ISSN: 2230-7346

Yellasubbaiah .N et al. / JGTPS / 6(2)-(2015) 2579– 2588

(Research Article)

Journal of Global Trends in Pharmaceutical Sciences

Journal home page: www.jgtps.com

EVALUATION OF ANTI-OXIDANT AND ANTHELMINTHIC ACTIVITY OF RHYNCHOSIA MINIMA (LINN) DC

N. Yellasubbaiah*, B. Nagasudha, S.V.Suresh Kuamr, B.Sowjanya, M.Haseena Sultana, P.Hasanthi Lakshmi, S.Ismail, Y.Yelleshwari Devi, M. Suresh Babu[3]

C.E.S. College of Pharmacy, Chinnatekur, Kurnool, A.P.

ABSTRACT

Free radicals can be defined as molecules or molecular fragments with one or more unpaired electrons in atomic or molecular orbitals. Antioxidants are the substance which inhibit or delay the oxidative process significantly at low concentration, while often being oxidized themselves. Body was protected by endogenous antioxidant defense mechanism which acts against free radicals to scavenge them and maintain them in normal physiological concentration.A large number of medicinal plants are claimed to possess anthelmintic activity in traditional systems of medicine and also utilized by ethnic groups worldwide. The aqueous and ethanol extracts of *Rhynchosia minima* (Linn.) DC (Fabaceae) screened for antioxidant activity by DPPH, Hydroxyl radical, Nitric oxide and Hydrogen peroxide scavenging methods respectively. The aqueous and ethanol extracts of *Rhynchosia minima* (Linn.) DC (Fabaceae) DC (Fabaceae) screened for the anthelmintic activity performed on adult Indian adult earthworms Pheretima posthuma. The aqueous and ethanol extracts shows prominent DPPH, Hydroxyl radical, Nitric acid and Hydrogen peroxide scavenging activity when compared with standard ascorbic acid. The aqueous and ethanol extracts shows significant anthelmintic activity at all concentrations when compared with the standard albendazole.

Key words: *Rhynchosia minima* (Linn.) DC, DPPH, Hydroxyl, Nitric oxide, H_2O_2, Ascorbic acid, anthelmintic activity, Albendazole.

1. INTRODUCTION

Free radicals can be defined as molecules or molecular fragments with one or more unpaired electrons in atomic or molecular orbitals. This unpaired electron(s) of free radicals make them highly reactive and unstable in nature[1]. Antioxidants are the substance which inhibit or delay the oxidative process significantly at low concentration, while often being oxidized themselves. Body was protected by endogenous antioxidant defense mechanism which acts against free radicals to scavenge them and maintain them in normal physiological concentration. Exogenous antioxidants are also used to neutralize free radical reactive species and they are work in synergy with endogenous antioxidant to protect the body from free radicals induced oxidative stress[2].

Address for correspondence

Yellasubbaiah. N*,
Assistant Professor
CES College of Pharmacy
Chinnatekur, Kurnool, A.P
E-mail ID:yellasubbaiah27@gmail.com

Human body system is enriched with natural antioxidants and can prevent the onset of diseases and treat diseases caused due to free-radical mediated oxidative stress. Antioxidants from plant origin with free-radical scavenging properties could have great significances as therapeutic agents in several diseases caused due to oxidative stress.[3] A large number of medicinal plants are claimed to possess anthelmintic activity in traditional systems of medicine and also utilized by ethnic groups worldwide. Following the folk claims, several medicinal plants, products thereof and isolated phytoprinciples have been scrutinized for their anthelmintic activity to achieve lead molecules in the search of novel anthelmintic drugs (Satyavati, 1990)[4]. Although the majority of infections due to worms are generally limited to tropical countries, they can occur to travelers, who have visited those areas and some of them can be developed in temperate climates[5]. The helminthes which infect the intestine are cestodes e.g. Tapeworms (*Taenia solium*), nematodes e.g. hookworm (*Ancylostoma duodenale*), roundworm (*Ascaris lumbricoids*) and trematodes or flukes (*Schistosoma mansoni* and *Schistosoma hematobolium)*. The plant *Rhynchosia*

minima Synonym(s): *Dolicholus minimus*, *Dolichos minimus*, *Rhynchosia minima* var. *diminifolia* Family: Fabaceae, locally known as Nela Alumu (Telugu) is an indigenous medicinal palnt used traditionally as abortifacient, antihelminthic, used in the treatment of wounds, asthma and piles. The seeds are bitter and poisonous and seed extract shows specific agglutinating action on human RBC[6]. Rangaswamy *et al*., (1974) [7] studied the phytochemistry of seed coat and pericarp and found to contain gallic acid, Hydroquinone diacetate and other phenolics. Elisabeth *et al*., (1977) studied phenolics and flavonoids in the leaves and reported that all flavonoids of the leaf extract were present in the form of C-glycosyflavones[8]. The hydroquinone present in the seeds of *R. minima* is supposed to be involved in seed germination (Krishnamurty *et al*., 1975). Flavonoid profiles of seven species of *Rhynchosia* including *R. minima* were reported by Adinarayana *et al*., (1985).[9] New flavonoids were identified in the leaf extract of *R. cyanosperma* (Adinarayana *et al*., 1980; 1981).[10] In all these studies the medicinal uses of the phytochemical principles were not discussed. However, Gundidza *et al*., (2009)[11] demonstrated range of 8 essential oils which showed high antibacterial activity against several bacterial and fungal species. There are no reports of phytochemical activity or pharmacological action of whole plant extract of *Rhynchosia minima*.

.

2. MATERIALS AND METHODS

2.1. Materials

Rhynchosia minima (Linn.) DC plants were procured in spring season, from Medicinal garden of CES College of Pharmacy, Chinntekur locality in Kurnool. Leaves were identified and authenticated by botanist Dr. M.Palanisamy, Scientist 'C' Botanical survey of India, Southern regional centre, Coimbatore. A specimen voucher of the plant has been deposited in the Department of Pharmacognosy, CES College of Pharmacy, Chinntekur, Kurnool.

2.2. Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium dihydrogen phosphate, sodium hydroxide (NaOH), deoxyribose, ferric chloride ($FeCl_3$), ethylene diamine tetra acetic acid (EDTA), ascorbic acid, trichloro acetic acid (TCA), thiobarbituric acid (TBA), sodium nitroprusside (SNP), sulfanilamide, phosphoric acid (H_3PO_4), naphthylethylenediaminedihydrochloride, sodium nitrite, Folin-Ciocalteau reagent, ethanol were procured from Sd Fine-Chem Ltd. Gallic acid was obtained from Nice chemicals Pvt. Ltd. Albendazole and saline solution was obtained from the local medical store.

2.3. Preparation of extracts

Whole plant of ***Rhynchosia minima (Linn.) DC*** was shade dried under room temperature for one week and whole plant was powdered mechanically. The finely powdered plant was kept separately in an air tight container until the time of use. About 60 gms of finely powdered plant was extracted with organic solvents in polar dependent manner by using ethanol and water solvents in Soxhlet apparatus for 28 hours individually and finally solvents were evaporated and concentrated by using distillation apparatus.[12] These extracts were screened for phytochemical screening, antioxidant and anthelminthic activities.

2.4. Qualitative phytochemical screening of aqueous and ethanolic extracts

Aqueous and ethanolic extracts of ***Rhynchosia minima (Linn) DC*** was screened for their chemical constituents. Phytochemical screening was done as explained in literature.[13, 14] A small amount of dried extract was used to determine the alkaloids, carbohydrates, flavonoids, phenols, steroids and triterpenoids using the following methods.

2.5. Determination of total phenolics (TP) content

The total phenolics content of the extracts was determined with the Folin–Ciocalteau method with little change[15]. Briefly, 0.5 ml diluted extract solution was shaken for 1 min with 100 ll of Folin–Ciocalteau reagent and 6 ml of distilled water. After the mixture was shaken, 2 ml of 15% Na2CO3 was added and the mixture was shaken once again for 0.5 min. Finally, the solution was brought up to 10 ml by adding distilled water. After 1.5 h, the absorbance at 750 nm was evaluated using a spectrophotometer. The results were expressed as gallic acid equivalents.

2.6. Determination *of total Flavonoid content.*

The total flavonoid content of the Aqueous and ethanolic extracts of ***Rhynchosia minima (Linn.) DC*** was estimated by reported method. Aluminium chloride method used for flavonoid content determination[16]. 25 mg of gallic acid was dissolved in 50 ml of distilled water. 10 gms of Aluminium chloride was dissolved in 100 ml of distilled water. 1 ml of sample (1 mg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420nm with UV-Visible spectrophotometer. The content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (50-150 µg/ml) in distilled water. Calibration curve for gallic acid was obtained by plotting absorbance on Y-axis and their corresponding concentration on X-axis.

The concentration of flavonoids was expressed in terms of µg/ml.

2.7. *In-vitro* antioxidant and radical scavenging activity

2.7.1. *DPPH radical scavenging activity.*

DPPH is widely used to test the ability of the compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity. The antioxidant activity of titled compounds assessed on the basis of the radical scavenging effect of the stable DPPH radical. DPPH is a stable free radical that can accept electron of hydrogen radical, to become a stable diamagnetic molecule[17]. To the 3ml of various concentrations of sample and standard solutions add 1ml of DPPH (2mg in 50ml methanol) in triplicate manner i.e. each concentration in 3 series of test tubes. 3 ml of sample and 1 ml of methanol used as blank solutions of each concentration. 3 ml of methanol and 1 ml of DPPH used as control test and 4 ml of methanol used as control blank. Ascorbic acid was used as standard for comparison. After incubation for 20minutes in dark, absorbance was recorded at 517nm. % scavenging activity was calculated using the formula. I% = [(A0 — Ae)/A0] ×100, where A0 is the absorbance of the blank sample and Ae is the absorbance of the standard/extract sample. The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition on Y-axis and concentration on X-axis.

2.7.2. *Hydroxyl radical scavenging activity.*

The hydroxyl radical is an extremely reactive species and reacts at a high rate with all surrounding molecules - proteins, lipids, nucleic acids and sugars, causing oxidative damage to tissues and biomolecules, eventually leading to degenerative diseases like cancer, inflammation and brain dysfunction[18]. To 0.4 ml of various concentrations of sample and standard solutions, 0.4 ml of 3mMdeoxy ribose solution, 0.4 ml of 0.1 mM $FeCl_3$ solution, 0.4ml of 0.1mM EDTA solution, 0.4 ml of 2 mM H_2O_2 in phosphate buffer (pH 7.4, 20 mM) solution, 0.4 ml of 0.1mMascorbic acid solution were added triplicate manner. Then these were incubated for 30 min at 37°C. After incubation 0.4 ml of ice-cold 15% w/v trichloro acetic acid and 0.4 ml of 1% w/v thiobarbituric acid in 0.25 N HCl were added. Add all reagents expect thiobarbituric acid (instead add vehicle same amount) used as test blank. In control test add all reagents expect standard or extract (instead add vehicle same amount). In control blank add all reagents expect standard or extract and thiobarbituric acid (instead add vehicle same amount). Ascorbic acid was used as standard for comparison. % scavenging activity was calculated using the formula. I% = [(A0 — Ae)/A0] × 100, where A0 is the absorbance of the blank sample and Ae is the absorbance of the standard/extract sample. The effective concentration of sample required to scavenge Hydroxyl radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition on Y-axis and concentration on X-axis.

2.7.3. *Nitric oxide radical scavenging activity*

Nitric oxide (NO) has also been involved in a variety of biological functions, including neurotransmission, vascular homeostasis, antimicrobial, and antitumor activities. Despite the possible beneficial effects of NO, its contribution to oxidative damage is also reported[19].

To 4ml of various concentrations of sample, standard solutions and 1 ml of 25mM sodium nitroprusside solution was added in triplicate manner and incubated at 37°C for 2 h. An equal amount of sample solution and 1ml phosphate buffer, pH 7.4 was added and used as control. After incubation 2.0 ml of the solution from each test tube was removed and 1.2 ml Griess reagent (i.e., 1% sulfanilamide in 5% H_3PO_4& 0.1% Naphthylethylenediamine dihydrochloride in equal amounts) was mixed with that solution. The absorbance of chromophore that produced during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was taken immediately at 570nm. Ascorbic acid was used as standard for comparison. % scavenging activity was calculated using the formula. I% = [(A0 — Ae)/A0] × 100, where A0 is the absorbance of the blank sample and Ae is the absorbance of the standard/extract sample. The effective concentration of sample required to scavenge Nitric oxide radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition on Y-axis and concentration on X-axis.

2.7.4. *Hydrogen peroxide scavenging activity*

Hydrogen peroxide (H_2O_2) is non-radical reactive species, formed *in vivo* by different oxidizing enzymes like SOD. It is the least reactive ROS, but has higher ability to penetrate biological membranes. Due to this activity it is highly important. H_2O_2also involved in the inactivation of different enzymes directly, generally by oxidation of essential thiol groups[20]. To the 4 ml of various concentrations of sample and standard solutions add 0.6 ml of 40mM H_2O_2 (0.136mg of 30% H_2O_2) in triplicate manner i.e. each concentration in 3 series of test tubes. An equal amount of sample and phosphate buffer of pH 7.4 and H_2O_2 were mixed and was used as control. Ascorbic acid was used as standard for comparison. After incubation for 10 min. in dark, absorbance was recorded at 230nm. % scavenging activity was calculated using the formula. I% = [(A0 — Ae)/A0] × 100, where A0 is

the absorbance of the blank sample and Ae is the absorbance of the standard/extract sample. The effective concentration of sample required to scavenge H_2O_2 radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition on Y-axis and concentration on X-axis.

2.8. Anthelminthic activity

Anthelminthic activity was carried out for the aqueous and ethanol extracts taking Albendazole as standard. The anthelminthic assay was carried as per the method of Mathew et al [21-23]. The assay was performed on adult Indian adult earthworms Pheretima posthuma due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [24-27]. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelminthic compounds *invitro*[28-30].Indian adult earthworms (Pheretima posthuma),collected from moist soil and washed with normal saline to remove fecal matter, were used for the anthelminthic study .The earth worms of 3-5cm in length and 0.1-0.2cm in width were used for all the experimental protocol. The earth worms were dividing into eight groups. Each group contains six worms were released into 50ml of formulations containing two different concentrations (50 and 100 mg/ml) of title compounds. Albendazole suspension (15mg/ml) was used as reference standard and normal saline is taken as a control. Observations were made for the time taken to paralysis and death of individual worms. The time taken to paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colours.

3. RESULTS AND DISCUSSION

3.1. Physical characteristics of extracts:

The Aqueous extract of *Rhynchosia minima (Linn.) DC.* was thick dark brown color, sticky in nature and the percentage yield of the extract was found to be 32% w/w. Ethanol extract of *Rhynchosia minima (Linn.) DC.*was slightly black green in color, sticky in nature and the percentage yield of the extract were found to be 22.42% w/w.

3.2. Preliminary phytochemical screening of extracts:

Qualitative phytochemical screening was carried out using several tests and results revealed that Aqueous and Ethanol extract of *Rhynchosia minima (Linn.) DC.* contains phenols, flavonoids, alkaloids, carbohydrates and steroids & triterpenoids. The results were represented in Table No. 1.

3.2. Determination of total phenolic and total flavonoid Content of extracts:

The quantity of total phenolic and flavonoid content was determined from gallic acid calibration curve using the regression equation y = 0.0043x + 0.0104, R^2 = 0.9968, The total phenolic content of the aqueous extract was 100.5±0.402, of ethanol extract was 89.5±0.358µg gallic acid equivalents/gm of dry material and total flavonoid content of the aqueous extract was 116.25±0.465, of ethanol extract was 58.2±0.233, µg gallic acid equivalents/gm of dry material. In these extracts aqueous contains high total phenolic and total flavonoid content and ethanol extract having less phenolic and flavonoid contents compared to aqueous extract. The results were represented in Table No. 2, Figure No. 1,2.

3.4. *In-vitro* antioxidant Activity

3.4.1. *DPPH radical scavenging activity of extracts*

This assay showed the abilities of the extract and standard ascorbic acid to scavenge DPPH radical at concentration range of 20-100µg/ml in a concentration dependent manner. Decrease in absorbance with increase in concentration indicates a concentration response relationship in DPPH$^{\bullet}$scavenging activity of extracts. The aqueous extract of *Rhynchosia minima (Linn.) DC.* had significant DPPH$^{\bullet}$ scavenging effect with an IC_{50}value of 14µg/ml. The IC_{50} value of ascorbic acid was 12 µg/ml. At 100µg/ml, the percentage inhibition value was 85.72% for aqueous extract, while ascorbic acid possesses 89.63% scavenging activity. Ethanol extract having activity with an IC_{50} value of 14µg/ml, the percentage inhibition value was 74.24%. The results were represented in Table No. 3, Figure No. 3.

3.4.2. *Hydroxyl radical scavenging activity of extracts*

The degradation of deoxyribose by Fe^{+3}-ascorbic acid-EDTA-H_2O_2system was significantly decreased by *Rhynchosia minima (Linn.) DC.* and ascorbic acid at concentration range of 20-100µg/ml in concentration dependent manner, proving the significant hydroxyl radical scavenging activity of extracts. The aqueous extract shown significant antioxidant activity (IC_{50}=18µg/ml)compared to standard (IC_{50}=14.5µg/ml). At 100µg/ml, the percentage inhibition value was 76.43% for aqueous extract, while ascorbic acid possesses 79.45% scavenging activity at same concentration. Ethanol extractIC_{50} value was 18.5µg/ml, the percentage inhibition value was 76.34%. The results were represented in Table No. 4, Figure No. 4.

3.4.3. *Nitric oxide radical scavenging activity of extracts*

The extracts showed a significant nitric oxide scavenging activity between concentration range of20 to 100µg/ml in a concentration dependent manner. Ethanol extract of *Rhynchosia minima (Linn.) DC.* showed significant nitric oxide scavenging activity, the (IC_{50}) value of the Ethanol

extract was found to be 44µg/ml and standard ascorbic acid (IC_{50}) value was 40µg/ml. At 100µg/ml, nitric oxide scavenging activity of Ethanol extract was 78.45% while at same concentration ascorbic acid possesses 83.64% scavenging activity. Aqueous extract IC_{50}values 44µg/ml with a percentage inhibition 72.87% respectively. The results were represented in Table No. 5, Figure No. 5.

3.4.4. *Hydrogen peroxide scavenging activity of extracts*

The extracts was able to neutralize H_2O_2in a concentration dependent manner at a concentration range of 20-100µg/ml. Ethyl acetate (IC_{50}=15µg/ml) extract shows better antioxidant activity than the standard ascorbic acid (IC_{50}=41 µg/ml) and the percentage scavenging activity of ethanol extract and ascorbic acid values are 73.16% and 80.45% respectively. Aqueous extract IC_{50}values was 80µg/ml with percentage inhibition values of 59.23%respectively. The results were represented in Table No. 6, Figure No. 6

3.5. Biological activity (Anthelmintic activity)

The results of anthelmintic activity revealed that aqueous and ethanol extracts exhibited varying degree of activity against *Pheretima posthuma* andcaused paralysis followed by death at all tested concentrations. However, aqueous extract of the plant exhibited more potent activity at higher concentration (100mg/ml) when compared to the reference standard albendazole (15 mg/ml). As shown in **Table 7** both the extracts showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 25, 50, 100 mg/ml concentration, for the *Pheretima posthuma* worm. The ethanolic extract of *R. minima* caused paralysis in 3.8, 2.00, and 1.25. min respectively and death in 4.13, 2.35, 1.55 min respectively while aqueous extract showed P and D in 3.27, 2.05, 1.13 and 3.48, 2.20, 1.61 min, respectively against the earthworm *P. posthuma*. The reference drug albendazole (15 mg/ml) showed the same at 1.44 and 2.20 min.

Table 1: Qualitative phytochemical screening of aqueous and ethanol extracts of *Rhynchosia minima*

S.No	Plant constituents	Inference	
		Aqueous extract	Ethanol extract
1.	Alkaloids	+	+
2.	Carbohydrates	+	+
3.	Phenols	+	+
4.	Flavonoids	+	+
5.	Steroids	+	+
6.	Triterpenoids	+	+

Table 2: Total phenolic and total flavonoid contents in aqueous and ethanol extractof *Rhynchosia minima*

S. No	Extract	Total phenolic content (Mean±SEM) (GAE µg/g of dry material)	Total flavonoid content (GAE µg/g of dry material)
1	Aqueous extract	100.5±0.402	116.25±0.465
2	Ethanol extract	89.5±0.358	58.2±0.233

Table 3: DPPH radical scavenging activity of aqueous and ethanol extracts of *Rhynchosia minima (Linn.)*

S. No	Sample	Concentration	Absorbance (Mean±SEM)	Percentage inhibition (Mean±SEM)	IC_{50}
1	**Aqueous extract**	20µg/ml	0.058±0.003	62.11±1.663	14µg/ml
		40µg/ml	0.047±0.001	69.16±0.610	
		60µg/ml	0.051±0.002	72.84±3.110	
		80µg/ml	0.037±0.003	73.09±0.881	
		100µg/ml	0.020±0.001	85.72±0.907	
2	**Ethanol extract**	20µg/ml	0.077±0.004	51.23±0.275	19µg/ml
		40µg/ml	0.059±0.002	64.80±0.062	
		60µg/ml	0.055±0.002	67.25±2.002	
		80µg/ml	0.047±0.002	70.95±1.082	
		100µg/ml	0.044±0.006	74.00±2.418	
3	**Ascorbic acid**	20µg/ml	0.056±0.003	65.84±0.607	12µg/ml
		40µg/ml	0.059±0.005	63.59±2.861	
		60µg/ml	0.048±0.002	70.01±0.775	
		80µg/ml	0.039±0.002	76.07±1.062	
		100µg/ml	0.057±0.002	89.63±1.005	

Table 4: Hydroxyl radical scavenging activity of aqueous and ethanol extractof *Rhynchosia minima (Linn.*

S. No	Sample	Concentration	Absorbance (Mean±SEM)	Percentage inhibition (Mean±SEM)	IC_{50}
1	**Aqueous extract**	20 µg/ml	0.187±0.001	53.89±0.654	18 µg/ml
		40 µg/ml	0.176±0.003	55.67±0.863	
		60 µg/ml	0.162±0.001	59.04±0.521	
		80 µg/ml	0.122±0.006	67.89±1.320	
		100 µg/ml	0.092±0.005	76.43±2.984	
2	**Ethanol extract**	20 µg/ml	0.171±0.002	59.40±0.765	18.5 µg/ml
		40 µg/ml	0.154±0.002	64.07±0.891	
		60 µg/ml	0.142±0.002	63.56±0.743	
		80 µg/ml	0.118±0.001	70.85±0.532	
		100 µg/ml	0.095±0.003	76.34±1.537	
3	**Ascorbic acid**	20 µg/ml	0.142±0.002	65.96±1.616	14.5 µg/ml
		40 µg/ml	0.117±0.001	71.52±0.768	
		60 µg/ml	0.106±0.003	73.47±0.775	
		80 µg/ml	0.090±0.004	76.47±2.082	
		100 µg/ml	0.081±0.002	79.45±0.567	

Table 5: Nitric oxide radical scavenging activity of aqueous and ethanol extract of *Rhynchosia minima*

S. No	Sample	Concentration	Absorbance (Mean±SEM)	Percentage Inhibition (Mean±SEM)	IC_{50}
1	**Aqueous extract**	20µg/ml	0.120±0.001	45.67±0.670	44µg/ml
		40µg/ml	0.113±0.003	49.98±1.327	
		60µg/ml	0.087±0.001	58.45±0.589	
		80µg/ml	0.070±0.003	64.14±0.873	
		100µg/ml	0.057±0.001	72.87±0.523	
2	**Ethanol extract**	20µg/ml	0.114±0.001	48.45±0.345	38µg/ml
		40µg/ml	0.111±0.001	51.64±0.781	
		60µg/ml	0.091±0.001	57.57±1.345	
		80µg/ml	0.074±0.002	65.62±0.539	
		100µg/ml	0.057±0.002	78.45±1.642	
3	**Ascorbic acid**	20µg/ml	0.120±0.002	49.38±0.340	40µg/ml
		40µg/ml	0.090±0.005	55.87±0.384	
		60µg/ml	0.067±0.001	65.59±0.850	
		80µg/ml	0.058±0.002	74.06±1.853	
		100µg/ml	0.035±0.001	83.64±0.629	

Table 6: H_2O_2 radical scavenging activity of aqueous and ethanol extractof *Rhynchosia minima (Linn.)*

S. No	Sample	Concentration	Absorbance (Mean ±SEM)	Percentage inhibition (Mean±SEM)	IC_{50}
1	**Aqueous extract**	20µg/ml	0.128±0.001	36.76±0.567	80µg/ml
		40µg/ml	0.121±0.006	39.65±2.368	
		60µg/ml	0.119±0.003	42.28±0.934	
		80µg/ml	0.103±0.002	48.43±1.478	
		100µg/ml	0.074±0.003	59.23±0.678	
2	**Ethanol extract**	20µg/ml	0.121±0.002	41.34±1.645	53µg/ml
		40µg/ml	0.112±0.001	44.89±1.091	
		60µg/ml	0.095±0.003	52.45±0.528	
		80µg/ml	0.083±0.002	58.58±0.931	
		100µg/ml	0.056±0.002	73.16±1.482	
3	**Ascorbic acid**	20µg/ml	0.113±0.001	43.89±0.689	41µg/ml
		40µg/ml	0.096±0.001	51.78±0.620	
		60µg/ml	0.056±0.004	69.49±1.403	
		80µg/ml	0.049±0.003	74.46±1.629	
		100µg/ml	0.037±0.002	80.45±1.054	

Table-7: Anthelminthic activity of Aqueous and Ethanol extract *of Rhynchosia minima* (Linn) DC

Test Substance	Concentration mg/ml	Time taken for paralysis(minutes)	Time taken for death(minutes)
Albendazole	15mg/ml	1.44±0.17	2.20±0.01
Aqueous Extract	25mg/ml	3.27±0.45	3.48±0.12
	50mg/ml	2.05±0.03	2.20±0.03
	100mg/ml	1.13±0.04	1.61±0.05
Ethnol Extract	25mg/ml	3.86±0.38	4.13±0.06
	50mg/ml	2.00±0.05	2.35±0.04
	100mg/ml	1.25±0.04	1.55±0.08

Results are expressed as a Mean ± SEM (n = 6) significant at p<0.05. P is calculated by Comparing with standard by one-way ANOVA.

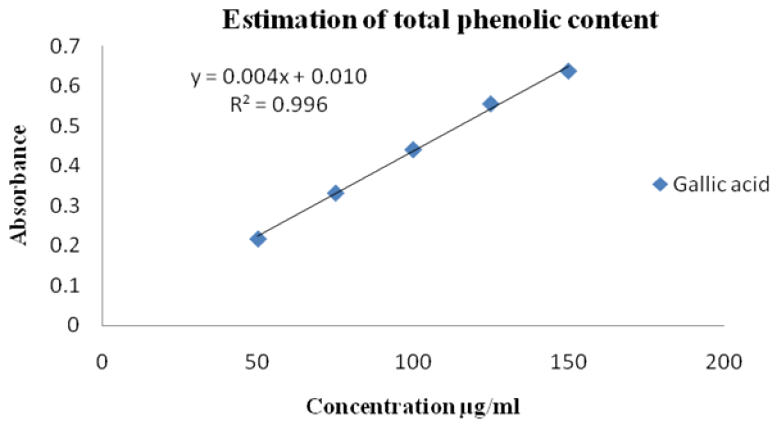

Figure1: Standard calibration curve of gallic acid for estimation of total phenolic content

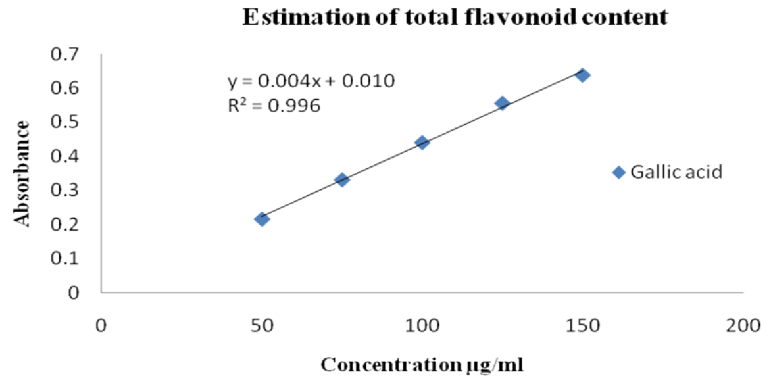

Figure 2: Standard calibration curve of gallic acid for estimation of total flavonoid content

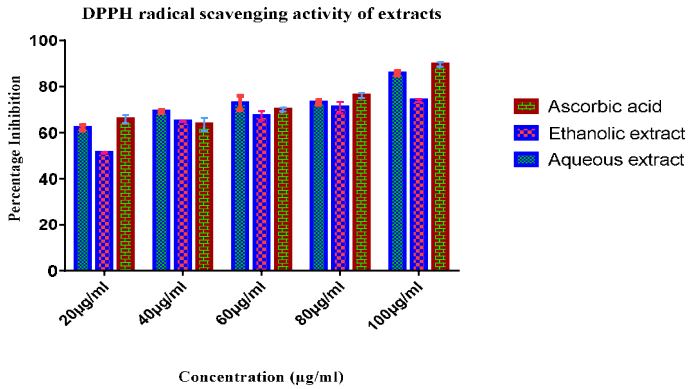

Figure 3: DPPH radical scavenging activity of aqueous and ethanol extracts of *Rhynchosia minima (Linn.) DC.* Values are expressed as the Mean±SEM, (n=3)**.**

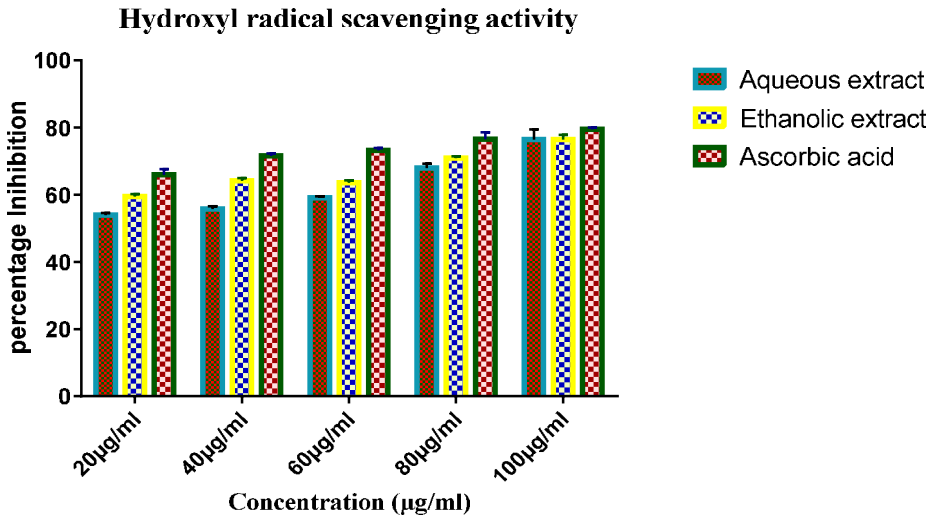

Figure 4: Hydroxyl radical scavenging activity of aqueous and ethanol extracts of *Rhynchosia minima (Linn.) DC.* Values are expressed as the Mean±SEM, (n=3).

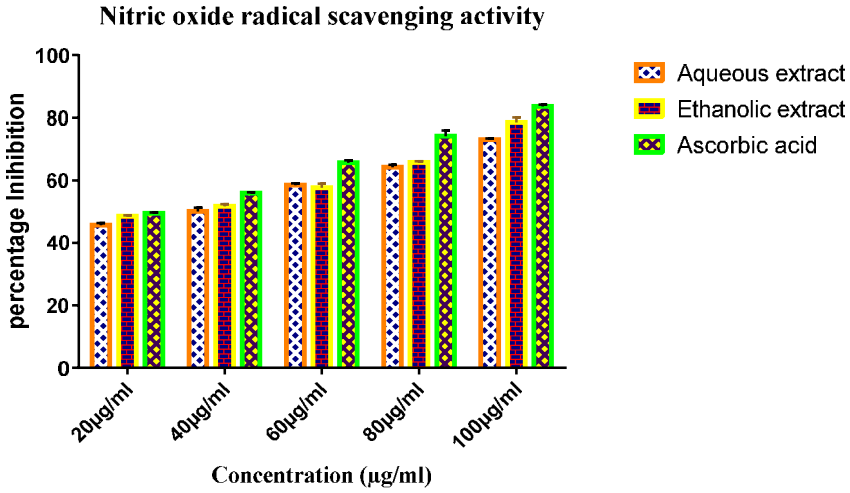

Figure 5: Nitric oxide radical scavenging activity of aqueous and ethanol extracts of *Rhynchosia minima (Linn.) DC.* Values are expressed as the Mean±SEM, (n=3).

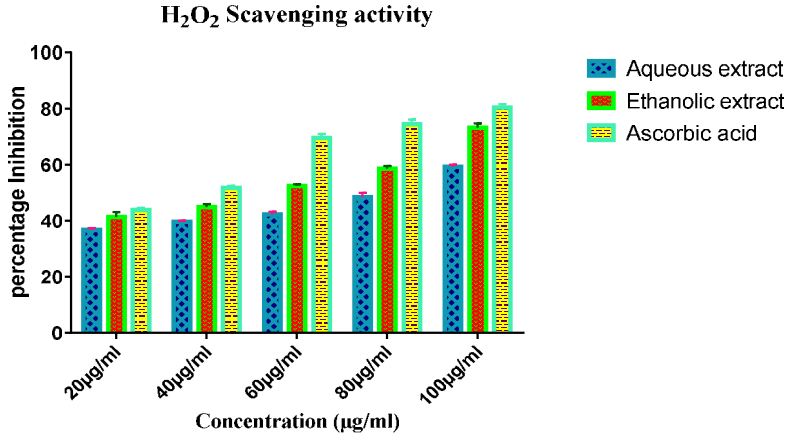

Figure 6: H_2O_2 scavenging activity of aqueous and ethanol extracts of *Rhynchosia minima (Linn.) DC.* Values are expressed as the Mean±SEM, (n=3).

4. CONCLUSION

The present work revealed that the extracts of *Rhynchosia minima (Linn.) DC.* by phytochemical screening of methanol and Aqueous and Ethanol extract of *Rhynchosia minima (Linn.) DC.* contains phenols, flavonoids, alkaloids, carbohydrates and steroids & triterpenoids. Estimation of total phenolic and total flavonoid contents methanol extract having high phenolics and flavonoids. By evaluation of *in-vitro* antioxidant studies aqueous extract shows significant antioxidant activity in DPPH radical scavenging activity and Hydroxyl radical scavenging activity when compared to standard. In nitric oxide method ethanol extract possess significant activity compared to standard and ethanol extract shows excellent activity than the standard in H_2O_2scavenging activity. The results of the anthelminthic activity revealed that aqueous and ethanol extracts exhibited varying degree of activity against Indian adult earthworms Pheretima posthuma and caused paralysis followed by death at all tested concentrations. Present study also indicates that the possible antioxidant mechanism of the extract can be due to hydrogen or electron donating and direct free radical scavenging activity of the extracts, but exact antioxidant mechanism and identification of antioxidant phytoconstituents should be further studied. The results justified the use of plant extracts in several anti-inflammatory, skin diseases, and antioxidant and antiulcer diseases traditionally. We suggest that the plant can be viewed as the potential source of natural antioxidant and anthelminthic can afford precious functional components.

5. BIBLIOGRAPHY

1. Sen S, De B, Ganesh T, Raghavendra HG. Analgesic and antiinflammatory drugs, a potential source of herbal medicine, *International Journal of Pharmaceutical Sciences and Research,* **2010**, 1, 32-44.
2. Samy RP, Pushparaj PN, Gopalakrishnakone P. A compilation of bioactive compounds from Ayurveda, *Biotransformation*, **2008,** 3, 100-110.
3. Tiwari AK. Imbalance in antioxidant defence and human disease, multiple approach of natural antioxidant therapy,*Current Science*, **2001**, 81, 1179-1187.
4. Satyavati GV, Use of plant drugs in Indian Traditional System of Medicine and their relevance to primary health care, *In*: Economic and Medicinal Plant Research by Farnworth NR and Wagner H (Eds), Academic Press Ltd, London, 1990, pp.190-210.
5. Bundy DAP, Immunoepidemiology of intestinal helminthic infection, *Trans Royal Soc Trop Med Hygiene*, 1994, **8,** 259-261.
6. Mali RG, Mahajan S and Patil KS, Anthelmintic activity of root bark of *Capparis spinosa*, *Indian J Nat Prod,* 2005, **21**(4), 50-51.
7. Krishnamurthy HG, Krishnaswami L and Rangaswamy NS. Hydroquinone diacetate from *Rhynchosia minima*. *Phytochemistry.* **14:** 2518-2519 (1975).
8. Krishnamurthy et al C-Glycosylflavones from *Rhynchosia Minima Phytochemistry, 16, P.498, 1977.*
9. Adinarayana D, Gunasekar D, Seligmann O and Wagner H. Rhyncosin- A new 5-deoxyflavonol from *Rhynchosia minima*. *Phytochemisry*; 1980; 19:483-484.
10. Adinarayana D, Ramachanraiah P and Rao KN. Flavonoid profiles of certain species of Rhychosia of the family Leguminosae (Fabeceae). *Experirntia*; 1985; 41:251-252.
11. M. Gundidza et al Phytochemical composition and biological acivities of essential oils of *Rhynchosia minima* (L) (DC) (Fabaceae). *African Journal of Biotechnology Vol. 8 (5), p 721-724, 6 March, 2009*.
12. Kurhade BB, Vite MH, Nangude SL. Antibacterial bacterial activity of *Rosa damascena* Mill. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, **2011**, 2(3), 1015-1020.
13. Kokate CK,Purohit AP, Ghokale SP.Pharmacognosy, 2nd edition, *NiraliPrakashan*, **2006**, 593-597.
14. Khandelwal KR. Practical Pharmacognosy, 15th edition,*NiraliPrakashan*, **2006**, 149-153.
15. Bonoli, M., Verardo, V., Marconi, E., & Caboni, M. F. (2004). Antioxidant phenols in barley (Hordeum vulgare L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. Journal of Agricultural Food Chemistry, 52, 5195–5200.
16. Olayinka A Aiyegoro and Anthony Okoh. Preliminary phytochemical screening and *In-vitro* antioxidant activities of the aqueous extract of *Helichrysumlongifolium* DC, *BioMedCentral Complementary and Alternative Medicine*, **2010**, 10, 21-29.
17. Harlalka VG, Patil CR, Patil MR. Protective effect of *Kalanchoepinnata*pers on gentamycin induced nephrotoxicity in rats, *Indian Journal of Pharmacology*, **2007**, 39, 201-205.

18. Ozsoy N, Can A, Yanardag R, Akev N. Antioxidant activity of *Smilax excels* L. leaf extracts, *Food Chemistry*, **2008**, 110, 571-583.
19. Yen GC, Lai HH, Chou HY. Nitric oxide scavenging and antioxidant effects of*urariacrinita root*, *Food Chemistry*, **2001**, 74, 471-478.
20. Bozin B, Mimica-Duki N, Samojlik I, Goran A, Igic R. Phenolics as antioxidants in garlic (*Allium sativam* L., Alliaceae), *Food Chemistry*, **2008**, 111, 925-929.
21. Szewezuk VD, Mongelli ER and Pomilio AB, Antiparasitic activity of *Melia azedarach* growing in Argentina, *Mol Med Chem*, 2003, **1**, 54-57.
22. Shivkar YM and Kumar VL, Anthelmintic activity of latex *Calotropis procera*, *Pharm Biol*, 2003, **41**(4), 263-265.
23. Mali RG, Mahajan S and Patil KS, Anthelmintic activity of root bark of *Capparis spinosa*, *Indian J Nat Prod,* 2005, **21**(4), 50-51.
24. Kaushik RK, Katiyar JC and Sen AB, Studies on the mode of the action of anthelmintics with *Ascardia galli* as a test parasite, *Indian J Med Res*, 1974, **62,** 1367-75.
25. Lal J, Chandra S, Raviprakash V and Sabir M, *In vitro* anthelmintic action of some indigenous medicinal plants on *Ascardia galli* worms, *Indian J Physiol Pharmacol*, 1976 **20**(2), 64-68.
26. Asuzu IU and Onu OU, Anthelmintic activity of the ethanolic extract of *Piliostigma thonningii* bark in *Ascardia galli* infected chickens, *Fitoterapia*, 1994, **65**(4), 291-297.
27. Asuzu IU, Gray AI and Waterman PG, The anthelmintic activity of D-3-*O*methylchiroinositol isolated from *Piliostigma thonningii* stem bark, *Fitoterapia*, 1999, **70,** 77-79.
28. Prakash V, Singhal KC and Gupta RR, Anthelmintic activity of *Punica granatum* and *Artemisia silversiana*, *Indian J Pharmacol*, 1980, **12**, 62-65.
29. Mali RG, Mahajan S and Patil KS. Anthelmintic activity of root bark of *Capparis spinosa. Indian J. Nat. Prod.* **21:** 50-51 (2005).
30. Martin RJ. Mode of action of anthelmintic drugs. *Vet. J.* **154:** 11-34 (1997).

How to cite this article:
N. Yellasubbaiah*,B. Nagasudha,S.V.Suresh Kuamr, B.Sowjanya,M.Haseena Sultana, P.Hasanthi Lakshmi, S. Ismail, Y.Yelleshwari Devi, M. Suresh Babu Evaluation of anti-oxidant and anthelminthic activity of rhynchosia minima (linn) DC, 6 (2): 2579 – 2588 (2015)