International Journal of Drug Development & Research
| October-December 2011 | Vol. 3 | Issue 4 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands


FULL Length Research Paper
Covered in Index Copernicus with IC Value 4.68 for 2010

# Free Radical Scavenging (DPPH) and Ferric Reducing Ability (FRAP) of *Aphanamixis polystachya (Wall) Parker*

**Saklani Sarla[1], Mishra Abhay Prakash[1]*, Rawat Apeksha[1], Chandra Subhash[1]**
Department of Pharmaceutical Sciences, H.N.B. Garhwal (A Central) University Srinagar, Pauri Garhwal, Uttarakhand, INDIA.

**Abstract**

Many plants possess antioxidant ingredients that provided efficacy by additive or synergistic activities. Present article highlights an antioxidant activity of a red listed medicinal plant *Aphanamixis polystachya* bark which has a strong astringent power. It is used for the treatment of rheumatism, tumours, liver and spleen diseases. Antioxidant activity of the crude extracts of *A. polystachya* (bark) were assessed using DPPH and FRAP assays. The alcohol, aqueous methanol and petroleum ether extracts exhibited potent antioxidant activity compared to known antioxidants. Due to its natural origin and potent free-radical scavenging ability *A. Polystachya* could be used as a potential preventive action taken to improve free radical-mediated diseases.

*Corresponding author, Mailing address:
Mishra Abhay Prakash
Department of Pharmaceutical Sciences, H.N.B. Garhwal (A Central) University Srinagar, Pauri Garhwal, Uttarakhand, INDIA
E-mail: abhaycimap@gmail.com

***Key words:***

*Aphanamixis polystachya,* Free-radical scavenging, Ferric Reducing Ability.

***How to Cite this Paper:***

**Saklani Sarla, Mishra Abhay Prakash*, Rawat Apeksha, Chandra Subhash** "Free Radical Scavenging (DPPH) and Ferric Reducing Ability (FRAP) of *Aphanamixis polystachya (Wall) Parker*", Int. J. Drug Dev. & Res., Oct-Dec 2011, 3(4): 271-274



***Article History:----------***
***Date of Submission: 12-09-2011***
***Date of Acceptance: 22-09-2011***
***Conflict of Interest: NIL***
***Source of Support: NONE***

## Introduction

*Aphanamixis polystachya (Wall) Parker* also familiar as Amoora Rohituka, belonging to the family Meliaceae is the large handsome tree continues to holding green leaves throughout the

year, with an umbrella shaped or dense spreading crown. It has a straight cylindrical bole up to 15m in height and 1.5-1.8m in girth. It is distributed in the sub-Himalayan tract from Gonda (Uttar Pradesh) eastwards to Bengal, Sikkim, Assam up to 6000ft and in Western ghats, chota Nagpur, Konkarn, Andamans and adjoining hill ranges from the Poona district southwards to Tinnevelly up to 3500ft [1].

The stem barks of *Aphanamixis polystachya* is used traditionally in treatment of tumors, cancer, skin and spleen diseases, leprosy, diabetes, opthalmopathy, intestinal worm, jaundice and rheumatism [2]. The hepatoprotective activity [3], antimicrobial, antiviral and antibacterial activity of isolated limonoid rohitukin [4,5] and cytotoxicity of amoorastatin were also established [6]. *Aphanamixis polystachya* has disclosed the presence of Aphanmixis a triterpenes [7], tetranortriterpenoid *i.e.* aphanamixinin, sterol, saponins **[8],** flavanone and anthraquinone glycosides [9].

## Material and Methods

### Plant Material

The stem bark of *Aphanmixis polystachya* family meliaceae was collected from Forest Research Institute, Dehradun (Uttarakhand) and authenticated by Dr. Arvind Bhardwaj, who is a Botanist. The stem barks were also authenticated by comparison of macroscopic and microscopic characteristics with monograph of Pharmacopoeia [10].

### Prepration of Extract

About 500gm of stem barks were washed, air dried and ground into course powder. It was extracted in soxhlet apparatus with alcoholic (95.0%), aqueous methanol (methanol: water, 50:50) and petroleum ether solvent for 36 hours. The solvents were then removed under pressure to yield brownish residue (yields sequentially: 12.51%, 05.82% and 02.41% w/w with respect to dried stem barks) [11]. Now the alcoholic, aqueous methanol and petroleum ether extracts were studied for *in vitro* antioxidant activity.

**DPPH free-radical scavenging activity:** DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical-scavenging activity was measured by the method of Szabo *et al.* [12]. The reaction mixture contained $1.5\times10^{-7}$M Methanolic solution of DPPH and various concentrations of the test substances and were kept in dark for 50min. Optical Density (OD) of the samples was measured at 517nm against a blank and IC50 values were calculated using linear regression analysis.

**FRAP assay:** The Ferric Reducing Antioxidant Power (FRAP) of various extracts of *A. polystachya* was performed based on the method of Benzie and Strain [13]**.** The assay mixture contained 2.5mL of 300mM acetate buffer at pH 3.6, 0.25mL of 10mM TPTZ solution in 40mM HCl, 0.25mL of 20mM FeCl3 and test substances in 0.1mL water or methanol. The absorbance was measured after 30min incubation at 593nm.

Standard graphs were constructed using known concentrations of ferrous salt in water/methanol to replace $FeCl_3$. All tests were run in triplicate and mean values were used to calculate EC1 values. EC1 is defined as concentration of an antioxidant having a ferric reducing ability equivalent to that of mM ferrous salt.

## Result and Discussion

**DPPH free radical scavenging activity:** The results of the DPPH scavenging activity of *A. polystachya* bark extracts are shown in [Fig. 1]. The IC50 values exhibited that the scavenging ability of alcohol (>25µg $mL^{-1}$), aqueous methanol (5.33µg $mL^{-1}$) and petroleum ether (>25µg $mL^{-1}$) extracts was comparable to Vitamin C (4.50µg $mL^{-1}$) [Table 01].

**FRAP assay:** *A. polystachya* bark extracts exhibited superior ferric reducing antioxidant power as

depicted in [Fig. 2], compared to that of vitamin C. The EC1 values indicated that the ferric reducing antioxidant potential of alcoholic extract was about 2 fold higher compared to vitamin C. The FRAP EC1 values were found to be 13.8, 18.6 and >25µg $mL^{-1}$ for alcoholic, aqueous methanolic extracts of *A. polystachya* and Vitamin C respectively whereas petroleum ether extract did not exhibited ferric reducing antioxidant power [Table 01].

The DPPH test provided information on the reactivity of test compounds with a stable free radical. Because of its odd electron, 2, 2-Diphenyl-Picryl Hydrazyl radical (DPPH) gives a strong absorption band at 517 nm in visible spectroscopy (deep violet colour). The efficacies of anti-oxidants are often associated with their ability to scavenge stable free radicals. In the present study, alcoholic, aqueous methanol and petroleum ether extracts exhibited >25µg $mL^{-1}$, 5.33µg $mL^{-1}$ and >25µg $mL^{-1}$ DPPH radical scavenging activity with IC50 values respectively in comparison to vitamin C (IC50 4.50µg $mL^{-1}$). FRAP assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. Electron donating anti-oxidants can be described as reductants and inactivation of oxidants by reductants can be described as redox reactions. Total antioxidant power may be referred analogously to total reducing power. In the current study methanol and aqueous methanol extracts of *A. polystachya* exhibited 13.8 and 18.6µg $mL^{-1}$ antioxidant power with EC1 values respectively whereas Vitamin C having EC1 >25µg $mL^{-1}$.

## CONCLUSION

In the present investigation, we evaluated comparative antioxidant activity of various *A. polystachya* extracts in comparison to Vitamin C. Alcohol and aqueous methanol extracts exhibited up to 3 fold better efficacy in various *in vitro* antioxidant assays. These antioxidant studies proved superior efficacy of *A. polystachya* extracts. It is a safe and effective intervention for free radical mediated diseases.

**Table 1**: Antioxidant Values of various extracts of *A. polystachya*.

| Plant Extracts | DPPH (µg/mL) | FRAP (µg/mL) |
|---|---|---|
| Alcoholic | >25.00 | 13.8 |
| Aq. MeOH | 05.33 | 18.6 |
| Pet. Ether | >25.00 | Nil |
| Vit-C | 04.50 | >25 |

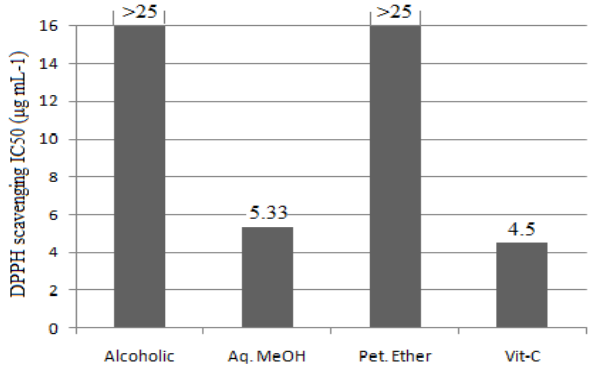


**Fig. 1:** DPPH free radical scavenging activity of *A. polystachya* extracts.

**Note:** Bar diagrammatic representations of *in vitro* DPPH radical scavenging activity. The bars represent *A. polystachya* bark alcoholic, aqueous methanol and pet. ether extracts and a positive control Vitamin C. Each bar represents 50% inhibitory concentration (IC50 in µg $mL^{-1}$)

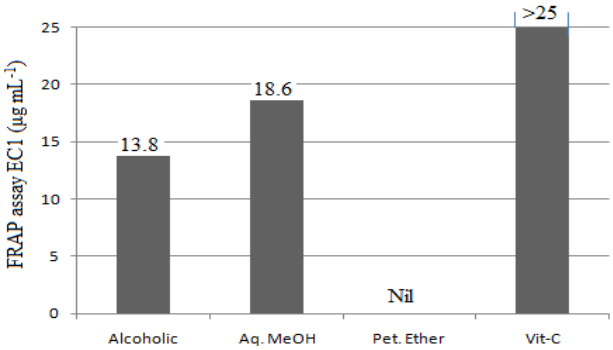


**Fig. 2:** Ferric reducing antioxidant power of *A. polystachya* extracts.

Note: Bar diagrammatic representations of *in vitro* Ferric Reducing Antioxidant Potential (FRAP). The bars represent *A. polystachya* bark methanol, aqueous methanol and water extracts and a positive

control Vitamin C. Each bar represents concentration of antioxidant required to reduce 1µM of Ferric ions (EC1 in µg $mL^{-1}$).

## REFERENCES


1) *Wealth of India, Raw material. CSIR, New Delhi 1973 (I).*
2) *Prajapati N, Purohit SS, Sharma AK Kumar T. A handbook of medicinal Plants, Agrobios (India), (2003).*
3) *Gole MK, Dasgupta S, Sur K, Ghosal J. Hepatoprotective effect of Amoora Rohituka. International Journal of Pharmacognosy 1997; 35(5): 318-322.*
4) *Choudhury R, Rashid RB. Effect of crude extract of Amoora rohituka stem bark on gastrointestinal transits in mice. Indian journal of pharmacology 2003; 35: 304-307.*
5) *Choudhury R, Hassan CM, Rashid MA. Guaiane sesquiterpenes from Amoora rohituka. Phytochemistry 2003; 62: 1213-1216.*
6) *Despande PJ, Gode JD, Prasad GC. Effect of Amoora rohituka on leucocytes in white rats. Journal of Research Medicine 1964; 2: 512-516.*
7) *Chatterjee A, Kundu AB. Isolation, structure and stereochemistry of Aphanmixis, A new triterpenes of Amoora Rohituka. Tetrahedron 1967; 26: 1471.*
8) *Agnihotri VK. Poriferasterol-3rhamnoside, a new saponine from the stem bark of Amoora rohituka. Indian Journal of Pharmaceutical Science 1987; 49(4):149-150.*
9) *Yadav R, Chauhan NS, Chouhan AS, Soni VK, Omray L. Antimicrobial screening of various extracts of Aphanmixis polystachya stems bark. International Journal of Advances in Pharmaceutical Sciences 2010; 1: 147-150.*
10) *Pandey G. Botanical identification and therapeutic efficacy of shwet Rohituka. Sachitra Ayurved, 1994; 46(9); 645-650.*
11) *Rabi T, Gupta RC. Antitumor and cytotoxic investigation of Amoora rohituka. International Journal of Pharmacognosy, 1995; 33(4):359-361.*
12) *Szabo MR, Iditoiu C¸ Chambre D, Lupea AX. Improved DPPH determination for antioxidant activity spectrophotometric assay. Chem. Pap., 2007; 61: 214-216.*
13) *Benzie IF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a measure of antioxidant power. The FRAP assay. Anal. Biochem., 1996; 239: 70-76.*