EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF *KYDIA CALYCINA* ROXB., AERIAL PARTS USING DPPH AND SUPEROXIDE RADICALS

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Abstracts

Back ground: Free radicals are chemical species possessing an unpaired electron which are generally very reactive. The role of free radicals has been implicated in the causation of several diseases such as liver cirrhosis, cancer, aging, arthritis, diabetes, etc. and the compounds that can scavenge free radicals have great potential in ameliorating these disease process. Medicinal herbs are considered to be good therapeutics since time immemorial. *Kydia calycina* Roxb., is a widely used ethnomedicinal plant in the north coastal districts of Andhra Pradesh, India, to treat various liver disorders, skin related problems and other common ailments.

Method: The DPPH radical and superoxide radical scavenging activity of hydro-alcoholic extract, hexane, ethyl acetate and methanol fractions of aerial parts of *Kydia calycina* Roxb., was determined by the IC_{50} values of the selected plant extracts and ascorbic acid. The lower the IC50 value, the higher the free radical scavenging activity.

Result: The mean IC_{50} values for DPPH radical with 80% hydro-alcoholic extract, hexane fraction, ethyl acetate fraction and methanol fraction of *Kydia calycina* aerial parts were found to be 73.5, 36.0, 23.0 and 25.9 µg. Among the test results, ethyl acetate fraction showed better activity. The mean IC_{50} values for Superoxide radical with 80% hydro-alcoholic extract, hexane fraction, ethyl acetate fraction and methanol fraction of *Kydia calycina* aerial parts were found to be 30.2, 21.6, 18.7 and 25.9 µg.

Conclusion: The test results revealed, the methanol extract in general and ethyl acetate fraction in specific showed better activity. The results were found to be significant, validating the free radical scavenging activity of *Kydia calycina*.

Keywords: Antioxidants, Free radicals, *Kydia calycina* Roxb, DPPH, Superoxide, Percentage inhibition, IC_{50} Value

1. Introduction

The human body has inherent mechanisms to reduce free radical induced injury by endogenous anti oxidative enzymes. Sometimes these protective mechanisms are found not to be sufficient when compared to the insult produced to the body. Hence, the search for exogenous antioxidants is continued. Recently, intensive research has been carried out either to characterize antioxidant properties of extracts from several plant materials and /or isolate and identify the compounds responsible for those activities[1,2].

The role of free radicals has been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, aging, arthritis, diabetes, etc and the compounds that can scavenge free radicals have great potential in ameliorating these diseases process. The reactive oxygen species (ROS) are continuously produced during normal physiological events and are removed by antioxidant defense mechanisms. These oxygen free radicals react readily with cellular phospholipids and proteins, causing lipid peroxidation and oxidation of thiol groups with subsequent alteration of membrane ultra structure and dysfunction of various cellular proteins. In addition to tissue injury, these free radicals may result in depression in contractile function, arrhythmias, and depletion of endogenous antioxidant network.

Under pathological conditions, the imbalance between ROS and antioxidant defense mechanisms leads to oxidative modification in cellular membrane or intracellular molecules. Consequently, antioxidants that can neutralize direct ROS attacks and terminate free radical-mediated oxidative reaction would have beneficial effect in protecting the human body from various diseases[3,4].

The removal of these free radicals is achieved through enzymatic and non-enzymatic antioxidant reactions. Antioxidants (e.g., glutathione, arginine, citrulline, taurine, creatine, selenium, zinc, vitaminE, vitamin C, vitamin A) and antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione reductase, and glutathionw peroxidase, glutathione S-transferase) exert synergistic actions in scavenging free radicals. Medicinal herbs are considered to be good therapeutics to counter act these disorders since time immemorial.

Kydia calycina Roxb. (Synonyms - *Kydia fraterna* Roxb. and *Kydia roxburghiana* Wight.) is native of Asia-tropical, India, Indo-china Pakistan, Nepal, Bhutan and Myanmar. In India they are distributed in tropical Himalayas and peninsular India. The plant is a deciduous tree, growing up to 10- 20m tall. Plants are mucilaginous, anti inflammatory, febrifuge; leaf[5] and root are anti rheumatic, paste of the leaves applied for body pains and leaves are used in poultices for skin diseases. Leaves are chewed for stimulating saliva. Stem is used for clarifying sugars. The plant extracts are used in treating liver disorders and skin related problems[6]. Studies on the application of leaf and bark extract of *Kydia calycina* showed phytotoxic effects on the germination and the radicle growth of some food crops[7].

Therefore the present study was carried out on the DPPH radical and superoxide radical scavenging activity of hydro-alcoholic extract, hexane, ethyl acetate and methanol fractions of aerial parts of *Kydia calycina,* a herb which is used in traditional medicine in the north coastal districts of Andhra Pradesh. Their activity was determined by the IC_{50} values of the selected plant extracts and ascorbic acid. The lower the IC_{50}, the higher the free radical scavenging activity.

2. Materials and Methods

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca *et al*[8]. An aliquot of 3ml of 0.004% DPPH solution in ethanol and 0.1 ml of plant extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. decolourization of DPPH was determined by measuring the absorbance at 517 m. a control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid.

Superoxide scavenging activity of the plant extract was determined by McCord and Fridocich method[9], which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tertrazolium. 0.1 ml of different concentrations of plant extracts, (20, 40, 80, 160, 320 & 640µg) and 0.1 ml of 6µM ethylenediamine tetraacetic acid containing NaCN, 0.1 ml of 50µM nitroblue tetrazolium, 0.05 ml of 2µM riboflavin were transferred to a test tube. And final volume was made up to 3 ml using phosphate buffer. Then the assay tubes were uniformly illuminated with an incandescent light (40 Watts) for 15minutes and thereafter the optical densities were measure at 560 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ ascorbic acid. The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes.

Calculation of percentage inhibition:

The percentage inhibition of superoxide production by the extract was calculated using the formula:

Inhibitory ratio = A0-A1x100/ A0

Where,

A0 is the absorbance of control:

A1 is the absorbance with addition of plant extract/ascorbic acid.

Calculation of 50% inhibition concentration:

The optical density obtained with each concentration of the extract/ ascorbic acid was plotted taking concentration on x- axis and percentage inhibition on Y-axis. The graph was extrapolated to find the 50% inhibition concentration of extract/ascorbic acid.

3. Results and Discussion

DPPH has been widely used to evaluate the free radical scavenging activity of various antioxidants substances because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations. The mean IC_{50} values for DPPH radical with hydro alcoholic extract, hexane, ethyl acetate and methanol soluble fractions of *Kydia calycina* were found to be 73.5, 36.0, 23.0 and 25.9., with ascorbic acid was found to be 15.8ug., respectively (Table-1; Fig: 1&2). Among the test results ethyl acetate fraction showed better activity than other extracts.

Superoxide anion plays an important role in the formation of more reactive species such as

hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA[10]. Therefore, studying the scavenging activity of plant extracts/ compounds on superoxide radical is one of the most ways of clarifying the mechanism of antioxidant activity.

Superoxide scavenging activity of the plant extract was determined by McCord and Fridocich method[9], which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tertrazolium. The IC_{50} values for superoxide radical with hydro alcoholic extract, hexane, ethyl acetate, methanol soluble fractions of *Kydia calycina* and ascorbic acid were found to be 30.2, 21.6, 18.7, 25.9µg., and 14.4ug., (Table-2; Fig: 3 & 4). Among the test results ethyl acetate fraction showed better activity than other extracts.

Antioxidant actions might be exerted by inhibiting generation of reactive oxygen species and reactive nitrogen species, or by directly scavenging free radicals or by raising the levels of endogenous antioxidant enzymes by up regulating expression of the genes encoding superoxide dismutase, catalase of alutathione peroxidase[11].

Possible mechanisms by which antioxidants may protect against reactive oxygen species (ROS) toxicity are (i) prevention of ROS formation (ii) interception of ROS attack by scavenging the reactive metabolites and converting them to less reactive molecules and / or by enhancing the resistivity of sensitive biological targets to ROS attack (iii) facilitating the repair of damage caused by ROS (iv) by regulating expression of the genes encoding them in treating such diseases (v) providing a favourable environment for effective functioning of other antioxidants.

Table-1: Percentage inhibition of DPPH radical scavenging activity *In vitro* by hydro alcoholic extract, hexane, ethyl acetate and methanol soluble fractions of *Kydia calycina* aerial parts

Extract / Fraction	Quantity in micro grams (µg)							50% Inhibition Conc.
	10	20	40	80	160	320	640	
Ascorbic acid	45.82 ± 0.69	52.60 ±0.80	88.08 ± 2.51	90.68 ±1.37	93.63 ± 1.45	94.21 ± 0.68	94.74 ± 0.75	15.8
Hydro alcoholic extract (80% methanol)	23.99 ±0.57	25.90 ± 0.85	35.95 ± 0.13	52.97 ± 1.04	78.88 ± 0.94	90.45 ± 0.28	93.55 ± 0.17	73.5
Hexane fraction	20.27 ±0.93	36.84 ± 1.48	54.31 ± 0.98	78.75 ± 0.82	89.03 ± 0.26	91.06 ± 0.78	92.40 ±0.80	36.0
Ethyl acetate fraction	25.83 ±0.21	45.17 ± 0.74	68.62 ± 0.49	76.38 ± 1.12	89.74 ± 0.54	93.05 ± 0.67	94.85 ± 0.38	23.0
Methanol fraction	39.26 ±0.60	44.17 ± 0.54	66.95 ±0.56	70.29 ±0.95	86.13 ±0.90	92.02 ± 0.31	94.65 ± 0.63	25.9

Figure 1: Percentage inhibition of DPPH radical scavenging activity *In vitro* by hydro alcoholic extract, hexane, ethyl acetate and methanol soluble fractions of *Kydia calycina* aerial parts

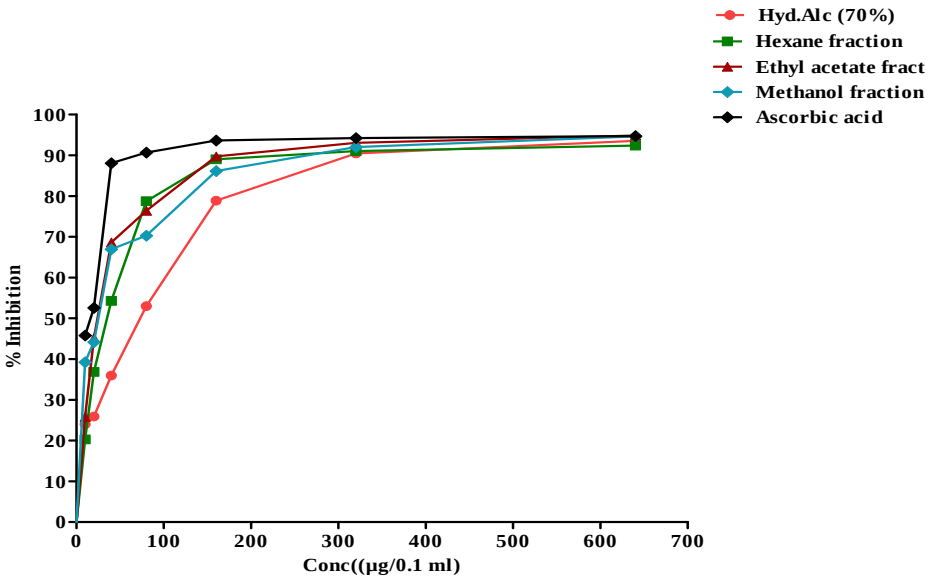


Figure 2: 50% inhibition concentration (IC_{50}) values for DPPH radical scavenging activity by various extracts and fractions of *Kydia calycina* aerial parts

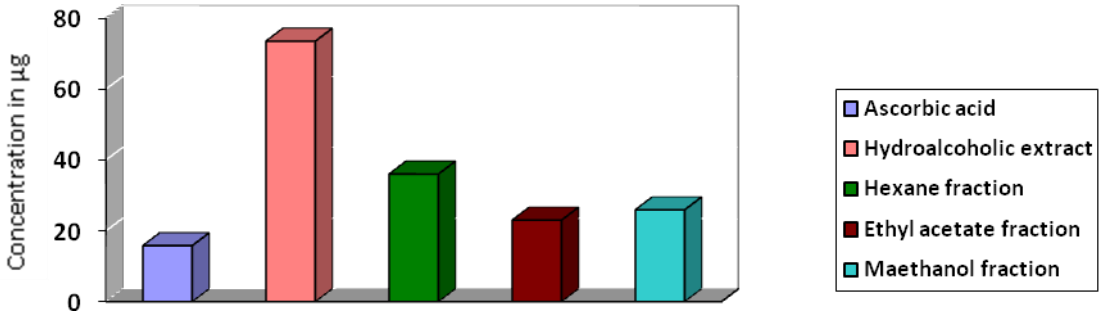


Table 2: Percentage inhibition of Super oxide radical scavenging activity *In vitro* by hydro alcohol extract, hexane, ethyl acetate and methanol soluble fractions of *Kydia calycina* aerial parts

Extract / Fraction	Quantity in micro grams (µg)							50% Inhibition Conc.
	10	20	40	80	160	320	640	
Ascorbic acid	46.45 ±1.80	55.57 ± 4.14	67.40 ±1.16	78.25 ± 0.78	84.08 ± 1.35	93.54 ± 1.25	95.85 ± 1.80	14.4
Hydro alcoholic extract(80%methanol)	26.28 ± 1.49	43.60 ± 1.18	57.07 ± 0.87	72.41 ± 0.60	77.55 ±1.03	90.89 ±1.27	91.94 ± 0.86	30.2
Hexane fraction	38.48 ± 0.92	54.75 ±1.41	65.01 ±1.34	73.26 ± 0.74	81.98 ± 0.33	92.33 ± 1.14	94.79 ± 0.85	21.6
Ethyl acetate fraction	40.86 ± 0.83	56.15 ± 1.41	67.96 ± 1.02	74.68 ± 0.80	83.07 ± 0.68	95.57 ± 1.30	97.35 ± 0.99	18.7
Methanol fraction	36.98 ±0.65	49.61 ±0.50	62.55 ± 0.81	69.57 ± 1.06	78.95 ± 0.58	90.13 ± 0.85	93.28± 0.79	25.7

Figure 3: Percentage inhibition of Super oxide radical scavenging activity *in vitro* by hydro alcohol extract, hexane, ethyl acetate and methanol soluble fractions of *Kydia calycina* aerial parts

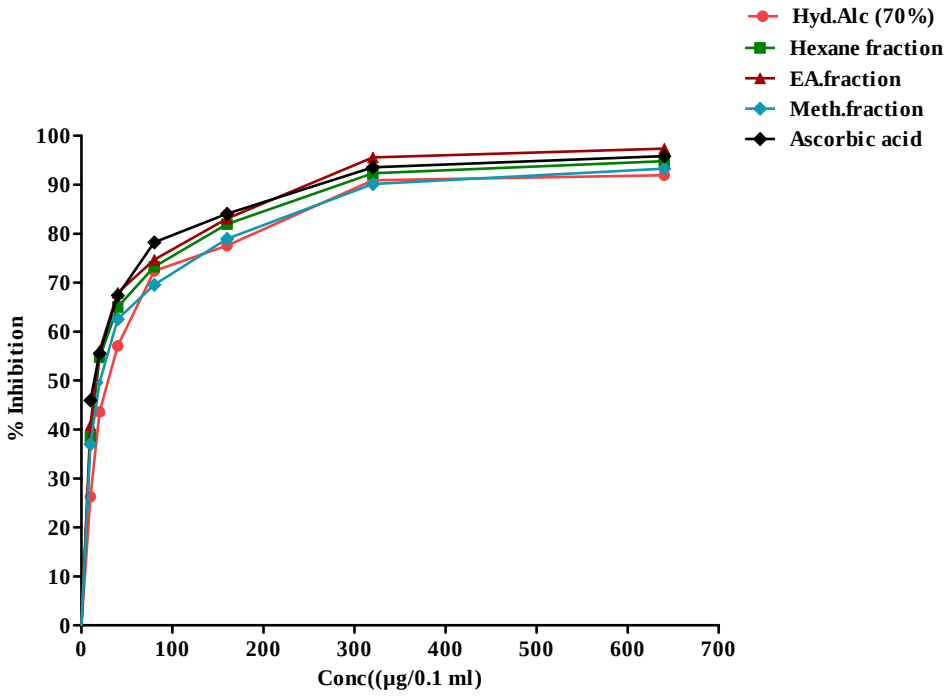
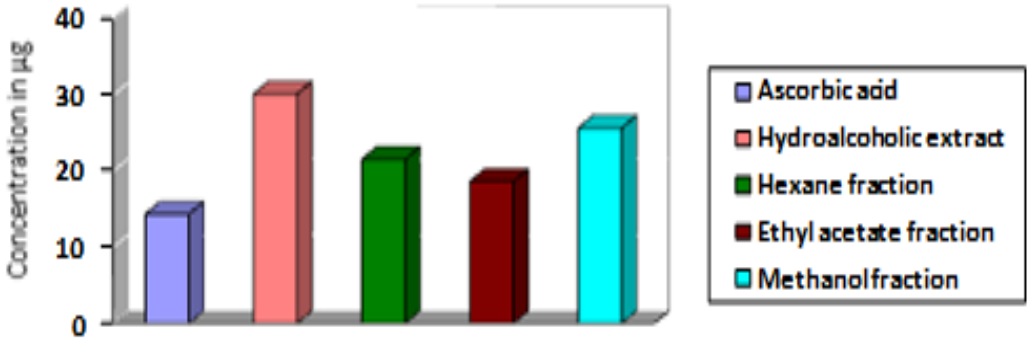


Figure 4: 50% inhibition concentration (IC_{50}) values for Super oxide radical scavenging activity by various extracts and fractions of *Kydia calycina* aerial parts



4. Conclusion

The free radical scavenging activity of the selected plant extracts of *Kydia calycina* whole plant were evaluated and found that the ethyl acetate fraction showed better DPPH scavenging activity than the hydro alcoholic extract, hexane and methanol soluble fractions. Whereas the ethyl acetate fraction showed better Superoxide radical scavenging activity than the hydro alcoholic extract, hexane and methanol soluble fractions which indicates that the plant as a whole or in fractions possess anti oxidant activity , thereby can be used as a herbal remedy against the diseases caused by free radicals.

Natural antioxidants such as phenolic acids, flavonoids and tannins possess potent antioxidant activity[12]. Sterols like β – sitosterol have been reported to have anti– oxidant activity. The triterpinoids are also reported to possess anti oxidant activity[13].

Qualitative phytochemical tests revealed that *K. calycina* contains phytosterols, triterpinoids, alkaloids, flavonoids, tannins and glycosides. These active constituents alone or in combination might be responsible for the observed anti oxidant activity in the present study.

Isolation and characterization of bioactive phytochemicals of the selected plant extracts revealed the presence of β-sitosterol, stigmasterol, lenoleic acid, squalin and tiliroside in the aerial parts of *Kydia calycina* might be responsible for the observed antioxidant activity either singly or in synergy.

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