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Research Article

PHYTOCHEMICAL SCREENING AND EVALUATION OF *IN-VITRO* ANTIOXIDANT ACTIVITY, TOTAL PHENOLICS AND TOTAL FLAVONOIDS OF HOLARRHENA ANTIDYSENTRICA LEAF EXTRACTS

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ABSTRACT

Preliminary phytochemical screening was carried out for pet ether, chloroform and ethanol extracts of *Holarrhena antidysentrica* leaves. The extracts showed the presence of various phytocompounds like alkaloids and flavonoids**.** In vitro antioxidant activity was carried out with all the three extracts using DPPH assay using ascorbic acid as a standard. All the extracts had shown significant antioxidant activity. The quantitative estimations showed that ethanolic extract contains higher total phenolic and flavonoid contents than the other extracts.

Keywords: *Holarrhena antidysentrica*, antioxidant, DPPH, phenolics, flavonoids.

INTRODUCTION

In living systems, free radicals are generated as part of the body's normal metabolic process and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias.

Plants are potent biochemical factories and have been components of phytomedicine since times immemorial. The plant based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant[1]. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, betacarotene and tocopherol are known to possess antioxidant potential. The relative antioxidant activity of extracts of some selected Iranian medicinal plant species was recorded.

With this background and abundant source of unique active components harbored in plants, the present study was taken up on the medicinal plant namely *Holarrhena antidysentrica* belongs to the family Apocyanaceae[2-9]**.** *Holarrhena* is a genus of trees or shrubs distributed throughout the tropical and subtropical regions of the world. It is a small deciduous tree with cream white flowers and found throughout the dry forests of India even as far as Travancore. The plant *Holarrhena antidysentrica* is a tall shrub or small tree, evergreen in nature. Flowers are cream coloured, fragrant and borne in bunches. The

fruits are thin and cylindrical, with two follicles attached together at distal ends. The different parts of the plant were used since antiquity in the indigenous system of medicine. It is one of the best drugs for diarrhoea. It is prescribed along with isabgol and castor oil to treat chronic diarrhoea and also to check blood coming from stool. According to Ayurveda, the bark is useful in treatment of piles, skin diseases and biliousness[10, 11]. Recent studies have shown that a number of plant products including polyphenols, terpenes and various plant extracts exerted an antioxidant action[12-14]. Considerable amount of data have been generated on antioxidant properties of food plants around the globe[15,16]. However, traditionally used medicinal plants await such screening. The literature survey showed scanty information available on the investigation of these activities so far. Thus the objective of this study is to investigate the antioxidant capacity and also to estimate the total phenolic and flavonoid content of the various extracts of the plant, which perhaps attribute for this activity.

MATERIALS AND METHODS

Plant materials

The fresh leaves of *H. antidysentrica* were collected from surrounding places of Shimoga, Karnataka. The plant material was authenticated by Prof. Pushpalatha, Retd, Dept. of Botany, Sahyadri Science College (Autonomous), Shimoga.

Preparation of extracts

Fresh leaves were washed, shade dried, powdered mechanically and subjected to soxhelt extraction using petroleum ether (60-80^{0}C), chloroform and ethanol in successive mode respectively for 48 h. The solvent was then recovered using Rotary Vacuum Evaporator and the concentrated extract was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation for potential antioxidant property and assay of phenolic and flavanoid contents.

Phytochemical screening

The extracts of the dry powdered leaves of *H.* antidysentrica were analyzed for the presence of various phytoconstituents using standard phytochemical procedures[17].

EXPERIMENTAL PROCEDURE

In-vitro Antioxidant assay

The antioxidant activity of plant extracts were determined by the *in vitro* method, the DPPH (1.1-diphenyl-2-picryl-hydrazyl) free radical scavenging assay.

DPPH Radical Scavenging Activity[18-20]

The free radical scavenging capacity of the crude extracts of *H. antidysentrica* was determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% methanol. The crude extracts of *H. antidysentrica* were mixed with 95% ethanol to prepare stock solution (1 mg/ml i.e. 1000 µg/ml concentration). From the stock solutions of pet ether, chloroform and ethanol extracts, 100 µl – 250 µl was taken in the test tubes respectively. The final volume was made up to 1 ml in all the tubes by the addition of ethanol. Then 3 ml of freshly prepared DPPH solution was added to all and were kept for 30 min incubation in the dark and absorbance was measured at 517 nm using UV- visible spectrophotometer (Shimadzu UV-1609, Japan). A stock solution of Ascorbic acid at the concentration 1000 µg/ml was prepared in distilled water. From this stock different concentrations ranging from 10-100µl was prepared and used as the reference standard. The solution containing only methanol and DPPH served as the blank.

% scavenging of the DPPH free radical was measured using the following equation:

% Inhibition = [Absorbance of control – Absorbance of test sample / Absorbance of Control] × 100

Total flavanoid determination

Total flavonoid content was determined by Aluminium chloride method[21] using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). 5 min after adding 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically (Shimadzu UV-1609, Japan). Results were expressed as catechin equivalents (mg catechin/g dried extract).

Total phenols determination

The total phenolics content in the water extract was determined with the Folin- Ciocalteu's reagent (FCR)[22]. In the procedure, different concentrations of the extracts were mixed with 0.4 ml FCR (diluted 1:10 v / v). After 5 min 4 ml of sodium carbonate solution was added. The final volume of the tubes were made upto 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer (Shimadzu UV-1609, Japan). A calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight.

STATISTICAL ANALYSIS

IC_{50} values were calculated by linear regression. Means ± SD were calculated. The data were analyzed for statistical significance using one way ANOVA followed by Tukey post test. P values less than 0.05 were considered significant.

RESULTS

Phytochemical screening of the extracts

The preliminary phytochemical screening had shown the presence of alkaloids, flavonoids, tannins & phenolic in the leaves extracts. The summary of the results is presented in the table 1.

***In-vitro* Antioxidant activity**

DPPH scavenging activity

The two extracts namely, chloroform and ethanolic had shown better free radical scavenging effect of DPPH than pet ether in a concentration dependant manner, however they are less in comparison to the standard ascorbic acid. The IC_{50} values were found to be 26 ± 0.03, 10 ± 0.04, 9 ± 0.05 and 8 ± 0.03 for ascorbic acid and the extracts of pet ether, chloroform and ethanol respectively. The comparision of IC_{50} values of the standard Ascorbic acid and the different leaf extracts of *Holarrhena antidysentrica* was graphically represented in the graph 1 and 2.

Total flavonoid contents of leaf extracts

The flavonoid content of the extracts was expressed in terms of catechin equivalent. The ethanolic and chloroform extracts of *H. antidysentrica* showed significant flavonoid content than the pet ether extract.

Total phenolic contents of leaf extracts

Total phenols were measured by Folin Ciocalteu reagent in terms of catechol equivalent in the extracts. The chloroform and ethanolic extracts showed comparatively good phenolic content in a dose dependent manner.

DISCUSSION

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins & phenolics in the leaves of *H. antidysentrica*. Although their specific roles were not investigated in this study, it has been reported that most active principles in plants are frequently alkaloids, flavonoids and phenols and these may be responsible for many of the pharmacological actions of the plant.

The free radical scavenging activity of the extracts was evaluated based on the ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet colour). As the electron became paired of in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stoichiometrically coincides with respect to the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the test drugs to scavenge free radicals independently. In the present study, the pet ether extract of *Holarrhena antidysentrica*, showed higher IC_{50} value as it had weaker DPPH scavenging activity. However, the chloroform and ethanol extracts showed less IC_{50} value comparable to the standard which had very less IC_{50} value.

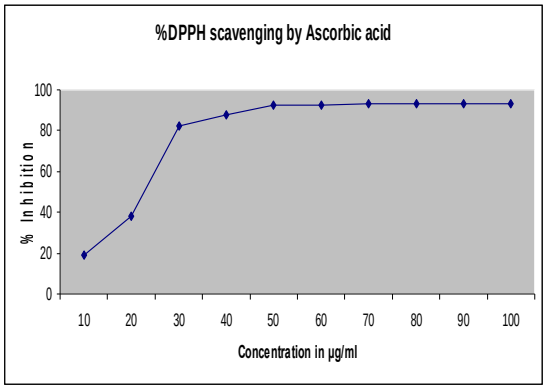
The chloroform and ethanolic extract of *Holarrhena antidysentrica*, which contain significant amount of flavonoid and phenolic compounds compared to the standards exhibited the significant antioxidant activity. These differences might be due to their different antioxidant mechanisms or variations in their ability to scavenge free radicals. All of the extracts in this work exhibited different extent of antioxidant activity.

Table 1: Result of phytochemical screening of leaves extract of *Holarrhena antidysentrica*

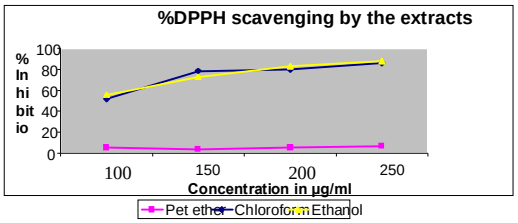
Tests	Pet ether extract	Chloroform extract	Ethanol extract
Tests for alkaloids:			
i. Mayer's test	-	++	+++
ii. Dragendorff's test	-	++	+++
Tests for flavonoids:			
i. NaOH solution test	+	++	++
ii. Shinoda test	+	++	++
Tests for steroids:			
i. Salkowski's test	++	-	++
ii. Libermann-Burchard's test	++	-	+
Tests for tannins & phenolics			
i. lead acetate test	-	+	++
ii. dilute HNO_3 test	-	+	++
Test for saponins			
i. Foam test	-	-	-
Tests for proteins & amino acids			
i. Biuret test	-	-	-
ii. Xanthoproteic test	-	-	-

- : absent; + : not significant; ++ : significant; +++ : very significant

Graph 1: %DPPH scavenging by the standard Ascorbic acid



Graph 2: %DPPH radical scavenging by Pet ether, Chloroform and Ethanol extracts



Graph 3: Total flavonoid content of the standard – Catechin

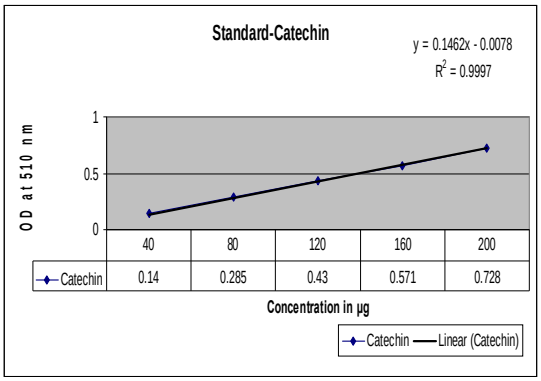


Table 2: Total flavonoid contents present in the extracts are expressed in mg of catechin equivalent/100 mg dry wt of the respective extract

Extracts	mg of Catechin equivalents/100mg dried extract
Pet ether extract	4.6
Chloroform extract	11.3
Ethanol extract	8.6

Graph 4: Total Phenolic content of the standard - Catechol

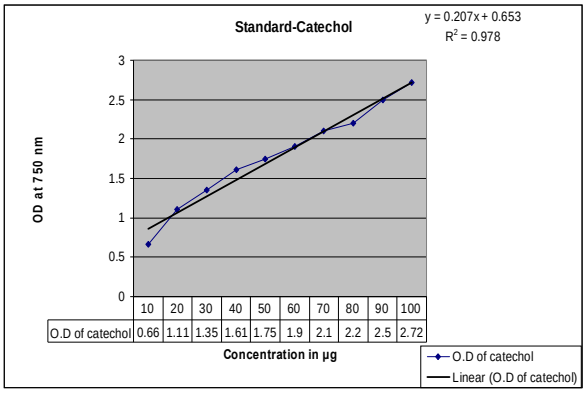


Table 3: Total phenolic contents present in the extracts are expressed in mg of catechol equivalent/100 mg dry wt of the respective extract

Extracts	mg of Catechol equivalents/100mg dried extract
Pet ether extract	2.15
Chloroform extract	2.3
Ethanol extract	4.5

CONCLUSION

In our present study we conclude that the chloroform and ethanol extracts of *Holarrhena antidysentrica* has potent antioxidant property than the pet ether extract. This could be attributed to the presence of various phytoconstituents viz., alkaloids, flavonoids, tannins and phenols in the respective extracts.

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