Research Article

Estimation of *In-vitro* Antioxidant activity and Total Phenolic, Flavonoid Content of Selected Medicinal Plants

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ABSTRACT

The nature has provided abundant sources for all the living creatures, which possess medicinal virtues. Natural products have been the basis for the treatment of human diseases. In many developing countries of the world, plant based drugs are used for primary healthcare. The main aim of present study is to open new avenues for the improvement of medicinal uses of some desired plants for the selected area for antioxidant activity. For this assessment dried methanol extract of those plants were subjected to free radical scavenging activity by using ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using 2,2diphenyl - 1-picryl hydrazyl (DPPH) free radical. The results revealed that leaf extracts of *Artabotrys hexapetalus*, *Oncoba spinosa* and *Cascabela thevetia* showed 83.17%, 63.46% and52.99% DPPH scavenging activity respectively.

Keywords: Natural product, scavenging activity, antioxidants, ascorbic acid.

INTRODUCTION

In present days a large number of world populations (nearly 80% people) cannot afford the products of the western pharmaceutical industries and have to relay up on the use of traditional medicines, which are mainly derived from plant material. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future. There are several medicinal plants with wide range of pharmacological, biological and very interesting phytochemical constituents. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although plants had been praised for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Antioxidants are loyal protectors and nurturers of our cells, repelling disease and promoting good health. Antioxidants can come from healthy eating or in the form of supplements and they include a family of naturally formed components like vitamin A, beta-carotene, lycopene, vitamin E and more. They are believed to protect cells from free radicals &harmful oxygen molecules. Free radicals are formed naturally in the body, but their production is increased by factors such as smoking, alcohol, air pollution, infection, stress, excessive sunlight and toxins like radiation and asbestos. Antioxidants act as defense mechanism that protects against oxidative damage and includes compounds to remove or repair the damaged molecules. It can prevent / retard the oxidation caused by free radicals and sufficient intake of antioxidants protects us fromdiseases.[1] Free radical reactions are important factors in the progression of chronic diseases such as cancers, hypertension, cardiac infarction, and atherosclerosis, as well as in rheumatism and cataracts.[2]These reactions are essential for several metabolic processes and could be in many cases harmful to health as well. It is also known that free radicals such as reactive oxygen species (ROS) e.g. superoxide anions, hydroxyl radicals, and hydrogen peroxide play an important role in the development of tissue damage in living organisms. Moreover, growing evidence relates the occurrence of cancer to the oxidative damage to DNA, proteins and lipid in the body caused by radicals and other carcinogens.[3] Many synthetic drugs protect against oxidative damage, but these drugs have adverse side effects.[4] Medicinal plants are rarely used as 'antioxidants' in traditional medicine, their claimed therapeutic properties could be due, in part, to their capacity for scavenging oxygen free radicals which may be involved in many diseases. Recently, many natural compounds with antioxidant and antimicrobial properties have been isolated from different plant materials.[5] Thus; the interest in natural antioxidants is on the increase, particularly from medicinal and dietary plants, which possess preventative oxidative damage capacity.

Natural compounds include Flavonoids and Phenols which are the most important groups of secondary metabolites and bio active compounds in plants and good sources of natural antioxidants in human diets.[6] They are also a kind of natural product and antioxidant substance capable of scavenging free superoxide radicals, reducing the risk of cancer and protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA.[7] The plants and herbs consumed by humans may contain different phenol and flavonoid components and they function as reducing agents and free radical scavengers so it will be necessary to examine the possible role of Flavonoids and Phenols in disease prevention especially in the current antioxidant activity.

Our previous investigations on these plants has evaluated the existence of several numbers of phyto-chemical compounds and also their antimicrobial activity.[8, 9] Plants with phenol& flavonoid compounds were further studied for total phenol& flavonoid contents and antioxidant activity.

METHODOLOGY

Plant material

Cascabela thevetia

Cascabela thevetia (L.) is a small evergreen plant species of shrub or small tree belonging to the family Apocynaceae. Common names are Be-still tree, Captain Cook tree, dicky plant, foreigner's tree, lucky nut, Mexican oleander, still tree, yellow oleander, Currant-tree. It is a large spreading shrub usually 2.5-3.5m tall. Leaves are spirally arranged.

Artabotrys hexapetalus

Artabotrys hexapetalus (Linn.f) bhandari belongs to family Annonaceae. It is globally distributed in India, Srilanka, Java and South china. Within India, it is indigenous to south India and very commonly cultivated in gardens throughout the country for its fragrant flowers. It is a climbing tree of 10m tall.

Oncoba spinosa

Oncoba spinosa belongs to family Salicaceae. It is a small to medium sized tree that has simple leaves usually not more than 5m height. The tree is widely distributed along the eastern side of Africa as far as South Africa, mainly in dry woodland or open savanna in a wide range of sites from river valleys to rocky hills. Its northernmost limits are reached on the eastern side of the Red sea in Arabia.

These three medicinal plants were collected from various areas of Guntur and Krishna districts of Andhra Pradesh, India. These plants were identified according to various literatures, including other pertinent taxonomic literature. Plant parts were collected on the basis of the information provided in the ethno botanical survey of India.

Chemicals and Reagents

DPPH (2,2-diphenyl-1- picryl hydrazyl radical), Ascorbic acid, Folin-Ciocalteu reagent, Gallic acid, Sodium carbonate, $AlCl_3$, Potassium acetate, $NaNO_2$, NaOH, Rutin solution and Methanol.

Preparation of extracts

The fresh and healthy leaves were collected and washed thoroughly 2-3 times with running tap water then leaf material was shade dried. After shade drying, the leaf material was grinded or pulverized and the powder was kept in small plastic bags with paper labeling.

Cold Extract

Methanol, Chloroform, Acetone, Hexane and Water extract were prepared by soaking 1gms of the dry powered plant material in 10 ml of respective solvent at room temperature for 48hours and filtered through a Whatmman filter Paper No: 1. The filtrate was stored in 4°C until further use.

Hot extracts

The grinded leaf material of 5gm was extracted with 900 ml of methanol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatmman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The extract was preserved at 2- 4°C. This crude extract of methanol was used for further biological studies.

Antioxidant activity

Determination of antioxidant activity by DPPH assay[10]

Principle

The DPPH assay is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl–picrylhydrazine.

1, 1- Diphenyl-2-picrylhydrazyl (DPPH, 0.004%) solution

4 mg of DPPH was dissolved in 100 ml of methanol and kept it overnight in dark place for the generation of DPPH radical.

In order to measure antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was used. This assay measures the free radical scavenging capacity of the investigated extracts. DPPH is a molecule containing a stable free radical. An aliquot of 3 ml of 0.004% DPPH solution in methanol and 0.1 ml of plant extract/ascorbic acid in DMSO at various concentrations (100, 200,300,400 and 500 µg/ ml) were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the

absorbance at 517 nm. Ascorbic acid was used as a positive control. The radical scavenging activity was calculated from the equation:

%radical scavenging activity $=[(A_0 - As) / A_0] \times 100$,

Where A_0 is the absorbance of the control and as is the absorbance of the plant sample.

Estimation of total phenol content by *Folin ciocalteu* method

Phenol constituents are very important in plants because of their scavenging ability due to their hydroxyl groups and also regarded as molecules with the highest potential to neutralize free radicals. A number of studies are focused on the biological activity of phenol compounds, which are potential antioxidants and free radical scavengers. In addition it has been reported that phenol compounds are associated with antioxidant activity. The content of total phenols in the extracts of *Cascabela thevetia, Artabotrys hexapetalus* and *Oncoba spinosa* were determined by using the Folin–Ciocalteu assay.[11] In this assay plant extracts of different concentrations from 100µl - 500µl were prepared and final volume of each extract was made up to 500µl with distilled water. Now add 0.1ml of Folin- ciocalteu reagent and 2.5ml of saturated sodium carbonate to each extract. The mixture was allowed to incubate for 2hours and absorbance was measured at 760nm. Gallic acid was used as standard and the total phenol values were expressed in terms of Gallic acid equivalent (mg/g of extracted compounds).

Estimation of Phenolics =Test O.D / standard O.D x standard concentration

Estimation of total flavonoid content by Aluminum chloride colorimetric method[12]

The total flavonoid content was measured with the Aluminum chloride colorimetric assay.

Principle

The principle involved in Aluminum chloride ($AlCl_3$) colorimetric method is that $AlCl_3$ forms acid stable complexes with the C-4 keto groups and either the C-3 orC-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids of or building the calibration curve, rutin is used as a standard material. Various concentrations of standard rutin solution were used for standard calibration curve.

Stock Solution of Extracts

100 mg of the each extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol.

Preparation of Test Solutions

0.5ml of each extract stock solution, 1.5 ml methanol, 0.1ml aluminum chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminum chloride with distilled water. Sample and sample blank of all three extracts were prepared and their absorbance was measured at 506 nm. All prepared solutions were filtered through whatmann filter paper before measuring.

Procedure:

The total flavonoid content was determined by the aluminum chloride calorimetric assay. In a test tube, 0.3ml of extracts, 3.4ml of 30% methanol, 0.15ml of $NaNO_2$ (0.5M) and 0.15ml of $AlCl_3.6H_2O$(0.3M) were mixed. 1ml of NaOH was added after 5min. The absorbance was taken at 506nm against the blank. The standard curve with the reference to rutin standard solution was made. The total flavonoid content was expressed with the rutin equivalents per gm of dried fraction.

RESULTS

DPPH free radical scavenging assay

The free radical scavenging activity of methanol extract of following plants *Cascabela thevetia*, *Artabotrys hexapetalus* & *Oncoba spinosa* leaves were studied and their IC_{50}values were calculated. These extracts has ability to reduce the DPPH, a stable free radical and any molecule that can donate an electron or hydrogen to DPPH, can react with it and there by bleach the DPPH absorption. DPPH is purple color dye having absorption maxima of 517nm and on reaction with a hydrogen donor the purple color fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease of absorbance. The results revealed that the methanolic extracts of *Cascabela thevetia, Artabotrys hexapetalus, and Oncoba spinosa* were found to possess concentration dependent scavenging activity on DPPH radicals. The IC_{50} value of ascorbic acid was found to be 107.02µg. The IC_{50} values for DPPH radical of methanolic extract of *Cascabela thevetia, Artabotrys hexapetalus, and Oncoba spinosa* were found to be 416.54µg, 57.30µg and 246.28 µg respectively. Among them highest free radical activity was found in methanol extract of *Artabotrys hexapetalus* than other plant extracts. The order of the scavenging activity for DPPH radical is in the following manner *Artabotrys hexapetalus>Oncoba spinosa>Cascabela thevetia.*

Estimation of total phenolic and Flavonoid contents

Total phenol and flavonoid content was calculated by folin ciocalteau method and aluminium chloride method respectively. Total phenol content in *Cascabela thevetia* is 126.595µg GAE/µg and flavonoid content is 90.107µg Rutin/µg. Total phenol content in *Artabotrys hexapetalus* is 123.138µg GAE/µg and flavonoid content is 279.640µg Rutin/µg. Total phenol content in *Oncoba spinosa* is 441.932µgGAE/µg and flavonoid content is 237.450µg Rutin/µg. The total phenolic content for the methanolic extract of three plants is in the following order *Oncoba*

spinosa >Cascabela thevetia>Artabotrys hexapetalus. The Total flavonoid content for the methanolic extract of three plants isin the following order *Artabotrys hexapetalus* > *Oncoba spinosa* >*Cascabela thevetia.*

Table 1: Antioxidant activity of *Cascabela thevetia*

Concentration(µg/ml)	% of inhibition	IC_{50}	Standard % of inhibition (Ascorbic acid)	IC_{50}
100	32.96±0.01	416.54	50.67 ±0.015	107.02
200	38.52±0.02		53.25±0.02	
300	44.76±0.02		79.07±0.015	
400	50.48±0.01		83.51±0.01	
500	52.99±0.02		91.26±0.01	

Table 2: Antioxidant activity of *Artabotrys hexapetalus*

Concentration(µg/ml)	% of inhibition	IC_{50}	Standard % of inhibition (Ascorbic acid)	IC_{50}
100	54.96±0.015	57.30	50.67 ±0.015	107.02
200	60.90±0.152		53.25±0.02	
300	71.90±0.01		79.07±0.015	
400	82.33±0.01		83.51±0.01	
500	89.17±0.01		91.26±0.01	

Table 3: Antioxidant activity of *Oncoba spinosa*

Concentration(µg/ml)	% of inhibition	IC_{50}	Standard % of inhibition (Ascorbic acid)	IC_{50}
100	36.8±0.1	246.28	50.67 ±0.015	107.02
200	51.78±0.02		53.25±0.02	
300	54.8±0.01		79.07±0.015	
400	60.88±0.015		83.51±0.01	
500	63.46±0.01		91.26±0.01	

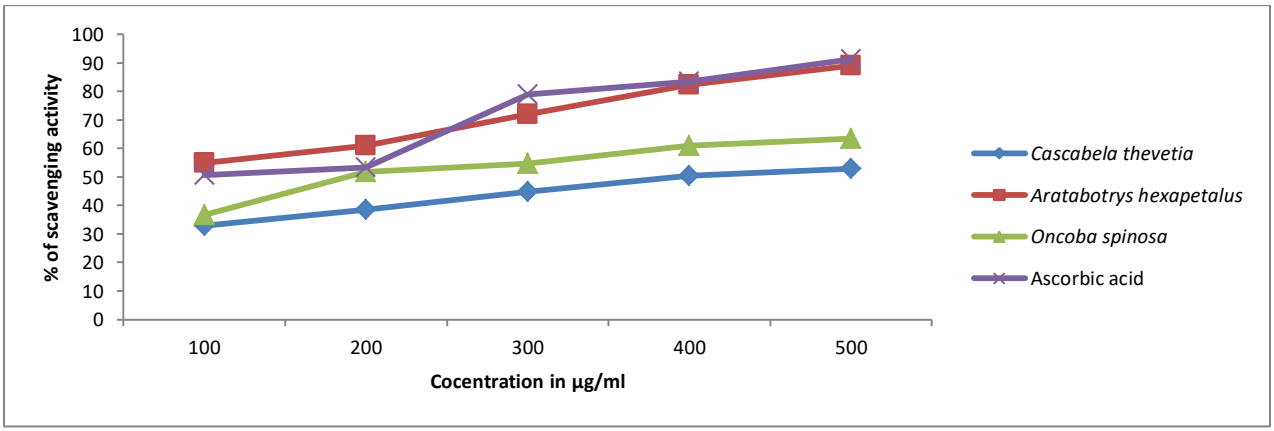

Figure 1: Graphical representation of % of scavenging activity of three plant extracts

Table 5: Estimation of total phenolic content by FolinCi-ocalteu method

S.NO	Concentration in µg/ml	% of Total phenolic contentµg GAE/µg		
		Cascabela thevetia	*Artabotrys hexapetalus*	*Oncoba spinosa*
1	100	6.189	12.056	36.993
2	200	17.257	38.514	114.9905
3	300	110.6088	75.855	186.752
4	400	169.953	136.013	265.217
5	500	126.595	123.138	441.932

Graphical representation for estimation of total phenolic content by FolinCiocalteu method

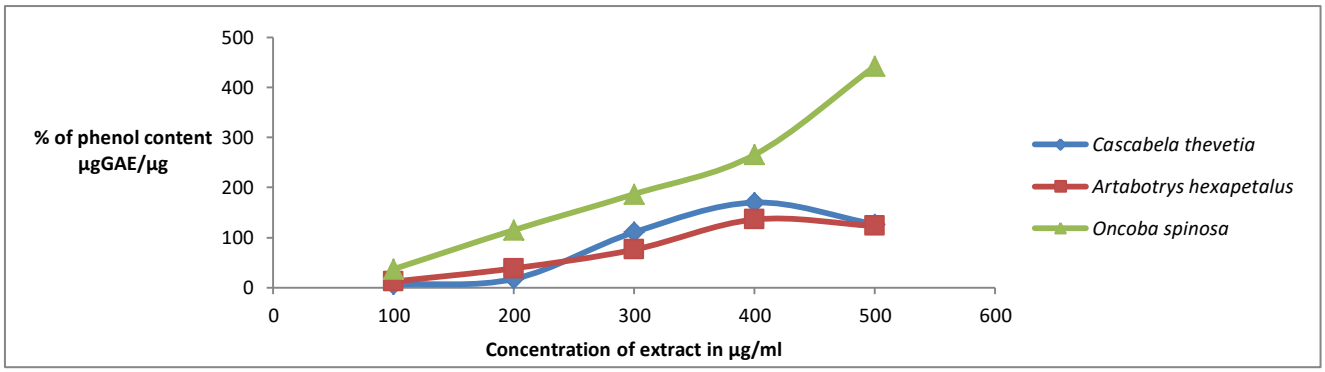

Table 6: Estimation of total flavonoid content by Aluminium Chloride Colorimetric Method

S.NO	Concentration in µg/ml	% of Flavonoid content µg Rutin/µg/mL		
		Cascabela thevetia	*Artabotrys hexapetalus*	*Oncoba spinosa*
1	100	4.423	30.134	20.345
2	200	23.517	120.526	50.416
3	300	40.374	171.632	147.809
4	400	70.265	226.423	184.732
5	500	90.107	279.640	237.450

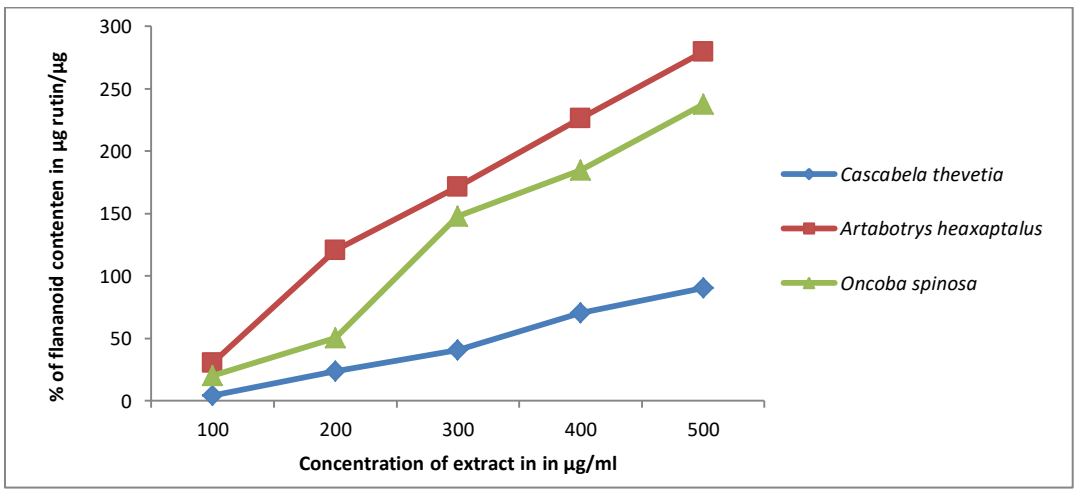

Figure 2: Graphical representation of estimation of flavonoid contentby Aluminium Chloride Colorimetric Method

DISCUSSION

Many medicinal plants have been analyzed and their DPPH Scavenging activity was reported. The DPPH scavenging ability of medicinal plants has been attributed to several components including Phenols[13] and Flavonoids[14]. In the present study attempts have been made to assess the antioxidant potential of methanolic leaf extract of desired medicinal plants by using DPPH assay; estimation of total phenolic and flavonoid content by Folin–Ciocalteu assay and Aluminium chloride ($AlCl_3$) colorimetric method respectively. Our investigations revealed that antioxidant activity of *Artabotrys hexapetalus, Oncoba spinosa* and *Cascabela thevetia* showed appreciable activity comparable to standard ascorbic acid. In present investigation *Artabotrys hexapetalus* was found to have maximum radical scavenging activity followed by *Oncoba spinosa* showing moderate activity and *Cascabela thevetia* exhibited lowest activity.

CONCLUSION

Preliminary phytochemical studies of these plants showed the existence of several numbers of phyto-chemical compounds including phenolics and flavonoids. Therefore the antioxidant activity might be due to the presence of flavonoids and phenolic compounds. Further we need to study the detailed chemical composition of the plant extracts and isolate the active constituents and formulate as biological antioxidants. The findings of this study support this view that some medicinal plants are promising sources of potential antioxidant and may be efficient as preventive medication sources in many diseases.

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