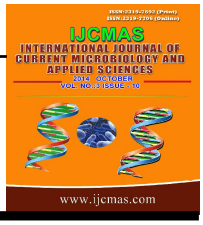
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 10 (2014) pp. 562-568**
http://www.ijcmas.com



Original Research Article

Evaluation of the Antioxidant Activities of the Seeds of *Holarrhena antidysenterica* grown in West Bengal, India

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Keywords

Holarrhena antidysenterica, free radicals, antioxidant, DPPH, tannins, polyphenols, flavonoids, ascorbic acid.

A B S T R A C T

Holarrhena antidysenterica plant is known in Ayurvedic medicine as a potent healer of several diseases from ancient times. The different parts of this plant have been used for various purposes. The present study has been carried out with the seeds of this plant. The water extract and ethanol extract were prepared with the seeds and the antioxidant and phytochemical activities were studied using suitable methods. The free radical scavenging activity was measured using DPPH method. The phytochemicals that were studied included total polyphenol, flavonoids, tannin and ascorbic acid. The ethanol extracts of the seeds were found to show higher results for all the parameters. The ascorbic acid contents were not very high in both the water and the ethanol extract. The seeds of *Holarrhena antidysenterica* have potent antioxidant properties and thus may be used for treatment of diabetes mellitus and several other diseases. Many studies have also been carried out to prove the ability of this plant in the treatment of such diseases.

Introduction

The *Holarrhena antidysenterica* plant is a deciduous lactiferous shrub or small tree 30-40 ft. high and upto 4 ft. in girth, with a clear bore of 10-20ft., occurring almost throughout the tropical and subtropical regions of the world. In India, this plant is found upto an altitude of 400 ft., often gregariously in deciduous forests and open waste lands. It is especially abundant in the sub-Himalayan tract. The bark of the plant is rather rough, pale brownish or greyish, peeling off in irregular flakes. The seeds are usually light brown in colour, 0.3-0.5 inch long (Ali Shah *et al.*, 2010).

The plant belongs to the family *Apocynaceae* and is known as a medicinal plant. During the past century, studies on the phytochemical and pharmacological nature of the plant have yielded important results regarding the chemical constituents present and have traditionally claimed properties associated with the plant viz. analgesic, antibacterial, anti-diarrhoeal, anti-amoebiasis, anti-inflammatory and anti-haemorrhoidal activities. Moreover recently it has been discovered that it also has anti-

malarial, anti-diabetic, antioxidant, anti-urolithic, anti-mutagenic, CNS-stimulating and other properties.

Very few reports of quantitative estimation of different antioxidant activities of the seeds, bark, flowers etc are available. Moreover there are variations in the antioxidant activities due to environmental (soil, climate) factors. This study was done to estimate the total antioxidant activity of the water extract and ethanol extract of the seeds of the plant and to also find out some of the phytochemical activities as well.

Materials and Methods

The seeds were collected from in and around Kolkata, West Bengal and the experiments were performed with these seeds in the months of March and April, 2013.

Chemicals

Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent, gallic acid, aluminium trichloride ($AlCl_3$), butylated hydroxytoluene (BHT), catechin and 2,6-dichloroindophenol (DCIP) were used. These were purchased from SRL India. Sulphuric acid (H_2SO_4), sodium nitrates ($NaNO_2$), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3) were purchased from Merck(India). Double distilled water was used for all the experiments.

Preparation of water and ethanol extracts from the seeds

The seeds were collected and water and ethanol extracts were prepared by mortar pestle using the respective solvents. The seeds were ground and water and ethanol extractions were done by shaking in a shaker for 24 hrs. The extracts were filtered through glass wool. This extraction process was repeated thrice. The collected filtrates were dried at room temperature and weighed by electronic balance, then diluted with distilled water to the desired concentration (10µg/ml). (Maneemegalai and Naveen, 2010). These extracts were stored in the refrigerator (4^0 C) for further use.

Antioxidant Measurement Assay Methods

All the experiments were performed in triplicate using the following procedures

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activities of extracts of the seeds were assessed by the method reported by Sasidharan *et al.* (2001) with some modifications. 0.002% DPPH was prepared in ethanol. 250µl of DPPH solution was mixed with 5µl of seed extracts (water and ethanol separately) and final volume of 1000µl was made up by adding ethanol. The mixtures were kept in the dark for 20 mins and the optical density (OD) was measured at 517 nm using Spectrophotometer (Systronics make, Model no 2202). 750µl of ethanol was used a control. The % of inhibition of DPPH activity was calculated (Chorage *et al.*, 2013) using the formula as given below:

$$\%\ of\ inhibition\ of\ DPPH\ activity: \frac{Absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control} x100$$

Total Phenolic Content Assay

The total phenolic content was measured using Folin-Ciocalteu's reagent based on procedures given by Singleton *et al.* (1999) with some modifications. 0.5 ml of sample was mixed with 1.5 ml (1:10 v/v diluted with distilled water). Folin-Ciocalteau's reagent and allowed to stand for 22^0C for 5 mins. Then 2ml of sodium carbonate

(Na_2CO_3, 7% w/v) was added and the mixture were allowed to stand for another 90 mins and kept in the dark with intermittent shaking. Then the absorbance of the blue colour that developed was measured at 725 nm using Spectrophotometer (Systronics make, Model no 2202). Gallic acid was used for constructing the standard curve (20-100µg/ml) and the total phenolic compound concentrations in the seed extracts (both water and ethanol extract) were expressed per gram of dry weight (mg GAE/g) of extract.

Total Flavonoid Content Assay

Total flavonoid content of the seed extracts were determined according to colorimetric method proposed by Zhishen *et al.* (1999), with some modifications. 0.5ml of the sample was mixed with 2ml of distilled water and 0.15 ml of sodium nitrite ($NaNO_2$ 5% w/v). This was allowed to stand for 6 mins and 0.15 ml aluminium trichloride ($AlCl_3$ 10% w/v) was added and allowed to stand again for 6 mins. This was followed by the addition of 2 ml of sodium hydroxide (NaOH 4%w/v). The final volume was made up to 5 ml by distilled water. The reaction mixture was mixed thoroughly and allowed to stand for another 15 mins. The absorbance of pink colour that developed was measured at 510nm using Spectrophotometer (Systronics make, Model 2202). Distilled water was used as the blank. The total flavonoid content was expressed in mg of catechin per gram of seed extract.

Tannin Assay

Content of tannin in the seed extracts was determined by Folin Denis method. (Polshettiwar and Ganjiwale, 2007). 1ml of sample or standard solution of tannic acid (5µg/ml-40µg/ml) was mixed with 0.25ml of Folin Denis reagent and 0.50ml saturated Na_2CO_3 solution was added to it. The volume was made up to 5ml with distilled water and absorbance was measured at 700nm after 30 min of incubation. The total tannic acid content was expressed as mg of tannic acid equivalent per gram of dry weight of the sample (Kalpana *et al.*, 2013).

Vitamin C Assay

Vitamin C of the seed extract was measured titrimetrically by 2,6-dichlorophenol (DCIP) solution. This solution was prepared by dissolving 52mg of 2,6-dichloroindophenol in about 500ml of water. 42 mg sodium bicarbonate ($NaHCO_3$) was then added and dissolved. The resulting solution was finally diluted to 1L with distilled water.

5ml of the sample or standard ascorbic acid solution was taken into 250 ml of Erlenmeyer flask. 2 ml of 3% metaphosphoric acid mixture and about 25 ml of distilled water were added to the flask and mixed. This mixture was then titrated with DCIP solution until a permanent (lasting more than 30 sec) light red or pink colour appeared. The volume of DCIP needed to oxidize the sample and standard ascorbic acid were correlated to find out the ascorbic acid content in the sample. The results were expressed in mg of ascorbic acid per gram of extract.

Statistical Analysis

All the analysis were carried out in triplicate and expressed as mean ±SD. Analyses of Variance were performed using one-way ANOVA. Significant differences between means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant.

Results and Discussion

In this study the antioxidant and some phytochemical activities of the water and ethanol extracts of the seeds of *Holarrhena antidysenterica* were studied and these have been given in Table 1.

The total scavenging activity of the seeds of Holerrhena antidysenterica is shown in Figure 1. The ethanol extract contains 87.32% and the water extract contains 83.01% of scavenging activity per gram of seed. Thus it is evident that the free radical scavenging action in the ethanol extract of the seeds is somewhat higher as compared to the water extract. A similar study was carried out by Pathak *et al.* (2011).

The total polyphenol contents of the water extract and ethanol extract of the sseds are respectively 1.32mg/g and 5.24mg/g as shown in Figure 2. From this it is evident that the total polyphenol content is significantly higher in case of the ethanol extract as compared to the water extract of the seeds. The total polyphenol of the bark and seeds of the same plant was evaluated by Pandey *et al.* (2011)

Flavonoids are important plant pigments which are now being associated with antioxidant activities. The flavonoid content of the water extract was found to be only 1.21mg/g as compared to 1.80mg/g in the ethanol extract. So it is evident that the ethanol extract of the seed contains a higher amount of the pigment and thus has greater antioxidant activity. This has been shown in Figure 3. A similar study was carried out by Ali *et al.* (2009)

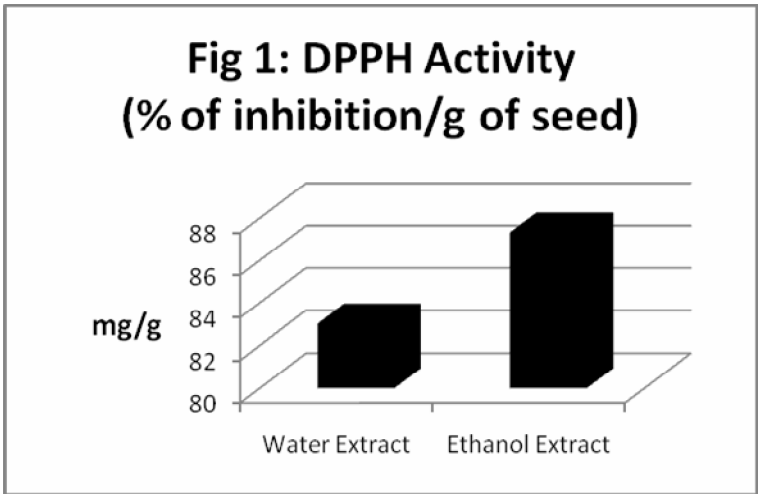
The tannin content also shows a similar trend as that of the other parameters i.e, the ethanol extract is found to show higher results as compared to the water extract. The water extract contains 0.52mg/g of tannin as compared to 0.941mg/g of tannin in the ethanol extract as shown in Figure 4. The evaluation of the tannin content was also done by Ali *et al.* (2011)

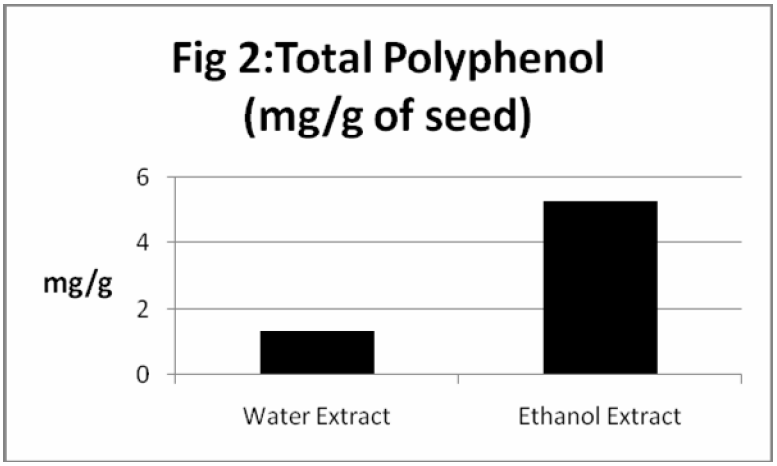
Total ascorbic acid or vitamin C content is not very high in both the extracts. The water extract contains 0.113mg/g whereas the ethanol extract contains 0.150mg/g of ascorbic acid. Thus from the study it is evident that the seeds of the plant are not very rich in the vitamin C content. The comparison has been shown in Figure 5.

Several studies have been conducted with different parts of *Holarrhena antidysenterica.* The seeds of the plant are believed to possess anti-diarrhoea and anti-dysentery properties (Dwivedi and Tripathi, 1991). Moreover the seeds of the plant have been found to possess anti-diabetic property, proved in a number of studies especially in rats. The antioxidant property of the seeds have also been studied under different conditions. The in vitro antioxidant activity of the ethanolic extract of the leaves of the plant was studied extensively (Ganapthy *et al.,* 2011). The antihyperglycaemic and antihyperlipidaemic properties have also been observed in rats by the administration of the methanolic extract of the bark of this plant.

Table.1 Antioxidant and Phytochemical Activity analysis of water and ethanol extract of the seeds of *Holarrhena antidysenterica*

Antioxidant properties	**DPPH Activity (% of inhibition/g of seed ±SD)**	**Total Polyphenol (mg/g of seed ±SD)**	**Flavonoid Content (mg/g of seed ±SD)**	**Tannin Content (mg/g of seed ±SD)**	**Ascorbic acid (mg/g of seed ± SD)**
Water Extract	83.01±0.015	1.32±0.02	1.21±0.015	0.52±0.01	0.113±0.005
Ethanol Extract	87.32±0.02	5.24±0.05	1.80±0.005	0.941±0.001	0.15±0.005





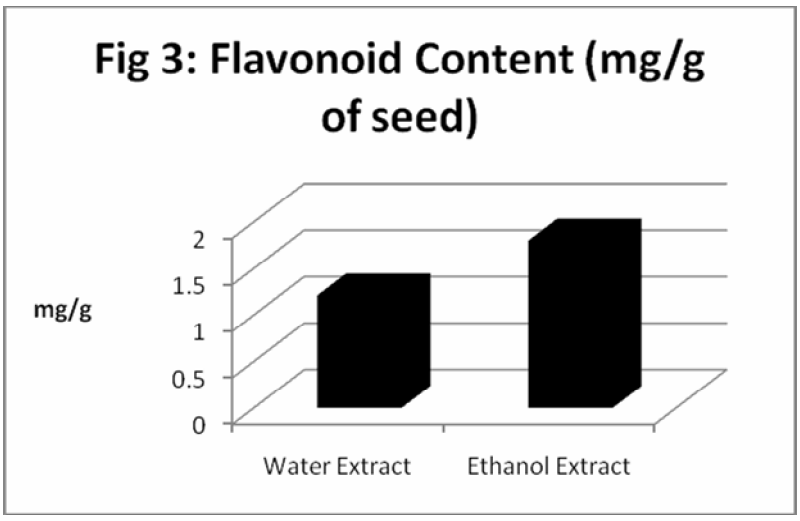


Fig 3: Flavonoid Content (mg/g of seed)
mg/g
2
1.5
1
0.5
0
Water Extract
Ethanol Extract

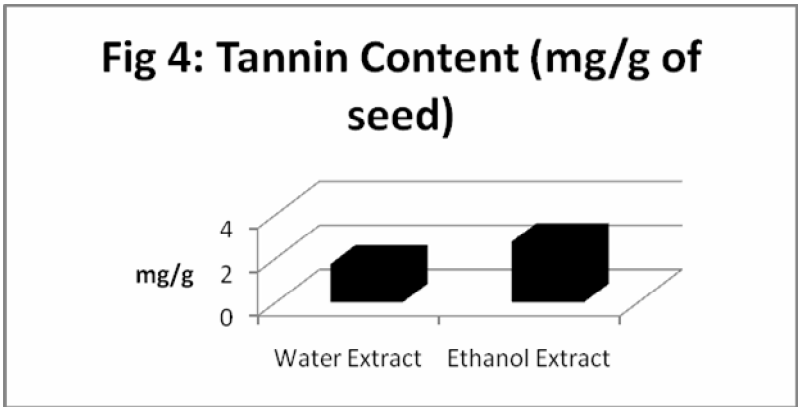


Fig 4: Tannin Content (mg/g of seed)
mg/g
4
2
0
Water Extract
Ethanol Extract

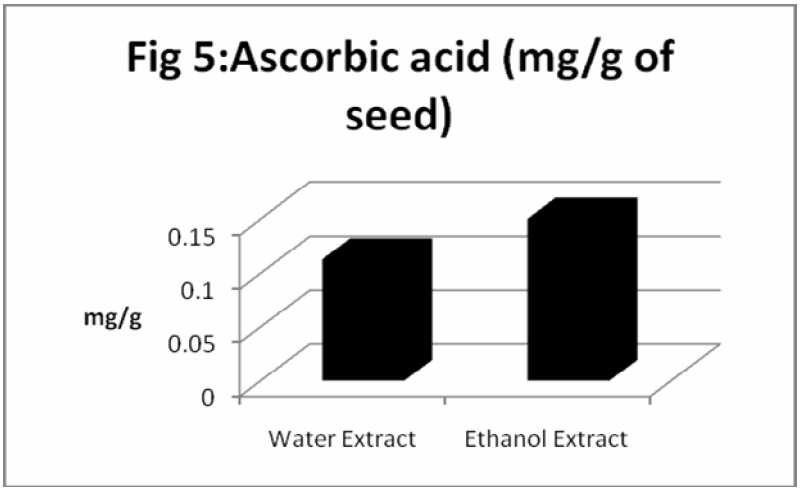


Fig 5:Ascorbic acid (mg/g of seed)
mg/g
0.15
0.1
0.05
0
Water Extract
Ethanol Extract

Acknowledgements

The author is thankful to the Dept. of Food & Nutrition of the University of Calcutta where the research was carried out.

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