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Studies on phyto chemical constituents, quantification of total phenol, alkaloid content and *In-vitro* anti-oxidant activity of *Coccinia cordifolia*

B. Rao Ganga[1], P. Rao Umamaheswara*, E. Rao Sambasiva, T. Rao Mallikarjuna and V. S. Praneeth D.

1, Dept. of Pharmacognosy and Phytochemistry, Consortium Principal Investigator, NAIP/ICAR Sub project, A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, (AP) - India

Research Scholar, A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, (AP) - India

Abstract

In the present study we have extracted dried plant of *Coccinia cordifolia* in hexane, ethyl acetate, ethanol (70%v/v) and methanol which was collected from Araku, Visakhapatnam Dist, Andhra Pradesh, India. These extracts were checked for its antioxidant and their phyto chemical constituents. The whole plant of *Coccinia cordifolia* revealed the presence of steroids, triterpenoids, glycosides, saponins, tannins, alkaloids, saponins, phenols and carbohydrates but extract of *Coccinia cordifolia* give the negative results for quinines and oils. The methanolic extract has more phenolic content than other extracts and the ethanol (70%v/v) extract has more alkaloid content than other extracts. The selected plant extracts were produced concentration dependent percentage inhibition of superoxide radical and produced maximum activity at a concentration of 160 µg and there after the percentage inhibition were raised gradually to its maximum level with higher concentrations. Among the four types of *C.cordifolia* extracts, the methanolic extract showed better activity than remaining extracts at 160 µg concentrations.

Key-Words: *Coccinia cordifolia,* Whole plant, Phenolic content, Alkaloid content, *In-vitro* anti oxidant activity

Introduction

Human civilization and all living organisms are dependent on plant kingdom to a great extend for many of their daily necessities of life. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Plants are unique in their ability to synthesize carbohydrates, fats and proteins that constitute three major food classes for human race. Scientific investigation and information of the therapeutic potential of the plant material is limited. With the possibility of traditional medical practitioners being integrated into the state health delivery system, there is an urgent need to systematically evaluate plants used in traditional medicine. Such research could also lead to new drug discovery or advance the use of indigenous herbal medicines for treatment of diseases.

*** Corresponding Author:**
E-mail: umaphd2011@gmail.com
Mob. :+91-9392505841

Besides, the plants also synthesize some compounds such as glycosides, alkaloids, sterols, toxalbumins, saponins, vitamins, essential oils, resins, tannins, coloring materials which posses medicinal values and used for the treatment of different diseases [1,2].

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent Pharmacological activities, no side effects and economic viability. There are several reports in the literature regarding the anti-oxidant activity of crude extracts prepared form plants [3-5]. As a result, some natural products have been approved as new anti-oxidant drugs, but there is still an urgent need to identify novel substances. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. The oxygen molecule produces a highly reactive oxygen species (ROS) by some exogenous factors and endogenous metabolic processes in human body. ROS include a number of

chemically reactive molecules such as hydrogen peroxide (H2O2), superoxide (O2-) and hydroxyl radical (OH) [6, 7]. Reactive oxygen species and reactive nitrogen species (RNS) have been widely implicated in the pathogenicity of several degenerative diseases such as Ulcer, Liver cirrhosis, Atherosclerosis, Alzheimer's disease, Parkinson's disease and Cancer[8]. Antioxidants are reducing agents and limit oxidative damage to biological structures by passivating free radicals[9]. Antioxidant compounds may function as free radical scavengers, completers of prooxidant metals, reducing agents and quenchers of singlet oxygen formation.

Coccinia cordifolia is a member of cucumber family, producing fruits that look like miniature versions of real cucumber. Kovakka or kovakkai, the fruit of koval is 2-3 inch long and has long been used as a vegetable in Kerala, India. The leaves and shoot are also used as vegetables. Koval also has medicinal properties and is useful for persons with diabetes.

In present study we have extracted dried plant of *Coccinia cordifolia* in hexane, ethyl acetate, ethanol (70%v/v) and methanol. These extracts were checked for its antioxidant and their phyto-chemical constituents. The extracts were found to be a potent antioxidant effect.

Material and Methods

Chemicals and Drugs

All chemicals and solvents were of the analytical grade obtained from S.D. Fine Chemical Pvt. Ltd., Mumbai, Sigma Chemical Company, U.S.A., Loba Chemic, Mumbai.

Preparation of extracts:

The plant *Coccinia cordifolia* was collected from Araku, Visakhapatnam Dist, Andhra Pradesh, India, during the month of December, 2009. The authentication of the plant was done by Dr. P. Prayaga Murthy, Department of Botany, Andhra University, Visakhapatnam. Shade dried powdered whole plant of *Coccinia cordifolia* was separately extracted in a Soxhlet apparatus for 6 hrs successively with hexane, ethyl acetate, methanol and ethanol (70%v/v) were concentrated to dryness under vacuum. The weight of the powdered plant materials taken for extraction and the weight of the crude extractives obtained are given in Table

Qualitative Phytochemical Screening

A systematic and complete study of crude drugs should include a thorough investigation of both primary and secondary metabolites derived as a result of plant metabolism. Different qualitative chemical tests and quantification of total phenolic and alkaloidal contents were performed for establishing the profile of a given extract for its nature of chemical composition.

Quantification of Total Phenolic content

Total phenolic content was determined using the Folin-Ciocalteau reagent[10]. Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765nm.The intensity of the light absorption at that wave length is proportional to the concentration of phenols. By using standard Gallic acid calibration curve, measure the concentration of phenolic content in Gallic acid total equivalents using unit's mg/gm. (GAE).

Quantification of Total Alkaloid Content

Total alkaloid content was determined by the Fazel *et al.,* 2008 method[11]. The plant extract (1mg/ml) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One ml of this solution was transferred to a separating funnel and then 5 ml of BCG solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice; the results were averaged and reported in the form of Mean ± S.E.M.

***In-vitro* anti oxidant activity:**

For the assessment of free radicals scavenging activity, the hexane, ethyl acetate, ethanol (70%) and methanol extracts were dissolved in water and 5% dimethyl sulphoxide (DMSO) respectively.

Superoxide radical Scavenging activity

Superoxide scavenging activity of the plant extract was determined by McCord & Fridovich, 1969 method[12], which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity is commonly used to evaluate the free radical scavenging effectiveness of various antioxidant substances (Elizabeth and Rao, 1990)[13].

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+}/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS) [14].

DPPH radical Scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca *et al*., 2003[10]. In DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl-picrylhydrazine. Lower the absorbance higher the free radical scavengin g activity[15].

Results and Conclusion

Qualitative phytochemical screening and quantified total phenolic and alkaloidal contents of whole plant of *Coccinia cordifolia* revealed the presence of steroids, triterpenoids, glycosides, saponins, tannins, alkaloids, saponins, phenols and carbohydrates but whole plant extract of *Coccinia cordifolia* do not contain the quinines and oils.

The Quantified phenolic contents of *Coccinia cordifolia* extracts were ranging from 38.25±0.86 to 72.58±0.36 (mg/gm). The methanolic extract has more phenolic content 72.58±0.36 (mg/gm) than other extracts and the alkaloid content was ranging from 12.52±0.48 to 24.45±0.19 (mg/gm). The ethanol (70%v/v) extract has more alkaloid content 24.45±0.19 (mg/gm) than other extracts.

Results of qualitative phytochemical screening and quantified total phenolic and alkaloid contents of all extracts are shown in Table 2 and 3.

The ethanol (70%v/v), methanol, ethyl acetate and hexane extracts of *C.cordifolia* were found to possess concentration dependent scavenging activity on superoxide generated by photoreduction of riboflavin and the results are given in fig -1. The mean IC_{50} values for superoxide radical of ethanol (70%v/v), methanol, ethyl acetate and hexane extracts *C.cordifolia* were found to be 147.54µg, 107.22µg, 195.35 µg and 326.22µg respectively. The mean IC_{50} value of ascorbic acid was found to be 53.5µg. The results were given in Table-4.

The ethanol (70%), methanol, ethyl acetate and hexane extracts of *C.cordifolia* were found to possess concentration dependent scavenging activity on hydroxyl radicals and the results were given fig-2. The mean IC_{50} values for hydroxyl radical of ethanol (70%v/v), methanol, ethyl acetate and hexane extracts *C.cordifolia* were found to be 155.00µg, 119.23µg, 215.14µg and 345.43µg respectively. The mean IC_{50} value of ascorbic acid was found to be 67.8µg. The results were given in Table-4.

The ethanol (70%v/v), methanolic, ethyl acetate and hexane extracts of *C.cordifolia* were found to possess concentration dependent scavenging activity on DPPH radicals and the results were given fig-3. The mean IC_{50} values for DPPH radical of ethanol (70%), methanolic, ethyl acetate and hexane extracts *C.cordifolia* were found to be 111.00µg, 86.30µg, 128.43µg and 139.45µg respectively. The mean IC_{50} value of ascorbic acid was found to be 18.5µg. The results were given in Table-4**.**

The selected plant extracts were produced concentration dependent percentage inhibition of superoxide radical and produced maximum activity at a concentration of 160 µg and there after the percentage inhibition were raised gradually to its maximum level with higher concentrations. Among the four types of *C.cordifolia* extracts, the methanolic extract showed better activity than remaining extracts at 160 µg concentrations.

Among the samples, better free radical scavenging activity was found in methanolic extract of *C.cordifolia*, the order of activity in the following manner: ascorbic acid >methanolic extract >ethanol (70%v/v) extract > ethyl acetate extract> hexane extract.

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Table 1: Percentage of the extractives obtained from whole plant of *Coccinia cordifolia*

Name of the solvent	Weight of the powdered material (kg)	% of Soluble extractives (Wt/Wt)
Hexane	3	8.48
Ethyl acetate	3	28.56
Methanol	3	29.25
Ethanol (70%)	3	39.85

Table 2: Nature of phytoconstituents present in different extracts of whole plant of *Coccinia cordifolia*

Name of the phytochemicals	*Coccinia cordifolia*			
	Hexane extract	Ethyl acetate extract	Methanol extract	Ethanol (70%)
Phytosterols	+	+	+	+
Triterpenes	-	+	+	+
Glycosides	+	+	+	+
Saponins	-	+	+	+
Flavonoids	-	+	+	+
Tannins		+	+	+
Carbohydrates	-	+	+	+
Alkaloids	-	+	+	+
Amino acids	-	-	-	-
Oils	-	-	-	-
Quinones	-	-	-	-

Abbr.: +=Present; - = Absent

Table 3: Total phenolic and alkaloid content (mg/gm) of whole plant extracts of *Coccinea cordifolia*

Name of the extract	Total Phenolic content (mg/gm)	Total alkaloid content (mg/gm)
Hexane	38.25±0.86	12.52±0.48
Ethyl acetate	55.44±0.52	18.26±0.74
Methanol	72.58±0.36	22.34±0.74
Ethanol (70%)	55.56±0.42	24.45±0.19

Table 4: 7 50% Inhibition concentrations (IC_{50}) of different extracts of *Coccinia cordifolia* whole plant against Superoxide, Hydroxyl and DPPH radicals

Extracts	50% Inhibition Conc (IC_{50})		
	Superoxide radical	Hydroxyl radical	DPPH radical
Ethanol (70%v/v)	147.54	155.00	111.00
Methanolic extract	107.22	119.23	86.30
Ethyl acetate extract	195.35	215.14	128.43
Hexane extract	326.22	345.43	139.45
Ascorbic acid	53.50	67.80	18.50

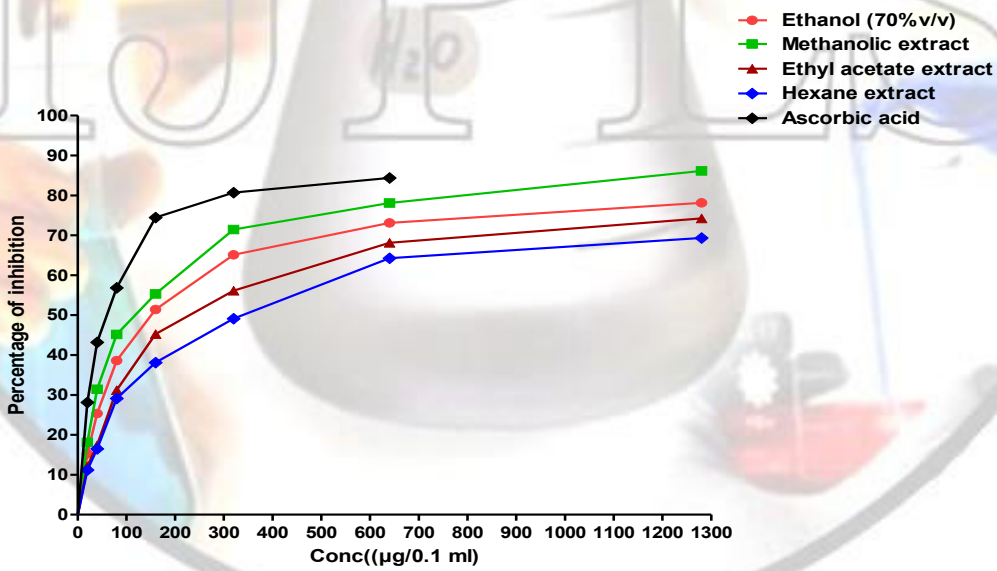

Fig 1: *In-vitro* concentration dependent percentage inhibition of Superoxide radical by different extracts of *Coccinia cordifolia* whole plant

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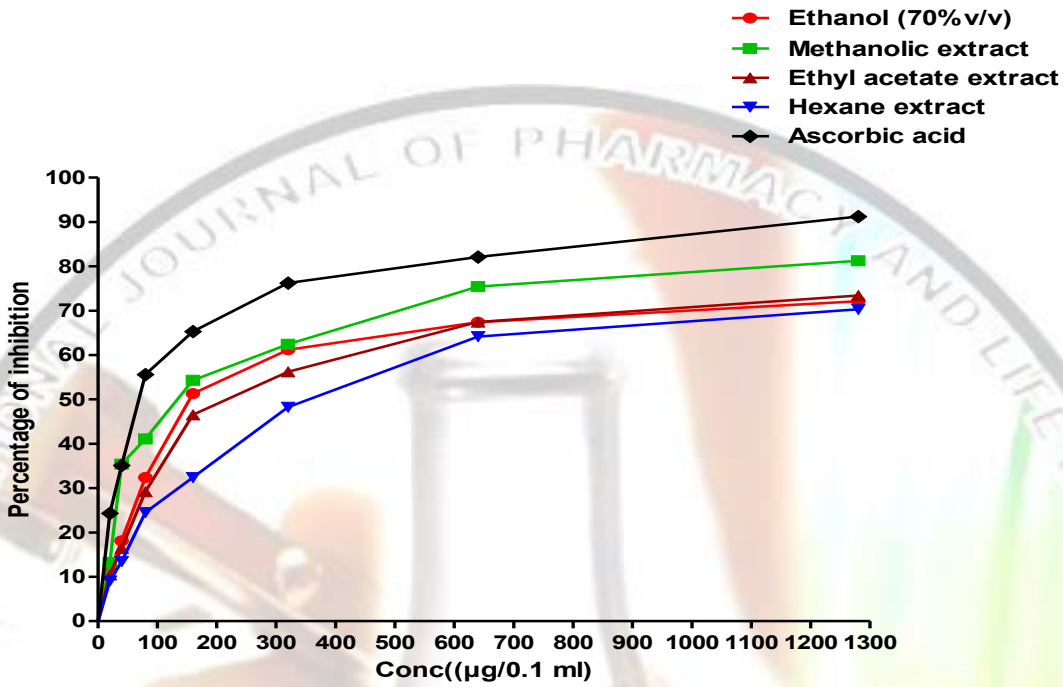

Fig 2: ***In-vitro*** **concentration dependent percentage inhibition of Hydroxyl radical by different extracts of** ***Coccinia cordifolia*** **whole plant**

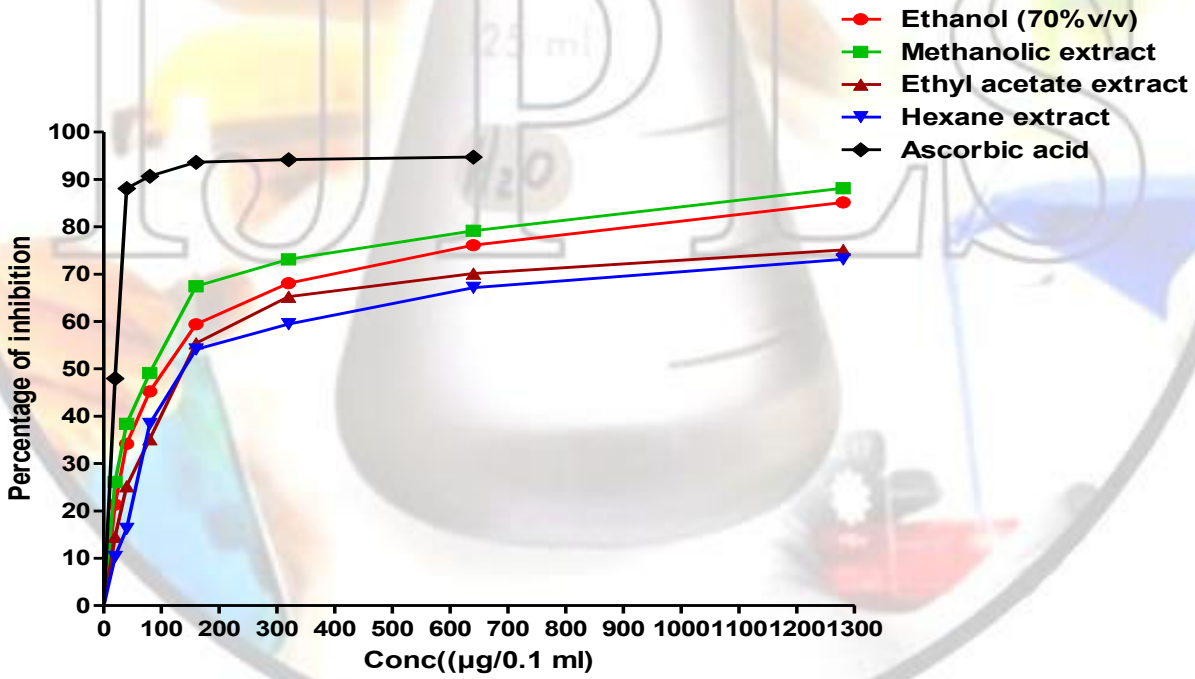

Fig 3: ***In-vitro*** **concentration dependent percentage inhibition of DPPH radical by different extracts of** ***Coccinia cordifolia*** **whole plant**