Phytochemical screening and *In vitro* antioxidant activity of *Paracalyx scariosa* (Roxb) Ali

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ABSTRACT:

Paracalyx scariosa (Roxb.) Ali. also called as *Paracalyx scariosus* and *cylista scariosa* is a woody twiners belongs to the family Fabaceae is one of the important medicinal plant distributed in Central Provinces, West and South India, and Upper Burma. It is woody twiners with tomentose branches and stems finely downy. Leaves are 3-foliate with rhachis prolonged 6-13mm. between the insertion of the leaflets and stipels of the terminal one.

The objective of this study is to explore the phytochemistry and the antioxidant potential of various extracts of *Paracalyx scariosa* (Roxb.) Ali which is considered traditionally as an important medicinal plant. The preliminary phytochemical analysis was done to find out the presence of various bioactive compounds and TLC was performed to identify the no of flavonoids present. In vitro antioxidant analysis of methanol, acetone, benzene extracts and ethyl acetate , aqueous fractions of methanol extract was carried out by DPPH assay and Nitric oxide assay.

It is observed from the phytochemical study, carbohydrates, proteins, saponins, triterpenoids, flavonoids and alkaloids are present in all the three extracts and Ethyl acetate fraction, benzene extract were separated in to 5 spots identified by bluish black colour confirming the presence of 5 types of flavonoids and the Acetone extract was not separated. Besides the extracts and fractions also possess strong antioxidant activity

Key words: *Paracalyx scariosa* (Roxb.) Ali, Phytochemical, Free Radicals, Antioxidant activity.

INTRODUCTION:

Plants are the basis of life on earth and are central to peoples livelihoods [1]. In recent times, there is an increasing interest in the role of free radical mediated damage in the etiology of human diseases. In normal metabolism, the levels of oxidants (i.e free radicals) and antioxidants in humans are maintained in balance, for sustaining optimal physiological conditions [2]. Overproduction of free radicals in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA, and proteins [3] and thus lead to a range of chronic diseases, such as cardiovascular disease, neuronal disease, cataracts and several forms of cancer [4]. It is established that the intake of antioxidant substances reinforces defenses against free radicals. The use of synthetic antioxidants has been limited because of their toxicity [5]. Therefore it is of great significant and necessity that research focuses on discovering potential natural, effective antioxidants to replace the synthetic ones.

Paracalyx scariosa (Roxb.) Ali. also called as *Paracalyx scariosus* and *cylista scariosa* is a woody twiners belongs to the family Fabaceae is one of the important medicinal plant distributed in Central Provinces, West and South India, and Upper Burma. In Karnataka it is distributed in Belgaum, Bellary, Chikmagalur, Coorg, Gulbarga, Hassan, Mysore, North Kanara, and Shimoga districts. It is woody twiners with tomentose branches and stems finely downy. Leaves are 3-foliate with rhachis prolonged 6-13mm. between the insertion of the leaflets and stipels of the terminal one.

Roots contain tannin. Leaves contain scariosin, isorynchospermin, kaempferol, quercetin, kaempferol-3-O-rutinoside and rutin. The fruit is acrid and bitter, improves taste, appetizer, and astringent to the bowels. The plant enriches the blood, cures biliousness, liver disorders and “kapha”. It is good for throat troubles, causes flatulence. The root is useful for treating dysentery and leucorrhoea and also applied externally along with other drugs to reduce tumors [6, 7].

Based on the above mentioned traditional uses of *Paracalyx scariosa* (Roxb.) Ali, the present study was undertaken to evaluate the in vitro antioxidant activity.

MATERIALS AND METHODS

Plant collection, preparation of extracts and fractions:

The fresh areal parts including leaves, flowers and stem were collected from Shimoga district of Gajanuru of Karnataka state. The plant was authenticated in Botanical Survey of India, Coimbatore, India. (BSI/SRC/5/23/2010-Tech. 1616)

The air dried plant material was coarsely powdered and extracted with methanol in soxhlet extractor with 100gms for 48 hrs and the solution was evaporated to dryness under reduced pressure and controlled temperature by using rotary flash evaporator. The dried methanol extract was dissolved in 30ml of distilled water and partitioned with double the volume of ethyl acetate successively for 3 times (60mlx3) in a separating funnel. The ethyl acetate soluble fraction and aqueous soluble fractions were collected and concentrated in vacuum using rotary flash evaporator. The solvent was removed completely over the water bath and finally desiccator dried.

Similarly, the dried areal parts were extracted with two different solvents, acetone and benzene with 100gms each for 48 hrs. The benzene extract was macerated with 150 ml of methanol and filtered and concentrated and the solvent was removed completely. The extracts and fractions so obtained were used for the study [8].

Phytochemical and TLC analysis:

The Various extracts of *Paracalyx scariosa* (Roxb.) Ali were subjected to the qualitative tests to detect the major chemical groups. And later the ethyl acetate fraction of methanolic extract, acetone extract and benzene was subjected to TLC analysis for the detection of flavonoids using anisaldehyde sulphuric acid as a spraying agent [8].

TLC of samples

Stationary phase : Silica gel 60 F_{254}

Mobile phase : Chloroform: Acetone: Formic acid

Proportion : 75:16.5: 8.5

Detection : Anisaldehyde sulphuric acid

***In vitro* antioxidant activities of *Paracalyx scariosa* (Roxb.) Ali.**

1. **DPPH Assay** [9,10].

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in color and upon reaction with hydrogen donor changes to yellow color. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490nm.

Procedure:

The assay was carried out in a 96 well microtitre plate. To 200 μl of DPPH solution, 10 μl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25 and 15.625, 7.812 μ g/ml. The plates were incubated at 37^{o} C for 30 min and the absorbance of each solution was measured at 490 nm, using a micro plate reader

2. **Scavenging of nitric oxide radical** [9,10].

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of modified Griess Ilosvay reaction. In the present investigation, Griess Ilosvay reagent is modified by using Naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). Nitrite ions react with Griess reagent, which forms a purple azo dye. In presence of test components, likely to be scavengers, the amount of nitrites will decrease. The degree of decrease in the formation of purple azo dye will reflect the extent of scavenging. The absorbance of the chromophore formed was measured at 540 nm.

Procedure*:*

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, pH 7.4, 1 ml) and extract in DMSO at various concentrations or standard was incubated at 25^{o} C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotization. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance of these solutions was measured at 540 nm.

RESULTS:

Qualitative chemical evaluation

The methanol, benzene and acetone extracts areal parts of *Paracalyx scariosa* were subjected to preliminary phytochemical evaluation. The various groups of phytochemical constituents found to be present in these extracts are shown in **Table No: 1.**

It is observed from the phytochemical study, carbohydrates, proteins, saponins, triterpenoids, flavonoids and alkaloids are present in all the three extracts.

Table No: 1: Phytochemical investigation of various extracts of *Paracalyx scariosa* (Roxb.) Ali.

Sl. No.	Name of the Phytoconstituents	Methanol extract	Benzene extract	Acetone extract
1	Carbohydrates	+	+	+
2	Proteins	+	+	+
3	Tannins	-	-	-
4	Saponins	+	+	+
5	Triterpenoids	+	+	+
6	Flavonoids	+	+	+
7	Quinones	-	-	-
8	Fixed oils	+	+	+
9	Glycosides	-	-	-
10	Alkaloids	+	+	+

Note: + = present; - = absent.

Thin layer chromatography:

The benzene, acetone extracts and ethyl acetate fraction from the methanol extract were subjected to thin layer chromatography for the presence of flavonoids in each extract using Anisaldehyde sulphuric acid as the spraying agent.

Ethyl acetate fraction, benzene extract were separated in to 5 spots identified by bluish black colour confirming the presence of 5 types of flavonoids and the Acetone extract was not separated The results were shown in the **Table No: 2**

Table No: 2: TLC of ethyl acetate fraction from methanol, benzene extract and acetone extract

Sl. No.	Extract	Observation		R_f values
		No of spots	Colour of spots	
1	Ethyl acetate fraction from methanol	5	Bluish black	0.07, 0.351, 0.611, 0.740, 0.77
2	Benzene extract	5	Bluish black	0.07, 0.351, 0.611, 0.740, 0.77
3	Acetone extract	-	-	-

Figure No: 1: Photograph showing the TLC of ethyl acetate fraction from methanol, benzene extract and acetone extract

Pharmacological evaluation:

***In vitro* antioxidant activities of *Paracalyx scariosa* (Roxb.) Ali.**

The benzene extract, acetone extract, methanol extract and the ethyl acetate fraction and aqueous fractions from the methanol extract were assayed for the DPPH and nitric oxide radical screening activity. Table no 4 and Figure No 2 represents the DPPH radical scavenging activity where as Table no 5 and Figure No 3 represents Nitric oxide radical scavenging activity.

The IC_{50} values were depicted in the **Table No: 3**

Table No: 3: *In vitro* antioxidant activity of *Paracalyx scariosa* (Roxb.) Ali

Sample	IC 50 values in µg/ml	
	DPPH	Nitric oxide
Benzene extract	162.5	125
Acetone extract	36	187.5
Methanol extract	19.5	109.4
Ethyl acetate fraction	42.22	200
Aqueous fraction	32.86	300
Standard	Quercetin- 15.4	Rutin-65.44

Table No: 4: DPPH radical scavenging activity of *Paracalyx scariosa (*Roxb.) Ali.

Sl. No.	Name of the sample	% inhibition (Mean ± SEM)
1	Quercetin	76.64 ± 4.127
2	Acetone	71.79 ± 9.964
3	Benzene	50.58 ± 13.24
4	Methanol	78.10 ± 6.26
5	Ethyl acetate fraction	73 ± 11.19
6	Aqueous fraction	71.72 ± 9.357

Data were analyzed by one way ANOVA fallowed by Dunnett's test.
Number of values in each sample n=7
P value not significant

Table No: 5: Nitric oxide radical scavenging activity of *Paracalyx scariosa (*Roxb.) Ali.

Sl. No	Name of the sample	% inhibition (Mean ± SEM)
1	Rutin	59.01 ± 10.58
2	Acetone	42.86 ± 11.07
3	Benzene	44.26 ± 11.08
4	Methanol	54.66 ± 10.72
5	Ethyl acetate fraction	40.33 ± 12.23
6	Aqueous fraction	39 ± 7.364

Data were analyzed by one way ANOVA followed by Dunnett's test.
Number of values in each sample n=7
P value not significant

Figure No: 2. Histogram showing DPPH radical scavenging activity of Paracalyx scariosa (Roxb.) Ali.

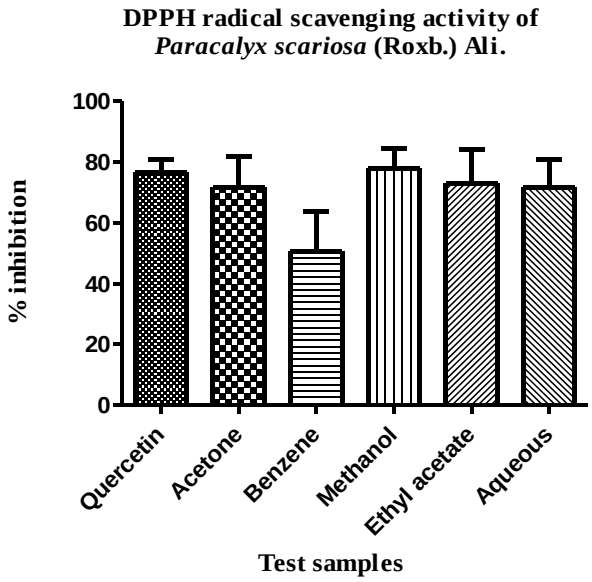
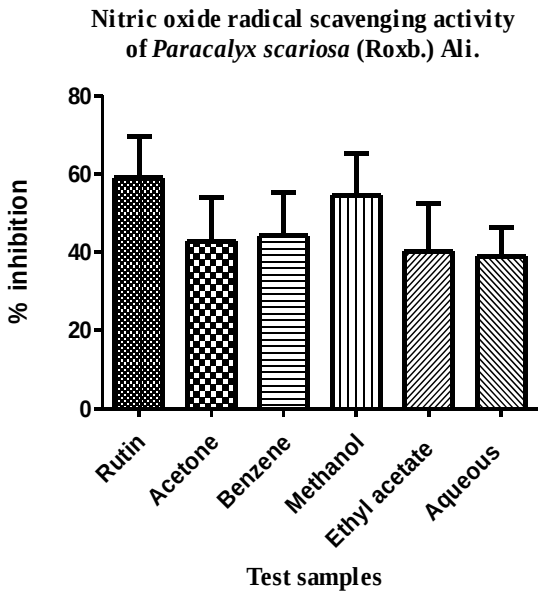


Figure No: 3.Histogram showing nitric oxide radical scavenging activity of Paracalyx scariosa (Roxb.) Ali.



DISCUSSION:

Paracalyx scariosa (Roxb) Ali. is a medicinal plant being used in folk medicine for treating liver disorders. Upon literature review it is found that the phytochemical and pharmacological profile of this plant is incomplete. In addition there are some reports that the leaves of this plant contain flavonoids like quercetin, rutin, kaempferol etc [11]. These polyphenolic compounds have antioxidant property and antioxidant have known to possess hepatoprotective activity, anti-inflammatory, anti ulcer, anti viral and anti- cancer properties [12].

Keeping the native knowledge and the above mentioned literature information this plant was selected for present study to screen the whole plant for the category of phytoconstituents and free radical scavenging activities.

The various extracts prepared were subjected to phytochemical tests and the outcome of these tests reveals that the presence of carbohydrates, proteins, saponins, triterpenoids, flavonoids and alkaloids in all the three extracts. Further, the methanolic extract is fractionated with ethyl acetate and ethyl acetate fraction, aqueous fraction were collected.

Mokhtar Ali Nia *et al* [11]. reported that the leaves of this plant contain 5 different types of flavonoids. Similar results were reported in TLC analysis of ethyl acetate fraction, benzene extract. They have got separated into 5 spots indicating the presence of 5 different types of flavonoids.

Since all the three extracts showed the presence of polyphenolic compounds, flavonoids they were subjects to *in-vitro* antioxidant studies. Ethyl acetate fraction and aqueous fraction were also considered for the further studies.

There are numerous antioxidant methods for evaluation of antioxidant activity out of which the present study used two assays.

The DPPH radical is considered to be a lipophilic radical. It is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic. DPPH radicals react with suitable reducing agents, as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colour stoichometrically depending on the number of electrons taken up. The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and DPPH radical and results in the scavenging of the radical by hydrogen donation [13].

It is visually noticeable as discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity at antioxidants. From the results it is evident that the extracts and fractions are acting as hydrogen donors and thus are able to scavenge DPPH free radical. Results also indicate that the IC_{50} values of the methanol extract and its fractions are comparable with IC_{50} value of standard drug quercetin.

In nitric oxide scavenging we generate the nitrate ions from sodium nitroprusside in aqueous solution at physiological pH. These nitrate ions reacts with Naphthyl ethylene diamine dihydrochloride (NEDD) forming a purple azo dye. In the presence of antioxidants likely to be scavengers the amount of nitrates will decrease resulting in the decrease in the purple azo dye colour intensity. From the results it has been proved that the extracts and fractions are acting as scavengers and thus are able to reduce the purple azo dye intensity [10].

Results also indicate that the IC_{50} values of the methanol extract and benzene extract are comparable with IC_{50} value of standard drug rutin.

It is evident from the present study that *Paracalyx scariosa* (Roxb) Ali could be a potential source of natural antioxidant that could have great importance as therapeutic agent in preventing or slowing the oxidative stress related degenerative diseases. Further work regarding isolation of bioactive compounds responsible for this potent antioxidant activity will provide more insight about the role of plant.

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REFERENCES:

[1] L. S. Albert, G. Kuldip, Traditional use of medicinal plants by the Jaintia tribes in North Cachar Hills district of Assam, northeast India. Journal of Ethnobiology and Ethnomedicine., 2006, 2: 33-39.
[2] N. J. Temple. Antioxidants and disease: More questions than answers. Nutr Res., 2000, 20: 449-459.
[3] R. H. Liu. Supplement quick fix fails to deliver. Food Technol Int., 2002, 1: 71-72.
[4] B . Halliwell. Antioxidants and human disease: a general introduction. Nutr Rev., 1997, 55: S44–S52.
[5] P.Valentao, E. Fernandes, Carvalho, et al. Antioxidative properties of cardoon (Cynara cardunculus L.) infusion against superoxide radical, hydroxyl radical and hypochlorous acid. J Agric Food Chem. 2002, 50: 4989–4993.
[6] S. N. Yoganarasimhan. Medicinal plants of India. Vol-1. Karnataka: Interline publishing pvt ltd; 1996, 349
[7] K. R. Kirtikar, B. D.Basu. Indian medicinal plants. 2nd ed. Vol-1. Periodical expert's book agency; 1991, 812-13.
[8] J. B. Harborne. Phytochemical Methods, 3rd edition. Chapman and Hall, London, U.K, 1998, 5-7.
[9] R. Manjula, B. Gunjan, C. Soumya, C. M. Subhash, K. Acharya. Evaluation of antioxidant and nitric oxide synthase activation properties of *Armillaria mellea* Quel. J Biol Sci. 2009, 1(1): 39-45.
[10] D. C. Garrat. The Quantitative Analysis of Drugs. 3rd Ed. Japan: Chapman and Hall Ltd 1964, p.456-58.
[11] A. N. Mokhtar, K. V. Rao, K. Sreeramulu, D. Gunasekhar. Two new prenylated flavonoids from *Paracalyx scariosa.* J Nat Prod, 1992., 55(8):1152-54.
[12] K . Raj narayana, R. M. Sripal, M. R. Chaluvadi, D. R. Krishna. Bioflavonoids classification, pharmacological, biochemical, effects and therapeutic potential. Ind J Pharmcol, 2001, 33: 2-16.
[13] S. M. Sabir, J. B. Rocha. Water extractable phytochemicals from *Phyllanthus niruri* exhibit distinct *in vitro* antioxidant and *in vivo* hepatoprotective activity against paracetamol induced liver damage in mice. Food Chemistry, 2008, 111:845-51.