PJN
ISSN 1680-5194

# PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 11 (7): 618-623, 2012
ISSN 1680-5194


# Evaluation of Polyphenolic Content and Antioxidant Activity of *Artemisia afra* Jacq. Ex Willd. Aqueous Extract

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**Abstract:** *Artemisia afra* Jacq. ex Willd. is widely used in South African traditional medicine for the treatment of many ailments and diseases. In this work, aqueous extract of the plant was screened for its phenolic profile and antioxidant activity. The results obtained revealed that the extract has considerable amount of polyphenolic compounds including phenol, flavonoid, flavonol and proanthocyanidin. The extract also exhibited significant inhibition of DPPH and ABTS radicals as well as ferric reductive ability in a concentration-dependent manner. These are indications of antioxidant activity of *A. afra* which could be attributed to the presence of phenolic compounds; and the data compared well with those of known standards like BHT, rutin and vitamin C. This study has, to some extent, justified the folkloric use of the herb in traditional medicinal practice of South Africa.

**Key words:** *Artemisia afra*, antioxidant, total phenolics, DPPH, ABTS, free radicals

## INTRODUCTION

The use of plant materials for medicinal purposes dates back to the history of mankind. In traditional African societies, Complementary and Alternative Medicines (CAM) continue to play an important role in the treatment of many diseases and ailments (Nweze and Eze, 2009). This is particularly true in the developing countries where most people have limited resources and do not have access to modern health facilities (Ali *et al*., 2006). The increase in demand for the use of plant based medicines may also be due to the side effects associated with orthodox drugs (Marles and Farnsworth, 1994). Another important factor that strengthens the use of herbs could be attributed to the belief that they do provide some benefits over and above allopathic medicine and allow users to feel that they have some control in their choice of medication (Joshi and Kaul, 2001). Therefore, it is not surprising why majority of the population use medicinal plants as their main source of primary health care or supplement to visiting western health care practitioners (Van Wyk *et al*., 1987).

Despite these success stories, the general acceptability of CAM has been limited by lack of dose regimen and adequate toxicity data to evaluate their safety (Saad *et al*., 2006). It is therefore appropriate that these herbs receive a holistic scientific scrutiny in order to determine their effective doses and possible toxicities. CAM derives great benefits from such research studies because they explain the scientific basis for treatment decisions and this has lent credibility to the folkloric use of many medicinal plants (Vojdani and Erde, 2006).

It is increasingly being realized that many of today's diseases are due to the oxidative stress that results from an imbalance between formation and neutralization of pro-oxidants (Hazra *et al*., 2008). Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells (Martinez-Cayuela, 1995). This results in protein and DNA damage along with lipid peroxidation. These changes cause cellular injury which leads to the development of chronic diseases such as cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases (Maxwell, 1995; Braca *et al*., 2002; Wong *et al*., 2006). In order to prevent these adverse effects, all human cells protect themselves against free radical damage with enzymes such as Superoxide Dismutase (SOD) and catalase; or compounds such as ascorbic acid, tocopherol and glutathione (Niki *et al*., 1994; Soto *et al*., 2003). Sometimes, these protective mechanisms are disrupted by various pathological processes and antioxidant supplements are vital to combat oxidative damage. Recently, the search for antioxidants from natural sources to replace synthetic ones has received much attention. This may be because medicinal plants especially those traditionally used in folk medicine have been found to offer protection against diseases (Halliwell, 1989; Moller *et al*., 1999).

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*Artemisia afra* Jacq. ex Willd. (Asteraceae) is one of the most commonly used medicinal plants in South Africa. It is an erect, shrubby, perennial plant growing up to 2 m tall with leafy and hairy stems. Its leaves are soft texture, narrowly ovate, feathery and finely divided; reaching a length of 8 cm and a width of 4 cm. The herb has an easily identifiable aromatic smell which turns pungent and sweet after bruising (Van Wyk *et al*., 1987). The plant grows in the mountain regions of Kenya, Tanzania, Uganda and as far north as Ethiopia. It is also widely distributed in Southern Africa including Namibia, Zimbabwe and South Africa. Locally known as *Unhlonyane* in Xhosa and African wormwood in English, it is widespread in the Northern Province, Gauteng, Limpopo, Western and Eastern Cape as well as KwaZulu-Natal Provinces of South Africa (Van Wyk *et al*., 1987).

*A. afra* has been documented to possess healing properties against many diseases and ailments. Syrup prepared from the plant is used for bronchial troubles, while infusion or decoction is applied as a lotion to bathe hemorrhoids and for earache. An infusion of leaves or roots of this species is also used for the treatment of diabetes in the Eastern Cape Province of south Africa (Erasto *et al*., 2005). Respiratory infections are treated by inhaling the vapour from boiling leaves. This vapour is also used to treat menstrual chill. Fresh tips are inserted into the nose for colds and headaches; and into hollow teeth to treat toothache. The poultice of the leaves is applied to relieve neuralgia, to treat swellings in mumps and is placed on the abdomen to treat infantile colic (Watt and Breyer-Brandwijk, 1932). In addition to these, the plant is also used to treat cough, dyspepsia, loss of appetite, gastric derangement, gout, asthma, malaria, bladder and kidney disorders, influenza, convulsion, fever, heart inflammation, rheumatism and as a purgative (Halliwell and Gutteridge, 1989; Thring and Weitz, 2006). These are indications that the herb possesses antiviral, antibacterial, anti-inflammatory and antioxidant activities. However, to the best of our knowledge as at the time of carrying out this study, there appears to be dearth of information on the antioxidant properties of this plant. Therefore, the present study was designed to evaluate the antioxidant activity, reducing power and total polyphenol contents of aqueous leaf extract of *A. afra*. This is with a view to justifying some of the therapeutic properties of the plant and to lend scientific credence to its general use for the treatment of many diseases and ailments in South Africa.

## MATERIALS AND METHODS

**Chemicals used:** Folin-Ciocalteu phenol reagent, methanol and ethanol were products of E. Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium ferricyanide, butylated hydroxytoluene (BHT), ascorbic acid, catechin, tannic acid, quercetin and ferric chloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA), vanillin from BDH Chemicals Ltd. (Poole, England). All other reagents were of analytical grade.

**Plant material:** Fresh matured leaves of *A. afra* were collected from around the University of Fort Hare, Alice (Eastern Cape Province of South Africa) in June 2009. The plant material was identified by Prof. DS Grierson, Department of Botany, University of Fort Hare, South Africa. A voucher specimen (Sunmed. 2009/01) was prepared and deposited at the Giffen Herbarium of the university.

**Preparation of the plant extract:** The leaves were thoroughly rinsed with distilled water, dried in the oven at 30°C and slightly crushed by hand. The dried leaves (100 g) were suspended in 1 L distilled water and the mixture was boiled for 30 min at 40°C to minimize loss of volatile constituents. The decoction obtained was cooled, filtered, frozen at -70°C and then freeze-dried (Vir Tis benchtop K, Vir Tis Company, Gardiner, NY) to give a yield of 17.4 g. The dried plant material was reconstituted in distilled water to give the required concentrations for the experiment.

**Measurement of polyphenolic contents**

**Total phenol:** The total phenolic content in the extract was determined by the modified Folin-Ciocalteu method (Wolfe *et al*., 2003). The extract (1 mg/ml) was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The mixture was vortexed for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured at 765 nm using the AJ-1C03 Spectrophotometer (Anjue Co. Ltd., Anqing, China). Samples of extract were evaluated at a final concentration of 1 mg/ml. Total phenolic content was expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$, $R^2 = 0.9365$, where x was the absorbance and y was the tannic acid equivalent (mg/g).

**Total flavonoid:** Total flavonoid content was determined using the method described by Ordon Ez *et al*. (2006). A volume of 0.5 ml of 2% $AlCl_3$ in ethanol solution was added to 0.5 ml of sample solution. After one hour at room temperature, the absorbance was measured at 420 nm using the AJ-1C03 Spectrophotometer. A yellow colour indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 1 mg/ml. Total flavonoid content was calculated as quercetin (mg/g), using the following equation based on the calibration curve: $y = 0.025x$, $R^2 = 0.9812$, where x was the absorbance and was the quercetin equivalent (mg/g).

**Total flavonol:** Total flavonol in the plant extract was estimated using the method of Kumaran and Karunakaran (2007). To 2 ml of sample (standard), 2 ml of 2% $AlCl_3$ in ethanol and 3 ml (50 g/l) sodium acetate solutions were added. The absorbance at 440 nm was read after 2.5 h using the AJ-1C03 Spectrophotometer. Extract sample was evaluated at a final concentration of 1 mg/ml. Total flavonol content was calculated as quercetin (mg/g) using the following equation based on the calibration curve: y = 0.0255x, $R^2$ = 0.9812, where x was the absorbance and y was the quercetin equivalent (mg/g).

**Total proanthocyanidin:** The procedure reported by Sun *et al.* (1998) was used to determine the total proanthocyanidin. A volume of 0.5 ml of 0.1 mg/ml extract solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid; the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm using the AJ-1C03 Spectrophotometer. The extract was evaluated at a final concentration of 0.1 mg/ml. Total proanthocyanidin content was expressed as catechin equivalents (mg/g) using the following equation based on the calibration curve: y = 0.5825x, $R^2$ = 0.9277, where x was the absorbance and y is the catechin equivalent (mg/g).

**Evaluation of antioxidant activity**

**ABTS radical scavenging assay:** The improved ABTS radical cation decolorization assay was adopted (Re *et al.*, 1999). The stock solution which was allowed to stand in the dark for 16 h at room temperature contained equal volumes of 7 mM ABTS salt and 2.4 mM potassium per sulfate. The resultant $ABTS^+$ solution was diluted with methanol until an absorbance of about 0.70±0.01 at 734 nm was achieved. Varying concentrations (0.025-0.2 mg/ml) of the methanolic plant extract (1 ml) was reacted with 1 ml of the $ABTS^+$ solution and the absorbance taken at 734 nm between 3-7 min using the AJ-1C03 Spectrophotometer. The $ABTS^{*+}$ scavenging capacity of the extract was compared with those of BHT and rutin and the percentage inhibition was calculated as:

$$\text{ABTS RSA (\%)} = [(Abs_{control} - Abs_{sample})]/ (Abs_{control}] \times 100$$

Where RSA = radical scavenging activity, Abscontrol was the absorbance of ABTS radical + methanol and Abssample was the absorbance of ABTS radical + sample extract/standard.

**DPPH radical scavenging assay:** The DPPH radical scavenging activity of the extract was estimated using the method of Liyana-Pathiranan and Shahidi (2005). A solution of DPPH (0.135 mM) in methanol was prepared and 1 ml of this solution was mixed with 1 ml of varying concentrations of the methanol extract (0.025-0.2 mg/ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm with AJ-1C03 Spectrophotometer using rutin and BHT as standards. The ability to scavenge DPPH radical was calculated as:

$$\text{DPPH RSA(\%)} = [(Abs_{control} - Abs_{sample})]/ (Abs_{control}] \times 100$$

Where RSA = Radical scavenging activity, Abscontrol was the absorbance of DPPH radical + methanol and Abssample was the absorbance of DPPH radical + sample extract/standard.

**Ferric reducing power:** The ferric reducing potential of the extract was assayed as described by Duh *et al.* (1999). Different concentrations (0.025-0.2 mg/ml) of the extract and the standards, vitamin C and BHT in methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1% w/v). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 ml, 10% w/v) was added to the mixture, and was then centrifuged for 10 min at 1000 rpm (CF-622-ANG-CENT, Labline Equipments PVT, LTD, Gujrat, India). The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1% w/v). The absorbance was measured at 700 nm using the AJ-1C03 Spectrophotometer. The reducing power of the extract was compared with those of BHT and vitamin C.

**Statistical analysis:** Data were analyzed using SPSS/10.0 with the results expressed as mean ± SD of three determinations. Statistical evaluations were assayed using one-way Analysis of Variance (ANOVA) and values were regarded as statistically significant at $p<0.05$.

## RESULTS

**Polyphenol contents:** The aqueous extract of *A. afra* was characterized by the presence of considerable amount of phenolic compounds (Table 1). The plant extract has a high level of total phenols (258.39 mg/g tannic acid equivalent) followed by proanthocyandins (199.21 mg/g catechin equivalent), flavonoids (29.68 mg/g quercetin equivalent) and flavonols (10.58 mg/g catechin equivalent).

**DPPH and ABTS radical scavenging activities:** Significant DPPH and ABTS radical scavenging activity was evident at all the tested concentrations of the extract and compared well with the standards (BHT and rutin). The inhibition of the two radicals by the extract occurred in a concentration dependent manner (Fig. 1 and 2).

Table 1: Polyphenolic contents of aqueous leaf extract of *A. afra* (n = 3, X±SD)

| Polyphenolic compounds | Quantity |
|---|---|
| Total phenol[a] | 258.39±2.49 |
| Total flavonoid[b] | 29.68±1.05 |
| Total flavonol[c] | 10.58±0.73 |
| Total proanthocyanidin[d] | 199.21±2.51 |

[a]Expressed as mg tannic acid/g of dry plant material.
[b]Expressed as mg quercetin/g of dry plant material.
[c]Expressed as mg quercetin/g of dry plant material.
[d]Expressed as mg catechin/g of dry plant material

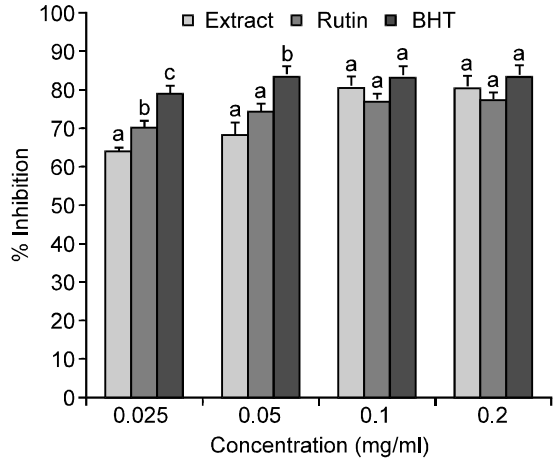


Fig. 1: Percentage inhibition of DPPH radical in the presence of *A. afra* aqueous extract, rutin and BHT. Results are mean of three determinations ± SD. Bars with different letters for each concentration are significantly different ($p<0.05$)

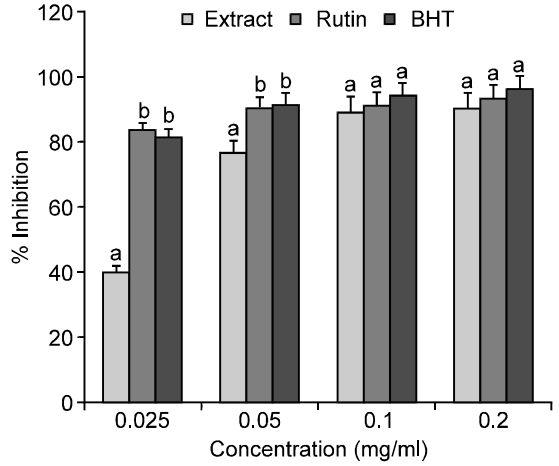


Fig. 2: Percentage inhibition of ABTS radical in the presence of *A. afra* aqueous extract, rutin and BHT. Results are mean of three determinations ± SD. Bars with different letters for each concentration are significantly different ($p<0.05$)

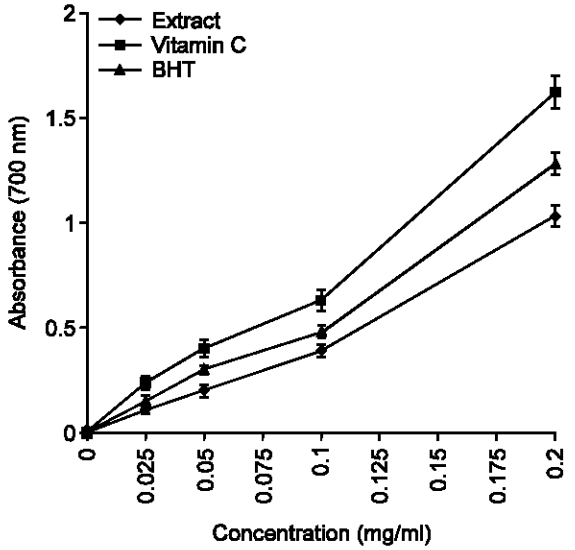


Fig. 3: Ferric reductive ability of *A. afra* aqueous extract, vitamin C and BHT. Each value represents mean of three determinations ± SD

**Reducing ability:** As illustrated in Fig. 3, Fe III was transformed to Fe II in the presence of the plant extract and the reference compounds. Although the reducing power of the extract was lower than those of BHT and vitamin C, the results revealed that the reductive capability of the extract was concentration dependent.

## DISCUSSION

There is increasing evidence that oxidative stress plays a vital role in the pathogenesis of many diseases; and antioxidants have been considered as effective treatments (Reaven *et al*., 1995; Cunningham, 1998). Research into the antioxidant properties of herbs may provide information on the mechanism of action of plant extracts, the utility of the medicines in fighting against oxidative damage and help to identify the presence of specific antioxidant constituents (Liao *et al*., 2007). The present study has clearly demonstrated that aqueous extract of *Artemisia afra* has significant antioxidant activity.

Polyphenolics are the major plant compounds with antioxidant activity and it is likely that the activity of the extract is due to the redox properties of these compounds (Tepe *et al*., 2006). These properties play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Zheng and Wang, 2001). The hydroxyl groups attached to the aromatic ring structure of flavonoid, which is a major phenolic compound present in the aqueous leaf extract of the plant, enabled them to undergo redox reaction and consequently scavenge free radicals (Brand-Williams *et al*., 1995). Plant phenolics can delay the onset of lipid oxidation and decomposition

of hydroperoxides in food products as well as in living tissues (Wettasinghe and Shahidi, 2002). Analyses of *A. afra* have revealed the presence of many phytochemicals which possess significant antioxidant properties that are associated with lower occurrence and lower mortality rates of several human diseases such as cancer, ageing, cardiovascular and inflammatory diseases. On the whole, the data obtained in this study indicated that phenolics are important components of *A. afra*, and most of the pharmacological effects attributed to this plant could be due to the presence of these valuable constituents.

The relatively stable organic radical, DPPH, has been widely used in the determination of antioxidant activity of compounds and different plant extracts (Katalinc *et al*., 2006). The mechanism of the reaction between antioxidants and DPPH is dependent upon on the structural conformation of the antioxidants which could be attributed to their hydrogen donating ability (Baumann *et al*., 1979). ABTS is a protonated radical with a characteristic absorbance at 734 nm which decreases with the scavenging of proton radicals (Mathew and Abraham, 2006). The scavenging of the ABTS* by the aqueous extract of *A. afra* was found to be higher than that of DPPH*. This could be attributed to the stereo selectivity of the radicals as reported by Yu *et al*. (2002). Similar observation was reported by Adedapo *et al*. (2009) using *Celtis africana*. Overall, the scavenging ability of the extract on the two radicals compared favourably with rutin and BHT, which is an indication of the antioxidant capability of the herb.

The reducing power of a compound is related to its electron transfer ability and may therefore serve as a significant indicator of its potential antioxidant activity (Meir *et al*., 1995). The Ferric Reducing/Antioxidant Power (FRAP) assay is widely used in the evaluation of the antioxidant component of dietary polyphenols (Luximon-Ramma *et al*., 2005). The plant extract showed very good reducing capacity which served as a significant indicator of its antioxidant activity (Hazra *et al*., 2008). This property enabled the herb to mop up noxious toxic metabolites released during pathological states and confer protection on the affected organs. The possible mechanism by which the extract exerts its effect may be by prevention of chain initiation or decomposition of peroxides (Yildidrim *et al*., 2000). The reductive capability of the extract was concentration dependent which is an indication that its antioxidant activity is likely to increase with higher concentrations.

In conclusion, our data revealed a positive correlation between the polyphenol contents and reducing/antioxidant power of the aqueous leaf extract of *A. afra*. These *in vitro* assays indicated that the extract is a significant source of natural antioxidant, which might be responsible for its ability to treat many ailments and diseases. These findings therefore, justified the folkloric use of the herb in South African herbal medicine.

## ACKNOWLEDGEMENT

This research was supported by grants from Govan Mbeki Research and Development Centre, University of Fort Hare and the National Research Foundation of South Africa. The authors are also grateful to the University of Ilorin, Nigeria, for supporting the postdoctoral fellowship of Dr. T.O. Sunmonu in 2009.

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