IJPSR (2018), Volume 9, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 February, 2018; received in revised form, 05 May, 2018; accepted, 13 May, 2018; published 01 November, 2018

PHARMACOGNOSTICAL, PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIOXIDANT ACTIVITY OF ETHANOL AND AQUEOUS EXTRACTS OF *RANDIA SPINOSA* LEAVES

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Keywords:

Pharmacognostical, Phytochemicals, Antioxidant activity, DPPH method, *Randia spinosa*

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ABSTRACT: *Randia spinosa* (Rubiaceae family), commonly known as 'Mainphal' is a large shrub or small tree. The plant grows in Brazil, Ceylon, China, East tropical Africa, India, Malaya and Sumatra. The leaves of *Randia spinosa* is used in traditional medicine to treat diarrhea and dysentery, snake bite, wound healing, inflammation, tumors, piles, gastrointestinal and skin diseases. The objective of this study is to investigate pharmacognostical, phytochemical features and antioxidant activity of ethanol and aqueous extracts of *Randia spinosa* leaves by using DPPH assay method. Pharmacognostical characteristics like microscopy, total ash, acid insoluble ash, water insoluble ash and sulphated ash and loss on drying of *Randia spinosa* leaves were determined. The preliminary phytochemical studies were performed with various reagents and chemicals on leaf extracts in order to determine the various secondary metabolites. The ethanol and aqueous extracts of *Randia spinosa* leaves were screened for *in-vitro* antioxidant activity by oxygen radical scavenging such as 1, 1-diphenyl-2-picryl hydrazyl (DPPH) method. Pharmacognostical studies reveal the microscopical and macroscopical characters of *Randia spinosa* leaves. Phytochemical screening of the ethanol and aqueous extracts of *Randia spinosa* leaves revealed the presence of secondary metabolites like alkaloids, carbohydrates, proteins and amino acids, tannins, saponins, flavonoids and glycosides. The ethanol and aqueous extracts showed good dose dependent free radical scavenging property. IC_{50} values for aqueous and ethanol extracts were calculated by DPPH method. Aqueous extract had shown more free radical scavenging power as compared to ethanol extract. Ascorbic acid was used as standard. The results indicate that ethanol and aqueous extracts of *Randia spinosa* leaf exhibited antioxidant activity, supporting its uses in traditional medicine. Further studies on the isolation of bioactive phytoconstituents of Randia spinosa leaves and their mechanism of action are strongly recommended before its application to humans.

INTRODUCTION: Majority of the people in developing countries still depend on traditional medicinal plants for their health care needs. Generally, plant-based medicines are used in the treatment of many diseases without adverse effects[1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(11).4854-58

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(11).4854-58

Randia spinosa (Rubiaceae Family) with many medical properties is being used traditionally. It is commonly known as 'Mainphal' is a large shrub or small tree up to 9 m height and 90 cm girth, with a bole 2 - 3 m. The plant grows in Brazil, Ceylon, China, East tropical Africa, India, Malaya and Sumatra [2].

According to ethnobotanical survey and traditional systems of medicine such as Ayurveda plant parts of *Randia spinosa* have great medicinal properties [3]. This plant contains carbohydrates, glycosides, proteins and amino acids, saponins, alkaloids, flavonoids and tannins [4]. The leaves of *Randia*

spinosa are used in traditional medicine to treat diarrhea and dysentery, gastrointestinal, snake bite, wound healing, inflammation, tumors, piles and skin diseases [2]. The *Randia spinosa* plant extracts exhibited anti-inflammatory, anti-tumor and antimicrobial activity [4, 5]. The present investigation was aimed to know the role of ethanol and aqueous extracts of *Randia spinosa* leaves as antioxidants by DPPH method.

MATERIALS AND METHODS:

Plant Collection: The leaves of *Randia spinosa* were collected from remote areas of Karimnagar, Telangana, India, in the month of August 2017. The plant was taxonomically identified by Dr. E. Narasimha Murthy, Department of Botany, Satavahana University, Karimnagar, Telangana.

Preparation of Plant Extracts: The plant material (leaves) was washed with distilled water, Shade dried and pulverized to coarse powder in a mechanical grinder, passed through a 40 mesh sieve. The powdered drug extraction was done with different solvents, ethanol and distilled water individually. A solid was obtained after complete elimination of solvent from plant extracts by rotary evaporator. The obtained extracts were subjected to phytochemical screening and antioxidant activity.

Determination of Physico-chemical Properties: The plant leaves were subjected to investigation of physico-chemical properties like organoleptic properties, microscopical studies, determination of ash values and loss on drying [6, 9].

Phytochemical Screening: The phytochemical screening was carried out according to standard procedures for identification of phytoconstituents *viz*. carbohydrates, glycosides, proteins and amino acids, saponins, alkaloids, steroids, flavonoids and tannins [6, 9].

Determination of Antioxidant Activity:

DPPH Assay: Free radical scavenging activity of different extracts were tested against a methanolic solution of 1, 1-diphenyl-2-picryl hydrazyl (DPPH). Antioxidants react with DPPH and convert it to 1, 1-diphenyl-2-picryl hydrazine **Fig. 1**. The degree of discoloration indicates the scavenging potential of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant activity [10, 11].

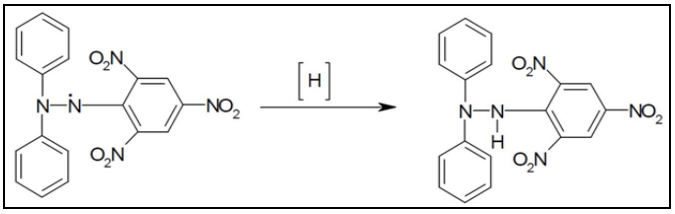

FIG. 1: REDUCTION OF 1, 1- DIPHENYL-2-PICRYL HYDRAZYL (DPPH) FREE RADICAL

Procedure: The samples of different extracts were prepared in various concentrations *viz*. 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 µg/ml in AR grade methanol. 1 ml samples of above concentrations were mixed with equal volume of 0.1mM methanolic solution of DPPH (0.39 mg in 10 ml methanol). An equal amount of methanol and DPPH was added and used as a control. Ascorbic acid solution of various concentrations *viz*. 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 µg/ml in distilled water were used as standard. After incubation for 20 min in dark, absorbance was recorded at 517 nm. Experiment was performed in triplicates. % scavenging was calculated by using the following formula:

$$\% \text{ Scavenging} = \frac{\text{Absorbance of control} \times \text{Absorbance of Test}}{\text{Absorbance of control}} \times 100$$

A graph was plotted with concentration (µg/ml) on X axis and % scavenging on y axis and IC_{50} values were calculated, which represents the concentration of the scavenging compound that caused 50% neutralization [10, 11].

Reducing Power Assay: The reducing power (ability) describes how easily one substance can give electrons to another. A powerful reducing agent is keen to donate electrons. This method measures the ability of antioxidants to reduce ferric ion. Reducing power was investigated using the method developed by Oyaizu [12].

Potassium ferricyanide + Ferric chloride $\xrightarrow{\text{Antioxidant}}$ Potassium ferrocyanide + Ferrous chloride

Procedure: The samples of different extracts were prepared in various concentrations *viz*. 250, 500 and 1000 µg/ml in distilled water. 1.25 ml of sample aliquots was mixed with 1.25 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 1.25 ml of

1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixtures were incubated at 50 ºC for 20 min. The resulting solution was cooled rapidly, mixed with 1.25 ml of 10% trichloro acetic acid and centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 ml) was taken out and immediately mixed with 2.5 ml of distilled water and 500 µl of 1.0 % ferric chloride ($FeCl_3$) was then added. After incubation for 10 min, the absorbance (abs) against blank was determined at 700 nm. All samples were assayed in triplicate. Ascorbic acid standard was utilized for comparison [12].

RESULTS:

Pharmacognostical Studies:

TABLE 1: ORGANOLEPTIC PROPERTIES OF *RANDIA SPINOSA* LEAF

Parameter	Inference
Appearance	Powder
Colour	Pale green
Odour	Characteristic
Taste	Bitter

TABLE 2: MICROSCOPICAL STUDIES OF *RANDIA SPINOSA* LEAF

Test	Observation	Inference
Phloroglucinol+ Conc. HCl	Pink colour observed	Lignified cells, Epidermal trichomes
Dil. Iodine + Conc. H_2SO_4	Blue colour observed	Starch grains

FIG. 2: PLANT OF *RANDIA SPINOSA*

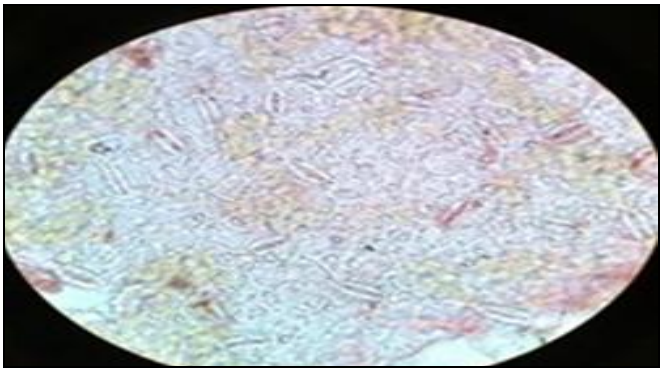

FIG. 3: TRANSVERSE SECTION OF *RANDIA SPINOSA* LEAF SHOWING PARACYTIC STOMATA

TABLE 3: DETERMINATION OF ASH VALUES OF *RANDIA SPINOSA* LEAF

Parameters	(%w/w) Leaf
Total ash	22
Acid insoluble ash	8.4
water soluble ash	4.2
Sulphated ash	2

TABLE 4: DETERMINATION OF LOSS ON DRYING

Plant parts	Loss on drying (%w/w)
leaf	52

Phytochemical Screening:

TABLE 5: PHYTOCHEMICAL SCREENING OF *RANDIA SPINOSA* LEAF EXTRACTS

Name of Phytoconstituents	Ethanol extract	Aqueous extract
Alkaloids	+	+
Carbohydrates	+	+
Amino acids	-	+
Tannins	+	+
Steroids	-	-
Saponins	-	+
Flavonoids	+	+
Proteins	-	+
Glycosides	+	+

(+) =Presence (-) = Absence

Determination of Antioxidant Activity:

DPPH Assay:

TABLE 6: DPPH FREE RADICAL SCAVENGING OF ETHANOL AND AQUEOUS LEAF EXTRACTS OF *RANDIA SPINOSA*

S. no.	Conc. (µg/ml)	% Scavenging		
		Aqueous extract	Ethanol extract	Ascorbic acid
1	2	7.25±0.23	4.22±0.51	9.56±0.47
2	4	22.56±0.51	12.34±0.56	28.24±0.28
3	8	38.41±0.55	21.24±0.37	52.08±0.37
4	16	44.22±0.37	31.11±0.66	89.76±0.43
5	32	57.64±0.35	39.64±0.52	93.27±0.72
6	64	71.28±0.47	44.56±0.23	95.73±0.37
7	128	95.26±0.44	57.12±0.38	95.92±0.81
8	256	96.24±0.64	66.31±0.29	95.81±0.49
9	512	96.57±0.28	71.22±0.51	87.17±0.43
10	1024	96.71±0.94	72.45±0.18	83.58±0.28

All values in this table represent the mean ±SD (n=3)

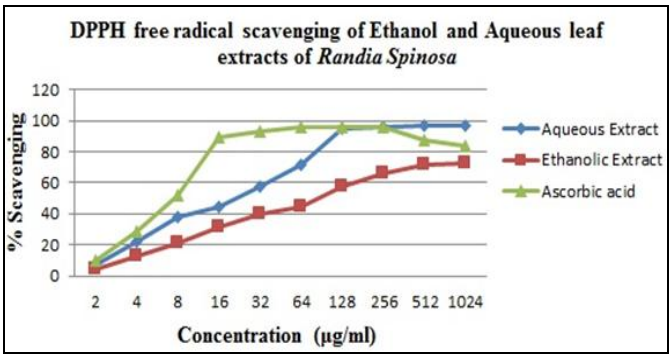

FIG. 4: GRAPH OF DPPH FREE RADICAL SCAVENGING OF ETHANOL AND AQUEOUS LEAF EXTRACTS OF *RANDIA SPINOSA*

TABLE 7: IC_{50} VALUES OF DPPH FREE RADICAL SCAVENGING ACTIVITY OF ETHANOL AND AQUEOUS LEAF EXTRACTS OF *RANDIA SPINOSA*

S. no.	IC_{50} (µg/ml)		
	Aqueous extract	Ethanol extract	Ascorbic acid
1	23.53	91.97	7.56

Reducing Power Assay:

TABLE 8: REDUCING POWER OF ETHANOL AND AQUEOUS LEAF EXTRACTS OF *RANDIA SPINOSA*

S. no.	Conc. (mg/ml)	Absorbance		
		Aqueous extract	Ethanol extract	Ascorbic acid
1	0.25	0.3201±0.34	0.2341±0.28	0.4122±0.94
2	0.50	0.6433±0.29	0.4695±0.94	0.8272±0.35
3	1.0	1.2862±0.33	0.8875±0.17	1.5945±0.49

All values in this table represent the mean ±SD (n=3)

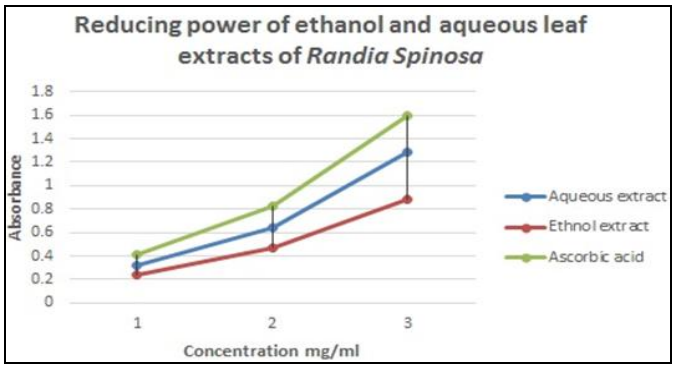

FIG. 5: GRAPH OF REDUCING POWER OF ETHANOL AND AQUEOUS LEAF EXTRACTS OF *RANDIA SPINOSA*

DISCUSSION: Plants are high source of antioxidants due to the presence of some phytochemical constituents like tannins, saponins, terpenoids and flavonoids having the capability to scavenge the free radicals. In the present study, the preliminary phytochemical screening of ethanol and aqueous leaf extracts of *Randia spinosa* revealed the presence of carbohydrates, glycosides, proteins and amino acids, saponins, alkaloids, flavonoids and tannins.[13]

The microscopic studies revealed lignified cells, epidermal trichomes, starch grains in the cells. The ash value of *Randia spinosa* leaves were performed. Acid insoluble ash, water soluble ash, sulphated ash and total ash were found be 8.4, 4.2, 2 and 22 %w/w respectively. The loss on drying value of *Randia spinosa* is 52 %w/w.

Free radicals/ oxidants/ reactive oxygen species (ROS) formed in the human body due to endogenous and exogenous factors are found to be responsible for many diseases [13]. The present investigation revealed the importance of phytochemical antioxidants as health benefit factors. This is due to their capability to scavenge the free radicals.

So many methods have been proposed to determine antioxidant activity. Total antioxidant activity, metal chelation, radical scavenging (DPPH) method and reducing power assay are widely used for this activity [14]. In the present investigation, radical scavenging (DPPH) method and reducing power assay were determined.

The DPPH method is preferred method because it is fast and reliable. The free radical scavenging activities of extracts depend on the ability of antioxidant compounds to lose hydrogen and the structural conformation of these components [14]. The DPPH free radicals after receiving an electron/hydrogen from the antioxidant molecules, become stable diamagnetic molecules [15]. Methanolic solution of DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radicals are converted into DPPH-H (1, 1 - diphenyl – 2 - picrylhydrazine) molecules in the presence of an antioxidant agent **Fig. 1** [16].

In the **Table 6**, the DPPH free radical scavenging effects of ethanol and aqueous leaf extracts of *Randia spinosa* and ascorbic acid are mentioned. The antioxidant activity of extracts were compared with ascorbic acid. Both extracts had radical scavenging effect at all concentrations. The radical scavenging effect of the aqueous extract showed better activity than ascorbic acid at concentrations of 256, 512 and 1024 µg/ml and produces similar action at a concentration of 128 µg/ml. Ethanol extract showed a lesser effect to that of ascorbic acid. IC_{50} values of ethanol and aqueous leaf extracts of *Randia spinosa* and ascorbic acid are mentioned in **Table 7**.

This effect was not as great as the half maximal inhibitory concentration (IC_{50}) of ascorbic acid. The reducing power of ethanol and aqueous leaf extracts of *Randia spinosa* and ascorbic acid are mentioned in **Table 8**. Ethanol extract showed lesser reducing power than ascorbic acid reducing power and aqueous extract reducing power appeared to be closer to that of ascorbic acid in comparison to the ethanol extract.

CONCLUSION: In this present investigation, the antioxidant ability of plant extracts were determined using DPPH free radical scavenging method and reducing power assay. Finally, it can be concluded that leaves of *Randia spinosa* have exhibited significant antioxidant activity with the presence of phytochemical constituents that leads to discovering new antioxidant agents in the pharmaceutical field.

ACKNOWLEDGEMENT: We are grateful to Management and Principal of Vaageswari Institute of Pharmaceutical Sciences, Karimnagar, Telangana, India for their support and providing institutional facilities.

CONFLICT OF INTEREST: There are no conflicts of interest.

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How to cite this article:
Sridhar V, Mamatha P and Prasad SH: Pharmacognostical, phytochemical screening and evaluation of antioxidant activity of ethanol and aqueous extracts of *Randia spinosa* leaves. Int J Pharm Sci & Res 2018; 9(11): 4854-58. doi: 10.13040/IJPSR.0975-8232.9(11).4854-58.