

Antioxidant Activity of Some Selected Medicinal Plants in Southern Region of India.

V Rathabai, and C Baskaran*

PG and Research Department of Zoology, Presidency College, Chennai-600005, Tamil Nadu, India.

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*For Correspondence

PG and Research Department of
Zoology, Presidency College,
Chennai-600005, Tamil Nadu,
India.

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ABSTRACT

The present study was undertaken to find the antioxidant value of certain medicinal plants in Tamilnadu region. Antioxidants have been reported to prevent oxidative damage caused by free radical and can be used in cardiovascular, anti-inflammatory, diabetes and cancer diseases. The amount of total phenols, flavonoids and radical scavenging activity has been studied. Major amount of phenols were determined in *Corchorusaestuans* followed by root of *Coleus Forskohlii*. Moreover, maximum flavonoid content was found to be present in the *Corchorusaestuans* followed by *Coleus Forskohlii*. However, high radical scavenging activity was observed in *Corchorusaestuans* followed by leaf of *Carica papaya* and *Coleus Forskohlii*.

INTRODUCTION

In response to the increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation [1]. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity, including *Ocimum sanctum*, *Piper cubeba* Linn, *Allium sativum* Linn, *Terminalia bellerica*, *Camellia sinensis* Linn., *Zingiber officinale* Roscoe and several Indian and Chinese plants. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins [2]. As antioxidants have been reported to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers [3]. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [4]. Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective [5]. They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions.

Coleus forskohlii (Family: Lamiaceae) is a perennial herb belongs to the part of the mint family of plants and has long been cultivated in India, Thailand and parts of South east Asia as a spice and as a condiment for heart ailments and stomach cramps. Mainly it is used in the treatment of eczema and psoriasis. The roots of the plant are a natural source of forskolin – the only plant-derived compound presently known to directly stimulate the enzyme adenylate cyclase, and subsequently cyclic AMP. Many other diterpenoids as deacetyl forskolin, 9-deoxyforskolin, 1, 9-deoxyforskolin, 1, 9-dideoxy-7-deacetylforskolin have been isolated. Other minor phytochemicals are Allylroyleanone, Barbatusin, Plectrin, Plectirion A, Acetoxycoleosol, Coleol, Coleonone, Coleosol, Deoxycoleonol, Crocetindialehyde, Naphopyrones [6]. *Carica papaya* and *P. nigrescens* leaf extracts may have anti-inflammatory property as a result of the phytochemicals that can exert that property possibly contained in the extracts, which may assist in relieving the pains associated with sickle-cell crisis and also may prevent opportunistic infections in sickle cell disease.

The study done on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems [7]. On continuation of our experimental work for the search of antioxidant activity of medicinal plants, we studied extracts of six medicinal plants. The free radical scavenging activity against 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was

evaluated during the course of work. The ascorbic acid, carotenoids and total phenol contents with antioxidant activity were also determined. The assessments of such properties remain an interesting and useful task, particularly for finding new sources for natural antioxidants.

MATERIALS AND METHODS

Plant materials

The five medicinal plants studied were collected during Apr-may of 2011 in and around Arakkonam, Tamilnadu were authenticated by Department of Botany. The voucher specimens were kept in the Department of Botany in C. Abdul Hakeem College, Mel Visharam, Vellore, and Tamilnadu, India. The botanical names, family names, English names and parts used are presented in Table 1.

Table-1

Botanical name	English name	Part used	Family name	Medicinal use
<i>Coleus Forskohlii</i>	Coleus	root	Lamiaceae	Asthma, hypertension, cancers
<i>Carica papaya</i>	papaya	leaves	Caricaceae	Diuretic, anthelmintic, cancer
<i>Corchorus estuans</i>	long-fruited jute	whole plants	Tiliaceae	Pneumonia, headaches, cancer
<i>Murraya koenigii</i>	curry leaves	leaves	Rutaceae	Anti-diabetic, hepatoprotective
<i>Urginea indica</i>	Indian Squill	Bulb	Liliaceae	Anticancer, asthma, hypoglycaemic

Extraction

All the laboratory works are done in Micro labs, Institute of Research and Technology, Arcot, Tamil Nadu, India. The collected plants materials were washed thoroughly 2-3 times with running water and with distilled water. The plants materials were air-dried under shade. The materials were crushed to make possible fine powder with the help of mortar and pestle and stored for further analysis. Then this powdered samples (15g/200ml) in Ethanol extracts for Overnight at room temperature. Soxhlet apparatus are used for this extraction. The extract from three consecutive soaking are pooled and evaporated under pressure. The percentage yield of extracts ranged from 5 - 20% (w/w). The crude samples were subjected to Antioxidant activity.

Antioxidant activity (DPPH free radical scavenging activity) determination

The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity [8]. Required amount of test solution was mixed with 500 µl of DPPH. The mixture was mixed properly, then the volume was made upto 1ml using methanol. The absorbance was determined at 517nm. The blank used contain 500µl of the sample with 500µl of methanol. The control used contained 500µl of DPPH with 500µl of methanol. The same process was repeated using different concentrations of test sample and the absorbance was noted after 15 mins. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation [9].

$$\text{Percent (\%)} \text{ inhibition of DPPH activity} = [(AB - AA) / AB] \times 100$$

Where AA and AB are the absorbance values of the test and of the blank sample, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC50 value for each of the test solutions. Standard curve was obtained using different concentrations of gallic acid. Gallic acid standard with a concentration of 1mg/ml was prepared. Different concentrations of this standard (2, 4, 6, 8, 10µl/ml) were used to prepare the standard graph.

Determination of phenol

Total phenols were recorded by Folin-Ciocalteu reagent [10] 10µl of the test solution was mixed with 20µl of Folin-Ciocalteu reagent and 50µl of 25% sodium carbonate solution, the mixture was shaken thoroughly and the volume was made upto 1ml. The mixture was allowed to stand for 1h in dark. Then the absorbance at 725nm was determined. These data were used to estimate the total phenolic content using the standard curve. Standard curve was obtained using various concentrations of gallic acid.

Determination of flavonoid

10mg of Quercetin was dissolved in 10 ml of 80% ethanol and then diluted to different concentrations (30-100 μ l/ml).the diluted standard solutions (200 μ l) were separately mixed with 750 μ l of 95% ethanol, 50 μ l of 10% aluminium chloride, 50 μ l of 1M potassium acetate and 1.4 ml of water, the mixture was shaken well and incubated for 30 mins at room temperature. The absorbance was determined at 415nm. The amount of aluminium chloride is substituted with the same amount of water for blank. The same process was repeated for the test samples. Standard curve was prepared using Quercetin.

Determination of total antioxidant capacity

10 μ l of the extract was mixed with 1ml of the reagent (1:1:1 ratio of 0.6M sulfuric acid: 28mM Sodium phosphate: 4mM Ammonium molybdate). The solution was mixed properly and the volume was made upto 1.1 ml using methanol. The tubes were incubated at 95oC for 90 mins. Then the absorbance was determined at 695nm against blank after cooling to room temperature.

A standard curve was obtained using different concentrations of ascorbic acid. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Statistical analysis

The statistical significance between free radical scavenging activity, total phenol, total flavonoid,and total antioxidant capacity values of the extracts was analyzed with a ANOVA test. P values less than 0.05 were considered to be statistically significant.

OBSERVATIONS AND RESULTS

In the present study several biochemical constituents and free radical scavenging activities of five medicinal plants were evaluated. Free radicals are involved in many disorders like neurodegenerative diseases, cancer, Anti-diabetic, hepatoprotective Anticancer, asthma, and hypoglycaemic. Antioxidants due to their scavenging activity are useful for the management of those diseases. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts [11, 12]. Figure 1 shows the different type of plants extract and percentage of radical scavenging activities. The free radical scavenging action of ethanol extracts of plant are in the order as *Corchorusaestuans*,*Carica papaya*,*Coleus Forskohlii*, *Urgineaindica*and*murrayakoenigii*. The extracts, which showed the strongest DPPH radical scavenging activity, are *Corchorusaestuans*and *Carica papaya*, while the others show moderate antioxidant properties. The therapeutic potential of natural medicinal plants asan antioxidant in reducing such free radical inducedtissue injury, suggests that many plants have antioxidantactivities that can be therapeutically useful [13].

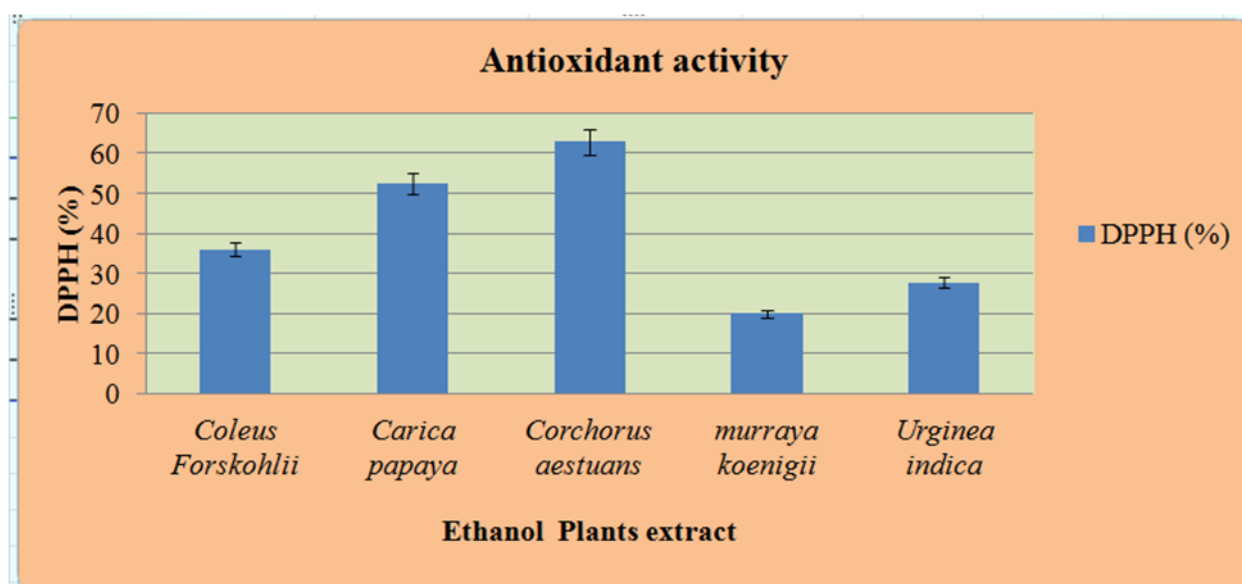


Figure 1: Different type of plants extract and percentage of radical scavenging activities

So far as plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts [14]. The content of phenols in ethanolic extracts expressed in gallic acid equivalents (GAE) varied between 1.21 \pm 0.10

and 6.28 ± 0.16 mg/gm (Figure 2). The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties [15]. According to our study, the high phenolic content in *Corchorusaestuans* can explain its high free radical scavenging activity. Common presence in plants, flavonoids is important components of human and animal diet. Due to the different biological activities of plant secondary metabolites, their regular consumption may have serious consequences for health, both positive and negative [16]. The content of Flavonoids in ethanolic extracts expressed in Quercetin acid equivalents (QAE) varied between 0.34 ± 0.01 and 4.12 ± 0.12 mg/gm (Figure 2).

According to our study, the high Flavonoids content in *Corchorusaestuans* can explain its high free radical scavenging activity.

Table-2: Antioxidant activity of investigated plant extracts

Sample name	DPPH (%)	phenol (mg/gm)	Flavonoids (mg/gm)	Total antioxidants (mg/gm)
<i>Coleus Forskohlii</i>	$36.0 \pm 0.50a$	$5.24 \pm 0.25a$	$2.41 \pm 0.08a$	$2.02 \pm 0.08a$
<i>Carica papaya</i>	$52.50 \pm 0.50b$	$2.11 \pm 0.11b$	$1.19 \pm 0.01b$	$4.92 \pm 0.08b$
<i>Corchorusa estuans</i>	$62.83 \pm 0.76c$	$6.28 \pm 0.16c$	$4.12 \pm 0.12c$	$4.0 \pm 0.09c$
<i>Murraya koenigii</i>	$20.0 \pm 0.50d$	$2.51 \pm 0.03d$	$1.37 \pm 0.04d$	$3.03 \pm 0.03d$
<i>Urginea indica</i>	$27.70 \pm 0.26e$	$1.21 \pm 0.10e$	$0.34 \pm 0.01e$	$2.0 \pm 0.10a$

The mean difference is significant at the .05 level.

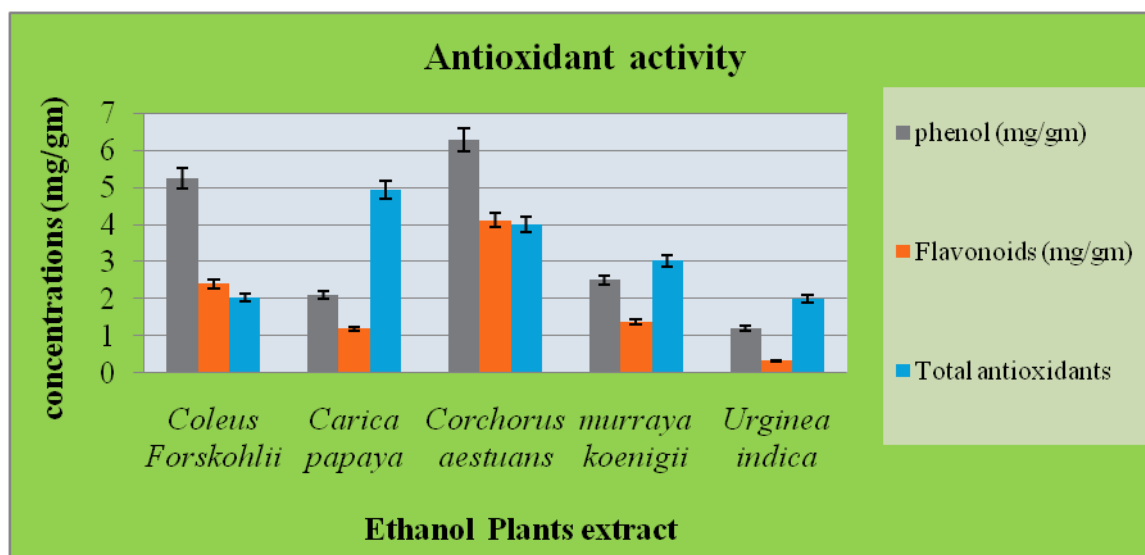


Figure 2: Antioxidant activity

This study reveals that tested plant materials have moderate to significant antioxidant activity and free radical scavenging activity. The result of the present study suggests that selected plants can be used as a source of antioxidants for pharmacological preparations which is very well evidenced by the present work.

CONCLUSIONS

Extracts of *Coleus Forskohlii*, *Carica papaya*, *Corchorusaestuans*, *Murraya koenigii*, *Urginea indica* extracts exhibited varying degrees of phenolic content, flavonoid content and antioxidant activity. The extracts, should be beneficial as an antioxidant protection system and they are required to isolate and identify the secondary metabolites responsible for their antioxidant activity.

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