavenging capacity is determined by the decrease in the absorbance at 546 nm, induced by antioxidants. The results of NO scavenging activity of the selected plant extracts are shown as per cent of NO scavenging in Table 1. The maximum per cent of NO scavenging activity of methanolic extract of bark, leaf were 85.29 and 79.44 with IC50 values of 5.15, 10.19 whereas for water extracts they were 93.17 for bark and 93.86 for leaf with IC50 value of 13.01, 9.41, respectively (Table 1). Maximum NO savenging activity was recorded from bark water extract at 600 µg/ml concentration. Water extracts of both bark and leaf showed better nitric oxide scavenging activity as compared to the methanolic extracts.

Total antioxidant activity:

The total antioxidant capacity of the different extracts was calculated based on the formation of the phosphomolybdenum complex which was measured spectrophotometrically at 695 nm. In the presence of different extract Mo (VI) is reduced to Mo (V) and forms a green coloured phosphomolybdenum V complex. Among different type of extracts, methanol bark extract had shown the maximum total antioxidant potential. The increase in the antioxidant potential of the extracts was observed with the increasing concentrations of bark and leaf of *A. latifolia*. The high total antioxidant potential was observed in methanolic extracts as compared to water extracts. Bark methanol extract show 215.75 mg AAE/g, leaf methanol 201.5 mg AAE/g, bark water 143.6

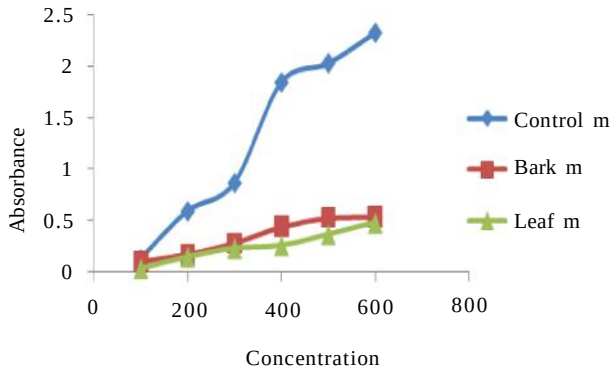

Fig. 1 : Total antioxidant activity of *A. latifolia* at athe different concentration of methanol extract

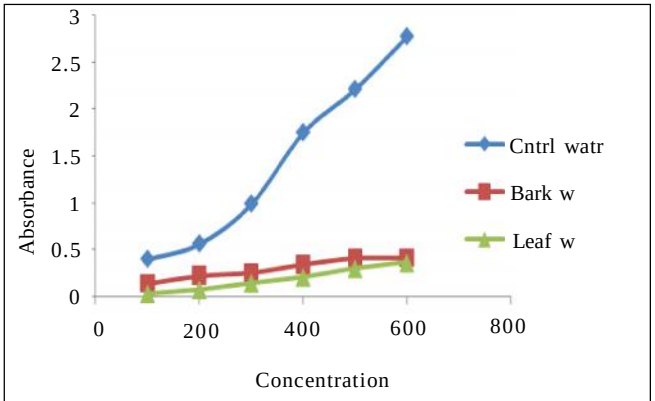

Fig. 2 : Total antioxidant activity of *A. latifolia* at the different concentration of water extract

Table 1 : Scavenging of nitric oxide by the different extracts of *A. latifolia*

Conc. µg/ml	% of scavenging of NO			
	Methanol extract		Water extract	
	Bark	Leaf	Bark	Leaf
100	50.22	52.54	49.1	52.06
200	68.37	62.88	71.133	64.97
300	71.33	64.27	73.8	81.25
400	74.15	70.49	80.373	91.18
500	84.36	76.88	81.416	92.09
600	85.29	79.44	93.171	93.86
IC 50	5.15	10.19	13.01	9.41

mg AAE/g and leaf water 134.5 mg AAE/g of total antioxidant potential.

Antioxidants are the molecules that provide the protection from oxidative stress by scavenging the free radicals. The ability of antioxidants to neutralize, free radicals depends on the structure of antioxidants (Loo *et al.*, 2008). The present study showed that, the methanolic and water extracts of *A. latifolia* bark and leaf exhibited the high nitric oxide scavenging and total antioxidant activity. A scavenging of free radicals may serve as a possible preventive intrusion for the diseases (Gyanfi *et al.*, 1999). The present study suggests that the bark and leaf of *A. latifolia* is a potential source of natural antioxidants. The antioxidant potential were also noticed in whole plant or part of some plants investigated *i.e. Torilis leptophylla* (Saeed *et al.*, 2012), *Indigofera cassioides* (Kumar *et al.*, 2012), *Gnidia glauca* (Rao *et al.*, 2013). The antioxidant potential of the extracts was increased markedly with the increase in concentrations of extract. The similar results of increase in antioxidant potential with concentration are also reported in *Panax ginseng* and *Lagerstroemia speciosa* (Saumya and Mahaboob Basha, 2011) *Ethulia conyzoides* (Aliyu *et al.*, 2012) *Withania somnifera* and *Aloe vera* (Patel *et al*., 2012), *Phyllanthus freternus, Triumfetta rhomboidae* and *Casuarina littorea* (Rozina *et al*., 2013).

The methanol extract of *A. latifolia* showed higher total antioxidant activity as compared to water extract of plant parts, Similar results of higher antioxidant activity in methanolic extract are reported in *B. vahlii* (Sowndhararajan and Kan, 2013), *Brugeiera gymnorrhiza and Aegialitis rotundifolia* (Reddy and Grace, 2016).

Nitric oxide is implicated in inflammation, cancer and other pathological conditions (Moncada *et al.*,1991). The metabolites present in plants may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body by scavenging activity to arrest the chain of reactions initiated by excess generation of NO (Kumaran and Karunakaran, 2006). Nitric oxide scavenging activity was observed in more in water extract comaprriable to methanolic extract. Nitric oxide scavenging activity has also been reported in *Coleus aromaticus* (Kumaran and Karunakaran, 2006), *C. zeyheri, C. platypetalum*and *P. curatellifolia* (Boora *et al.*, 2014).

Conclusion:

As a conclusion, the methanol and water extracts of *A. latifolia* showed nitric oxide scavenging and powerful total antioxidant activities when compared to standards such as ascorbic acid. The results of this study show that the methanol and water extracts of *A. latifolia* can be used as an easily accessible source of natural antioxidants used in pharmaceutical industry.

LITERATURE CITED

Aliyu, A.B., Ibrahim, M.A., Ibrahim, H., Musa, A., Lawal, A.Y., Oshanimi, J.A., Usman, M., Abdulkadir, I.E., Oyewale, A.O. and Amupitan, J.O. (2012). Free radical scavenging and total antioxidant capacity of methanol extract of *Ethulia conyzoides* growing in Nigeria; *Romanian Biotechnological Letters*,**17**(4): 7458-7465.

Banerjee, A. and Dasgupta, N. (2005). *In vitro* study of antioxidant activity of *Syzygium cumini* fruit, *Food Chemistry*, **90**(4): 727-733.

Boora, F., Chirisa, E. and Mukanganyama, S. (2014). Evaluation of nitrite radical scavenging properties of selected zimbabwean plant extracts and their phytoconstituents. *J. Food Process.*, **2014** : 7.

Garrat, D. C. (1964). *The quantitative analysis of drugs*. Chapman and Hall Ltd., Japan, **3**: 456.

Gyanfi, M.A., Yonamine, M. and Aniya, Y. (1999). Free radical scavenging action of medicinal herbs from Ghana Thonningla sanguine on experimentally induced liver injuries. *General Pharmacol.*, **32** : 661-667.

Jung, T., Höhn, A., Catalgol, B. and Grune, T. (2009). Age-related differences in oxidative protein-damage in young and senescent fibroblasts. *Archiv. Biochem. & Biophysics*, **483** : 127-135.

Kumaran, A. and Karunakaran, R. J. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus. Food Chem.*, **97**(1): 109-114.

Kumar, R.S., Rajkapoor, B. and Peruma, P. (2012). Antioxidant activities of *Indigofera cassioides* Rottl. Ex. DC. using various in vitro assay models. *Asian Pacific J. Tropical Biomedicine*, **2**(4): 256–261.

Loo, A.Y., Jain, K. and Darah, I. (2008). Antioxidant activity of compounds isolated from the pyroligneous acid, *Rhizophora apiculata*. *Food Chem.*, **107** : 1151–1160.

Moncada, A., Palmer, R.M.J. and Higgs, E. A. (1991). Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacological Reviews*, **43** : 109–142.

Patel, D.S., Shah, P. B. and Managoli, N. B. (2012). Evaluation of *in-vitro* anti-oxidant and free radical scavenging activities of *Withania somnifera* and *Aloe vera*; *Asian J. Pharm. Tech.*, **2**(4):143-147.

Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, **269** : 337-341. http://dx.doi.org/10.1006/abio.1999.4019.

Rao, B.S., Jayanthi, Yogeetha, M., Ramakrishnaiah, H. and Nataraj, J. (2013). Free radical scavenging activity and reducing power of *Gnidia glauca* (Fresen.). *J. Appl. Pharmaceutical Sci.*, **3** (6) : 203-207.

Reddy, A.R.K. and Grace, J.R. (2016). *In vitro* evaluation of antioxidant activity of *Brugeiera Gymnorrhiza* and *Aegialitis Rotundifolia*. *Medicinal & Aromatic Plants*, **5** : 2.

Ross, R. (1993). The pathogenesis of atherosclerosis: a perspective for the 1990's. *Nature*, **362** : 801.

Rozina, P.,Kundu, S.K. and Saha, P. (2013). *In vitro* nitric oxide scavenging activity of methanol extracts of three Bangladeshi medicinal plants, *Pharma Innovation J.*, **1** : 12.

Saeed, L. N., Khan, M.K. and Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla*;*BMC Complementary & Alternative Medicine*,**12** : 221.

Saumya, S. M. and Mahaboob Basha, P. (2011). *In vitro* evaluation of free radical scavenging activities of Panax Ginseng and *Lagerstroemia speciosa*: a comparative analysis, *Internat. J. Pharm. Pharm. Sci.*, **3** (1) : 165- 169.

Shoji, H., Yamashiro, Y., Koletzko, B., Packer, L. and Sies, H. (2008). Oxidative stress and antioxidants in the perinatal period, In: *Oxidative stress and inflammatory mechanisms in obesity, diabetes and the metabolic syndrome*, CRC Press Taylor & Francis Group, Boca Raton, FL, pp.72.

Sowndhararajan, K. and Kan, S.C. (2013). Free radical scavenging activity from different extracts of leaves of Bauhinia vahlii Wight & Arn. *Saudi J. Biological Sci.*, **20** (4) : 319–325.

Srivastav,P. K., Isa, Md., Siddiqui, A.A., Beck, S. and Prasad, D. N. (1990). Genetic resources of silk producing non-mulberry plants. *Indian J. Plant Genet. Resour.*, **3**: 107-110.

Whistler, R.L. (1982). Industrial gums from plants: Guar and Chia; *Economic Botany*, **36** : 195-202.

Wiseman, H. and Halliwell, B. (1996). Damage to DNA by reactive oxygen and nitrogen species: Role of inflammatory disease and progression to cancer. *Biochem. J.*, **313**: 17-29.

Yoshikawa, T., Toyokuni, S., Yamamoto, Y. and Naito, Y. (2000). Free radicals in chemistry biology and medicine. OICA International, LONDON, UNITED KINGDOM.