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Research Article

ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *SCOLOPIA CRENATA* (FLACOURTIACEAE) STEM BARK

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ABSTRACT

This study explored the chemical constituents of *Scolopia crenata* (Flacourtiaceae) stem bark and their role as antioxidant agents. Initially dried powder of stem bark from *Scolopia crenata* was extracted successively in hexane, ethyl acetate and methanol and screened for *in vitro* antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and preliminary phytochemical analysis was done according to standard procedures. This survey revealed the presence of phenolics & tannins, alkaloids, cardiac glycosides and steroids. The IC_{50} value obtained for DPPH inhibition were 406.425, 414.377 and 420.545 µg/ml for methanol, ethyl acetate and hexane extracts, respectively. The crude ethyl acetate and methanol fractions were subjected to column chromatography and were pooled together into five major fractions (F1 to F5) after monitoring with thin layer chromatography. The fractions thus obtained were concentrated and screened for DPPH radical scavenging activity. Fractions, F4 and F5 showed higher % inhibition of DPPH absorbance and lower IC_{50} values 366.221µg/ml and 375.963 µg/ml, respectively. In an attempt that was made to identify the chemical nature of pooled fractions F1 to F5, the results confirmed the presence of phenols, alkaloids, cardiac glycosides and steroids in different solvent elution mixtures which may be responsible for their antioxidant activity.

Keywords: *Scolopia crenata*, Antioxidant activity, Phytochemical analysis, Column chromatography, TLC

INTRODUCTION

Oxidation is essential to many living organisms for the production of energy to fuel biological process. However, oxygen-centre free radicals and other reactive oxygen species (ROS), which are continuously, produced *in vivo,* results in cell death and tissues damage. Scientific evidence has suggested that under oxidative stress conditions, oxygen radicals such as superoxide anions ($O2^-$), hydroxyl radical (OH) and peroxyl radicals (H_2O_2) are produced in biological system[1]. Moreover, this oxidative stress involving enhanced generation of reactive oxygen species (ROS) has been implicated in the etiology of over one hundred human diseases including inflammation, metabolic disorders, cellular aging and atherosclerosis, heart disease, neurodegenerative disorder, stroke, diabetes mellitus, cancer, malaria, rheumatoid arthritis and HIV/AIDS[2-4]. Antioxidants are substances that when present in foods or body at low concentrations compared with that of an oxidizable substrate significantly delay or prevent the oxidation of that substrate[5]. Antioxidants will help to minimize oxidative damage as the most important approaches to the primary prevention of age-related diseases, since antioxidant terminate direct ROS attacks and radical-mediated oxidative reactions[6]. There has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical induced damages rather than looking for synthetic ones[7]. Search for plant-derived antioxidants has been received much attention and effort in order to identify the compounds that have high capacity in scavenging free radicals related to various diseases[8]. Medicinal plants contain a variety of chemical components such as Alkaloids, Terpenes, Carbohydrates, Glycosides, Saponins etc. Phytochemical screening of the plants is primarily an important aspect. From very early times chemical plant product had received adequate attention on account of the economic importance of medicinally important active constituents. Preliminary phytochemical analysis is helpful in finding the chemical constituents in plant materials. These studies also useful for the development of small-scale industry engaged in extraction of crude herbal drugs[9].

Scolopia crenata (Flacourtiaceae family) is a rare small tree along the hill stream edges. Leaves are used for treating musco-skeletal pain[10], bark is a good diuretic, and leaf juice is an antidote for water snake bite. Tribal people use the green fruit for the treatment of night blindness. The flowers are used in the treatment of eye diseases; the seed is a rich source of rutin[11]. The biological activity of *S. crenata* has not yet been reported earlier. Therefore, our results are the first evidence demonstrating its antioxidant activity by DPPH method. The present study was designed to investigate chemical components of *Scolopia crenata*, which may contribute to its antioxidant effects.

MATERIALS AND METHODS

Collection and identification of plant

S. crenata stem bark was collected from Maredumilli forest located near Rampachodavaram (Mandal), East Godavari (district), Andhra Pradesh (State), South India with the help of a local Ayurvedic doctor. The plant was identified at Kovel Foundation, Visakhapatnam, A.P., India with collection no. KF VS 7313 and accession no. 33 with reference to passport data book of NBPGR (National Bureau of plant genetic resources) under the Division of plant exploration and collection, New Delhi.

Extraction of plant material

Plant material was brought to the laboratory and washed under running tap water and blotted dry with filter paper and then shade dried on laboratory benches on top of newspaper. After having completely dried, the plant material was then ground into powder with a hand mill. 100 g of the powder was extracted successively in hexane, ethyl acetate and methanol by using 250 ml of each solvent for soaking. Maceration was carried out in each solvent for four days at room temperature (35 ± 2 °C). The solvent of each extracted material were concentrated *in vacuo* at 40 °C using a rotary evaporator (PBU 6D model; Superfit). The crude extracts were preserved in a freezer at -20 °C until use. The successive extract weights were 0.40, 2.42 and 13.29 % (w/w) for hexane, ethyl acetate and methanol, respectively.

Chemicals

1, 1-diphenyl-2-picrylhydrazyl were purchased from Sigma Chemical Company, St. Louis, USA), Silica gel G (Qualigens, India for TLC) Silica gel G (Qualigens, India for column chromatography). All the chemicals and reagents used were of analytical grade.

Preliminary phytochemical screening

All extracts were analyzed for the presence of alkaloids, saponins, cardiac glycosides[12], tannins, phenols and flavanoids[13-14], and steroids[15].

DPPH free radical scavenging activity

Hexane, ethyl acetate and methanol fractions of *Scolopia crenata* were screened for DPPH radical scavenging activity. DPPH radical scavenging activity was measured according to the method of Braca *et al* [16]. An aliquot of 3ml of 0.004% DPPH solution in ethanol and 0.1ml of plant extract at various concentrations were mixed and incubated at 37 ºC for 30 min. and absorbance of the test mixture was read at 517nm. All experiments were performed three times in time and in space the results were averaged.

The percentage of inhibition of DPPH radical was calculated as

$$\text{Percentage of Inhibition} = ((A_0 - A_1)/ A_0) \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance with addition of plant extract/ascorbic acid.

The optical density obtained with each concentration of test sample plotted taking concentration on X-axis and percentage inhibition on Y-axis, the graph was extrapolated to find the 50% inhibition concentration (IC_{50}) of the test sample.

Column chromatography

The fractionation of ethyl acetate (2.42 g) and methanol extract (13.29 g) was carried out on silica gel G-60 (100-200 mesh). Column was packed using n-hexane. Then the column was eluted first with hexane followed by polarity of the system was raised by increasing the quantity of ethyl acetate in hexane, methanol in ethyl acetate and H_2O in methanol. 5 ml fractions were collected and the fractions having similar compounds were pooled together after monitoring with thin layer chromatography. Visualization of the TLC chromatograms was achieved by iodine vapors. Eleven number of ethyl acetate fractions were obtained and were labeled (EaA to EaK). Seven number of methanol fractions were obtained and were labeled (MeA to MeG). The fractions were further subjected to phytochemical screening by using TLC method, those fractions that gave similar spots and Rf with specific TLC methods were again pooled together into five major fractions (F1 to F5).

Separation of secondary metabolites by thin layer chromatography (TLC)

The ethyl acetate and methanol fractions of *Scolopia crenata* obtained from column fractionation were subjected to thin layer chromatographic analysis, to find the presence of number of chemical constituents to support the chemical test.

TLC is used to separate wide range of compounds of biochemical interest. It can be utilized for quantitative assays as well as for qualitative and preparative work[17]. The chromatographic separation of compounds occurs because of differing in their Rf values with respect to the solvent used in the mobile phase.

Thin Layer Chromatography (TLC) was done on analytical plates over silica gel G (TLC-grade; Qualigens, India) in appropriate solvent system:

TLC study of alkaloids

The alkaloids were separated using the solvent mixture chloroform and methanol in the ratio of 15:1. The color and Rf values of the separated alkaloids were recorded under visible light after spraying with Dragendorff's reagent[18].

TLC study of glycosides

The glycosides were separated using EtOAc-MeOH-H_2O (80:10:10) solvent mixture. The color and Rf values of separated glycoside were recorded by heating the developed plate at 110 to 120 ºC for 5 minutes and observed under ultraviolet light (UV 254 nm) after spraying with Marquis reagent when plate was in hot condition.

TLC study of phenols

The phenols were separated using chloroform and methanol (27:0.3) solvent mixture. The color and Rf values of these phenols were recorded under visible light after spraying the plates with Folin-Ciocalteu's reagent heating at 80 ºC/10min[19].

TLC study of sterols

The sterols were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The colour and Rf values of these spots were recorded under visible light after spraying the plates with anaisaldeyde - sulphuric acid reagent and heating (100 ºC for 6 min)[18].

RESULTS AND DISCUSSION

The phytochemical screening of the crude extracts of *Scolopia crenata* (Table 1) revealed the presence of phenolics & tannins, alkaloids, cardiac glycosides and steroids. Phenolics & tannins and alkaloids were only present in methanol extract where as cardiac glycosides and steroids were present in all the three extracts.

Table 1: Results of preliminary phytochemical screening of three crude plant extracts of *Scolopia crenata*.

Phytochemical tests	HX	EA	ME
Tannins and phenols	-	-	++
Saponins	-	-	-
Alkaloids	-	-	+
Steroids	+	+	+
Cardiac glycosides	+	+	+
Flavonoids	-	-	-
Carbonyls	-	-	-

+ = presence; - = absence

HX = Hexane extract, EA = Ethyl acetate extract, ME = Methanol extract

Percentage inhibition of DPPH and IC_{50} are parameters widely used to measure antioxidant/ free radical scavenging power[20-23]. The higher the percentage inhibition of DPPH absorbance the higher the FRSA (free radical scavenging assay) and the lower the IC_{50} value the higher the FRSA/antioxidant power, ascorbic acid was used as positive control. Results from the DPPH inhibition shows that methanol extract is more potent than ethyl acetate followed by hexane as a free radical scavenger (Fig 1). The IC_{50} values obtained for DPPH inhibition were 406.425, 414.377, 420.545 and 394.564µg/ml for methanol, ethyl acetate, hexane and ascorbic acid, respectively.

Eleven number of ethyl acetate fractions (EaA to EaK) and 7 number of methanol fractions (MeA to MeG) that gave similar spots and Rf with specific TLC methods were again pooled together into five major column fractions (F1 to F5). Where F1 and F2 are of ethyl acetate fractions obtained with solvent elution mixture, for F1 (hexane: ethyl acetate) and F2 (ethyl acetate: methanol). F3, F4, and F5 are of methanol fractions obtained with solvent elution mixture, for F3 and F4 (ethyl acetate: methanol) and F5 (methanol: water). These fractions were concentrated and the residues weights obtained were 0.36 g, 0.30 g, 0.21 g, 4.79 g and 4.68 g for fractions F1 to F5, respectively. The fractions thus obtained were screened for DPPH radical scavenging activity and phytochemical analysis was carried using specific TLC methods.

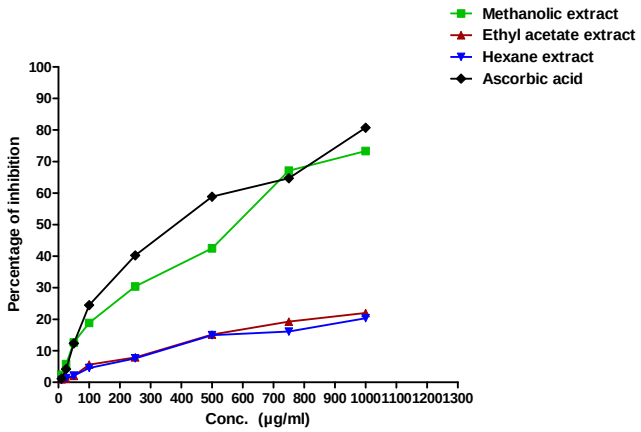

Fig. 1: Concentration dependent percentage inhibition of DPPH radical by different crude extracts of *Scolopia crenata* and Ascorbic acid in *in vitro* studies

The results of the free radical scavenging activity of the DPPH assay of five major column fractions were shown in (Fig 2). The IC_{50} values obtained for DPPH inhibition of five major column fractions are 402.178 µg/ml, 454.327 µg/ml, 409.342 µg/ml, 366.221 µg/ml, 375.963 µg/ml and 394.564 µg/ml for first fraction (F1), second fraction (F2), third fraction (F3), fourth fraction (F4), fifth fraction (F5) and ascorbic acid, respectively. The results revealed that fractions F4 and F5 are having higher percentage inhibition of DPPH absorbance and lower IC_{50} values of 366.221 µg/ml and 375.963 µg/ml, respectively when compared to IC_{50} value of ascorbic acid (394.564 µg/ml). The total antioxidant activity of some Australian *Flacourtiaceae* species were assessed based on scavenging activity of stable ABTS free radicals, among them the leaf extract of *Casearia* sp. (RB 3051), the mature stem extract of *Casearia grayi* and the stem extract of *Scolopia braunii* had the highest antioxidant activity (IC_{50} = 2.9 µg/ml)[24].

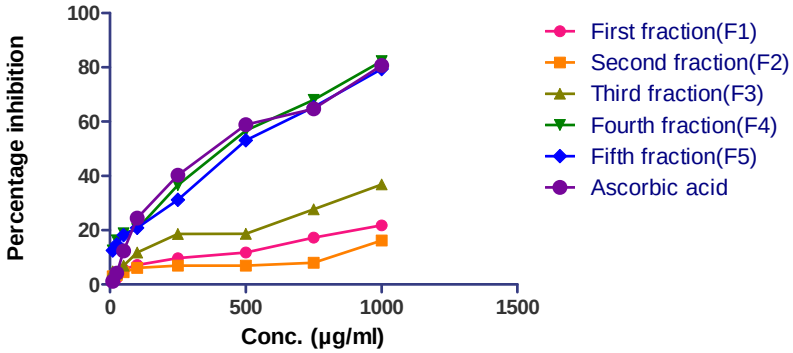

Fig. 2: Concentration dependent percentage inhibition of DPPH radical by five major column fractions of *Scolopia crenata* and Ascorbic acid in *in vitro* studies

The phytochemical screening of five major column fractions F1 to F5 (Table 2) confirmed the presence of phenols, alkaloids, cardiac glycosides and steroids in different fractions based on the difference in solvent elution mixtures which may be responsible for their antioxidant activities.

Phytochemicals are an integral part of the human diet due to their wide abundance in fruits and vegetables. They have attracted considerable interest as potential anticancer agents. They prevent oxidative damage as a result of their ability to scavenge reactive oxygen species. By, contrast the prooxidant properties of these compounds could contribute to tumor cell apoptosis and anticancer action[25]. Enzymatic antioxidants and non enzymatic antioxidants and oxidant detoxifiers have the ability to inhibit tumor initiation and promotion *in vivo* and *in vitro*[26].

Table 2: Detection of Phytochemicals in five major column fractions by Thin Layer Chromatography Study

Compounds	Silica gel G column fractions									
	F1		F2		F3		F4		F5	
	Color	Rf	Color	Rf	Color	Rf	Color	Rf	Color	Rf
Alkaloids	Brown	0.41	No spot	-	Brown	0.51	Brown	0.48	Brown	0.50
	Brown	0.59			Brown	0.81			Brown	0.95
	Brown	0.79								
Cardiac glycosides	Brown	0.95	No spot	-	No spot	-	Light Brown	0.5	Brick red	0.91
Steroids	Pink	0.7	No spot		No spot	-	Orange	0.92	Orange	0.95
Phenols	No spot	-	Blue	0.5	Blue	0.6	green	0.7	Blue	0.7

Rf = Retardation Factor

Considerable emerging evidence supports the inhibitory actions of phytosterols on lung, stomach, as well as ovarian and breast cancer[27]. Phytosterols seem to act through multiple mechanisms of action, including inhibition of carcinogen production, cancer-cell growth, angiogenesis, invasion and metastasis, and through the promotion of apoptosis of cancerous cells. Phytosterol consumption may also increase the activity of antioxidant enzymes and thereby reduce oxidative stress[27].

The β-carboline alkaloids found in medical plants and in a variety of foods, beverages and cigarette smoke have a range of action in various biological systems. *In vitro* studies have demonstrated that these alkaloids can act as scavengers of reactive oxygen species. Antioxidant properties of β-carboline alkaloids are related to their antimutagenic and antigenotoxic activities[28].

Cardiac glycosides are naturally cardio active drugs used in the treatment of congestive heart failure and cardiac arrhythmia[29]. The class of steroid-like compounds designated cardiac glycosides includes well-known drugs such as digoxin, digitoxin, and ouabain. Their continued efficacy in treatment of congestive heart failure and as anti-arrhythmic agents is well appreciated. New findings within the past five years have revealed these compounds to be involved in complex cell-signal transduction mechanisms, resulting in selective control of human tumor but not normal cellular proliferation. As such, they represent a promising form of targeted cancer chemotherapy[30].

Phenolics have been reported to have a capacity to scavenge free radicals. They are commonly found in both edible and non-edible plants and have multiple biological effects, including antioxidant activity[31-32]. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential[33]. Phenolics, such as flavonoids, phenolic acids, stilbenes, lignans, lignin, and tannins, are especially common in leaves, flowering tissues, and woody parts, such as stems and barks[34]. The role of phenols in possessing antioxidant activity is reviewed. The potent scavenging property of different extracts of *Phyllanthus emblica* can be due to hydroxyl groups present in the phenolic compounds[35].

CONCLUSION

The extracts of stem bark of *Scolopia crenata* showed potent antioxidant activity and contain biologically active compounds, including alkaloids, steroids, cardiac glycosides and phenols in different fractions obtained with different solvent mixtures. In conclusion, the study suggests that these achieved compounds may probably have a role as antioxidant agents. However, further investigations are required for identification of active principle(s), responsible for these effects.

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