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In Vitro Antioxidant Activity Of *Mussaenda Frondosa*

E.N.Siju[*1], G.R.Rajalakshmi[2], Kavitha V.P[1], Anju Joseph[1]

[1]Academy of Pharmaceutical Science, Pariyaram Medical College, Kannur, 670503, India

[2]College of Pharmaceutical Science, Govt Medical College, Calicut, 673008, India

Corres. Author :siju_ellickal@rediffmail.com

ABSTRACT: In vitro antioxidant effects of the ethyl alcohol and aqueous extracts of whole plant of *Mussaenda Frondosa* were tested.The ethyl alcohol extract of *Mussaenda Frondosa* had shown good DPPH(1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity.The ethyl alcohol and aqueous extract of *Mussaenda Frondosa* also exhibited promising result at higher concentration.BHA was used as standard antioxidant and positive control.The DPPH radical scavenging activity of the extract was increased with the increasing concentration.The reducing power of extract was carried out with ascorbic acid as a standard reducing agent.All the analysis was made with the use of UV-Visible spectrophotometer(Shimadzu 1700,INDIA).The study suggest that the *Mussaenda Frondosa* could be pharmaceutically exploited for antioxidant properties.

KEYWORDS Antioxidant activity,DPPH scavenging,Reducing power,*Mussaenda Frondosa.*

INTRODUCTION

Plants have been an important source of medicine for thousands of years.Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischaemia,asthma,arthritis,inflammation,neurodegeneration,Parkinson's disease,mongolism,ageing process and perhaps dementias[1].Flavanoids and flavones are widely distributed secondary metabolite with antioxidant and antiradical properties. Plant based natural constituents can be derived from any part of plant's bark ,leaves ,flowers,roots,fruits,seeds etc that is any part of the plant may contain active components[2].Antioxidant based drugs or formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetis, Alzheimer's disease and cancer have appeared during the last three decades.This has attracted a great deal of research interest in natural antioxidants.Recently there has been a surge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury.The main characteristics of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources.These free radicals may oxidize nucleic acids,proteins,lipids and DNA and can initiate degenerative diseases.Antioxidant compounds like phenolic acids,polyphenols and flavanoids scavenge free radicals such as peroxide,hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases[3].

In the current study, in- vitro experiment were conducted to determine possible antioxidant effect of ethyl alcohol and aqueous extract of the whole plant of *Mussaenda Frondosa*(Family: Rubiaceae*)*. *Mussaenda Frondosa* syn *Mussaenda glabrata* is a rambling shrub seen in Western ghats,Andamans,Konkan,Malabar and Tirunelvelly hills.Leaves are broadly elliptic, shortly accuminate and more or less pubescent.Flowers are terminal open cymes berries 10-13cm, subglobose or obovoid ,glabrous.Half a tola of the root is given with cow's urine in leprosy.In jaundice two tolas of the whole leaves are given in milk.In Indochina the flowers considered pectoral and diuretic, they are given in asthma,intermittent fever and dropsy.Externally they are given as a detergent to ulcers.It has been in Chinese folk medicine as a diuretic,antiphlogestic and

antipyretic.It is also used to detoxify mushroom poisons and terminate early pregnancy[4,5].

Iridoids,flavanoids and triterpenes are the common chemical ingredients distributed in Mussaenda species.Phytochemicals like astragalin, isoquercetin,kaempferol-3-o-beta drutinoside were isolated from leaves[6].A new compound sanzhilactone along with mussaenside,barlerine lupeol and beta-d-glucose has been obtained from the stem[7]. Quercetin,rutin,hyperin,ferulic acid, synaptic acid, beta sitosterol,saponins has also been isolated[8].*Mussaenda Frondosa* distributed in Central Nepal,India and Srilanka.The juice of the root is used in blemishes on tongue and sepals are diuretics.*Mussaenda Frondosa* has been found to possess antibacterial effect[9].

MATERIALS AND METHODS

Plant material The leaves of *Mussaenda Frondosa* were collected from Kannur district in Kerala, India in January 2009 and got authenticated by Dr.Radhika.C.P,Dept of Dravya Guna,Govt Ayurveda college,Kannur,Kerala.A voucher specimen PC-05/2009 was submitted at Academy of Pharmaceutical Sciences ,Pariyaram Medical College,Kannur for future reference.Dried whole plant were ground to coarse powder,passed through sieve no.24 and stored in air tight container and used for further extraction.

Preparation of extract

Ethyl alcohol extract

The powdered material was exhaustively extracted with 95% ethyl alcohol using a soxhlet apparatus.The extract was concentrated in vaccum to a syrupy consistency.The percentage yield of extract was found to be 4.26%.

Aqueous extract

The dried powders(200g) were kept for maceration with 1000ml of distilled water for 24hrs.The extract was double filtered by using muslin cloth and Whatmann no.1 filter paper and concentrated by evaporation on water bath.The extract was dried and used as a powder.The percentage yield of extract was found to be 6.31%.

Antioxidant assay

The antioxidant activity of plant extracts were determined by different in-vitro methods such as the DPPH free radical scavenging assay and reducing power methods.The different extracts were dissolved in ethanol at the concentration of 2mg/ml.All the assays were carried out in triplicate and average value was considered.

(a) DPPH Radical scavenging activity

DPPH scavenging activity of the plant extract was carried out according to the method of Koleva I.I *et al* 2002[10]; Mathiesen *et al* 1995[11]. 0.2 ml of ethyl alcohol solution of plant extract samples at different concentration (20- 100µg ml-l) was mixed with 0.8 ml of Tris Hcl buffer (100Mm, pH 7.4). One ml DPPH (500 M in ethanol) solution was added to above mixture. The mixture was shaken vigorously and incubated for 30min in room temperature. Absorbance of the resulting solution was measured at 517nm UV-Visible Spectrophotometer (Shimadzu 1700, INDIA). All the assays were carried out in triplicates with BHA (Butylated Hydroxy Anisole) as a positive control. Blank was prepared without the addition of DPPH and for control 0.2 ml of ethyl alcohol (without plant extract) was added. Percentage of DPPH scavenging activity determined as follows.

$$\% \text{ DPPH radical-scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbnce of test sample})}{(\text{Absorbance of control})} \times 100$$

Control was the DPPH solution without plant extract. Purified sample 2mg/ml in ethyl alcohol of *Mussaenda Frondosa* extracts were taken for antioxidant activity with a standard BHA (Butylated Hydroxy Anisole) antioxidant. Decreased absorbance of the reaction mixture indicates stronger DPPH radical-scavenging activity. In this study ethyl alcohol and aqueous whole plant extracts of *Mussaenda Frondosa* were used.

(b) Reducing power

This was carried out as per the method of Yildrim *et al* 2001[12]; Lu and Foo[13]. 1ml of plant extract solution (final concentration 100-500 mg/l) was mixed with 2.5 ml phosphate buffer(0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN_6)$] (10g/l), then mixture was incubated at 50 degree C for 20 minutes. Two and one-half, 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml Fecl3 (1g/l) and absorbance measured at 700nm in UV-Visible Spectrophotometer (Shimadzu 1700, INDIA). As a control, ascorbic acid was used (final concentration 10 mg/ml).

RESULT AND DISCUSSION

The phytochemical screening subjected to detect the presence of some secondary plant metabolites following standard procedure shown in Table 1. Ethyl alcohol extract revealed the presence of carbohydrates, steroids, alkaloids, terpenoids, flavanoids, tannins and poly phenols, while aqueous extract showed presence of carbohydrates, alkaloids, flavanoids, tannins and poly phenols.

It has been recognized that alkaloids and flavanoids shows antioxidant property and their effects on human nutrition and health care are considerable[14].Mechanism of action of alkaloids are through inhibition of peroxidation[15,16].Compounds such as flavanoids are responsible for inhibition of lipid peroxidation[17].

Scavenging activity of free radicals of 1,1 diphenyl-1,2-picryl hydrazyl(DPPH) has been widely used to evaluate the antioxidant activity of plants.Free radical scavenging activity of ethyl alcohol and aqueous extracts was quantitatively determined using DPPH and reducing power assay.

The DPPH radical scavenging activity increases with increasing concentration.Therefore,in this study, the antioxidant properties of ethyl alcohol and aqueous extract of whole plant of *Mussaenda Frondosa* were examined for DPPH radical scavenging activity according to the method described and the results of the screening are shown in Table 2a and 2b as comparable with known antioxidant BHA.In term of antioxidant activity,all the extracts investigated exhibited a rather high degree of ability.

Ethyl alcohol and aqueous extract of *Mussaenda Frondosa* exhibited good reducing power.The reducing power of both extracts of *Mussaenda Frondosa* along with that of ascorbic acid at concentration between 100-500mg/l.High absorbance indicates high reducing power.The reducing power of ethyl alcohol and aqueous extracts of *Mussaenda Frondosa* increases with increase in the concentration.(Table 3a and 3b)

Table 1:Phytochemical constituents of *Mussaenda Frondosa*

Phytochemical constituents	Ethyl alcohol extract	Aqueous extract
Carbohydrates	+	+
Steroids	+	-
Alkaloids	+	+
Saponins	-	-
Terpenoids	+	-
Tannins	+	+
Flavanoid	+	-
Polyphenols	+	+

(+): Present (-): Absent

Table 2a: Antioxidant activity of ethyl alcohol extract of *Mussaenda Frondosa*

Concentration	OD 517nm			% of activity	
	Sample	Standard		Sample	Standard
50µl	1.032	2µl	0.865	7.85	22.7
100µl	1.060	4µl	0.681	5.3	39.1
150µl	0.911	6µl	0.487	18.6	56.5
200µl	0.901	8µl	0.353	19.5	68.4
250µl	0.826	10µl	0.271	26.2	75.8

Control OD AT 517nm – 1.120

Table 2b: Antioxidant activity of aqueous extract of *Mussaenda Frondosa*

Concentration	OD 517nm			% of activity	
	Sample	Standard		Sample	Standard
50µl	1.052	2µl	0.899	5.2	19.7
100µl	0.981	4µl	0.686	11.6	38.1
150µl	0.932	6µl	0.486	16	56.2
200µl	0.876	8µl	0.371	21	66.5
250µl	0.786	10µl	0.274	29.1	75.3

Control OD AT 517nm – 1.110

Table 3a:Reducing power of ethyl alcohol extract of *Mussaenda Frondosa*

Sample	Concentration(mg/l)	Absorbance(700nm)
Control	0	0.07±0.06
Ethyl alcohol extracts of *Mussaenda Frondosa*	100	0.14±0.013
	200	0.30±0.047
	500	0.45±0.07
Ascorbic acid	5	0.39±0.006
	10	0.76±0.006
	15	1.10±0.006

Control was test sample without plant extract.High absorbance indicates high reducing power

Table 3b: Reducing power of aqueous extract of *Mussaenda Frondosa*

Sample	Concentration(mg/l)	Absorbance(700nm)
Control	0	0.09±0.06
Aqueous extract of *Mussaenda Frondosa*	100	0.20±0.012
	200	0.40±0.047
	500	0.58±0.07
Ascorbic acid	5	0.39±0.006
	10	0.76±0.006
	15	1.10±0.006

The control was test sample without plant extract.High absorbance indicates high reducing power.

CONCLUSION

There is a strong need for effective antioxidants from natural sources as alternatives to synthetic food additives inorder to prevent deterioration of food, drug and cosmetics.The results of the present study showed that the extract of *Mussaenda Frondosa* contains flavanoids and alkaloids which corresponds to higher lipid peroxidation.It has further confirmed that plant extracts endowed with potentially exploitable antioxidant activities.

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