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Research Article

Phytochemical Screening and Evaluation of Antioxidant Activity of *Symploco Racemosa Roxb*

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ABSTRACT

Use of Antioxidant in cancer treatment is a rapidly evolving area. Antioxidants have been extensively studied for their ability to prevent cancer in humans. *Symplocos racemosa roxb* is an indigenous plant having ample medicinal application. Ethanolic extract of *Symplocos racemosa roxb* was subjected to various phytochemical screening tests. Antioxidant activity of the plant was evaluated against swiss albino mice. Result indicates that plant contains flavonoids, steroids, terpenoid, saponins, tannins, proteins and essential oil. Moreover ethanolic extract of Symplocos racemosa roxb had exhibited very good antioxidant activity.

Keywords: *Symplocos racemosa roxb,* Antioxidant activity, Ethanolic extract

INTRODUCTION

Oxidative stress has been implicated in tumor initiation and progression. Reactive Oxygen Species (ROS) have been demonstrated to participate in regulation of cell proliferation and differentiation, as well as to be implicated in apoptosis induced by diverse stimuli, such as cytotoxic antitumor drugs, ionizing radiation and TNF-□ . Antioxidant tends to reduce free radical formation and scavenge free radical. Despite the fact that humans have evolved with antioxidant system to protect against free radicals, which may be endogenous or exogenous, some ROS still escape in quantities sufficient enough to cause damage. Therefore exogenous antioxidants that scavenge free radicals, especially, those from the relatively harmless natural sources play an important role in cardiovascular disease, aging, cancer and inflammatory disorders as well as in ameliorating drug-induced toxicity. This has accelerated the search for potential antioxidants from traditional medicinal plants. The present study aims to investigate the free radical scavenging activities of the plant to evaluate its anticancer potential as an antioxidant.

Symplocos Racemosa Roxb (*Symplocaceae*) is distributed throughout North East India, up to 2,500 ft., from the terai of Kumaon to Assam and Pegu, Chota Nagpur, Burma. It is a small evergreen tree with stem up to 6 m. height and 15 cm diameter.

Bark is useful in bowel complaints such as diarrhea, dysentery, in dropsy, eye disease, liver complaints, fevers, ulcers, scorption-string etc. Bark is often employed in the preparation of plasters and is supposed to promote maturation or resolution of stagnant tumors.[1] In cases of menorrhagia due to relaxation of the uterine tissue, given two to three times a day for three to four days alcoholic extract and watery extract of "lodh" as astringent for looseness of the bowels. A decoction of the bark or wood is used as gargle for giving firmness to spongy and bleeding gums and relaxed uvula. It is one of the constituent of a plaster or lap used to promote maturation of

boils and other malignant growths. Knowledge of chemical constituent of the plant is not only essential for the discovery of therapeutic agents but also for economical source of the alkaloids carbohydrates etc.[2-6]

MATERIALS AND METHODS

Plant *Symplocos racemosa roxb* belonging to the family Asteraceae, is an herb commonly found in hilly districts. It was identified by the survey of medicinal plant and collection unit of R.B. Patel Pharmacy College, Atkot.

Extraction

The leaves and flowering top of *Symplocos racemosa roxb* was dried under shade and then crushed into powder with a mechanical pulveriser and passed through sieve No. 8 and then sieve No-16.The dried powder material of plant was extracted with 95% ethanol in soxhlet apparatus about 55 hours. The solvent was evaporated by vacuum distillation under reduced pressure

Preliminary Phytochemical Screening

The petroleum ether extract and ethanol extract were subjected to chemical tests to identify chemical constituent of the plant. [7-10]

Test for Sugars: Small quantity of extract was dissolved in 4 ml of distilled water and filtered and the filtrate was subjected to Molisch's test and Iodine Test.

Test for Glycosides:A few mg of residue was dissolved 4 ml of distilled water and filtrated and the filtrate was subjected to Legal Test and Borntrager's test

Test for Flavonoids (Shinoda test): The extracts were dissolved in alcohol. One piece of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta color demonstrated the presence of flavonoids.

Test for Sterols (Salkowaski test): 10 mg of extract was dissolved in 2 ml of chloroform and 2ml of concentrated sulphuric acid was added from the side of the test tube. Test tube was shaken for few minutes. The development of red color in chloroform layer indicated the presence of sterols.

Liebermann–Burchard Test: 1 ml of concentrated sulphuric acid was added to 10 mg of extract in 1ml of chloroform. A reddish–blue color exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of sterols.

Test for Alkaloids: Few mg of extract was taken in 5 ml of 1.5% v/v hydrochloric acid and filtered. These filtrates were then used for testing alkaloids.

Dragendorff's test: Dragendorff's reagent was added in 2ml of filtrate. Formation of orange-brown precipitate indicated the presence of alkaloids.

Mayer's test: To 1ml of test filtrate in a watch glass, a few drops of mayers reagent were added. If the formation of cream colored precipitate it shows the presence of alkaloids.

Test for Tannins: The test extract was taken in water, warmed and filtered. 5 ml of filtrate was allowed to react with 1ml of 5% ferric chloride solution. If dark green or deep blue color is obtained, tannin is present.

Test for Saponins: (a) Foam test**:** 1ml solution of extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Development of stable foam suggests the presence of saponins.

b) 1ml extract was treated with 1% lead acetate solution. Formation of white precipitates indicates the presence of saponins.

Test for Terpenoids (Knollar's test): 5 mg of extract is treated with 2ml of 0.1% anhydrous stannic chloride in pure thionyl chloride. A deep purple color that changes to red indicates the presence of terpenoids.

Test for Protein and Amino Acids: Small quantity of the extract was dissolved in few ml of water and filtered. Filtrate was subjected to Millons test and Biuret test

Test for Resins: Few mg of extract was treated with caustic soda a red color was developed if resins are present.

ANTIOXIDANT ACTIVITY

Animals

Swiss albino mice weighing between 20-25 gm were used. They were fed with standard pellet diet of water and *ad-libtum*. The mice were acclimatized and laboratory condition for 10 days before commencement of experiment.

Tumor Transplantation

Ehrlich's Ascitic carcinoma was maintained by serial transplantation from tumor bearing Swiss Albino mice. Ascitic fluid was drawn out from tumor bearing mice at the log phase (day 7-8 of tumor bearing) of the tumor cells and used for transplantation intrperitoneally.[11]

Drug Treatment Schedule

Swiss albino mice were divided into 5 groups (n = 6). First normal group, second control group, third standard group and fourth and fifth group with doses 100mg/kg and 300mg/kg with plant extracts respectively. All the groups were injected with Ehrlich ascites carcinoma (EAC) cells (0.2 ml of 2 X 10^6 cells/mouse) intraperitoneally except the normal group. This was taken as day zero. From the first day normal saline 5 ml/kg/mouse/day and propylene glycol 5 ml/kg/mouse/day was administered to normal and EAC control groups respectively for 14 days orally. Standard drug vitamin C was administered in third group. Similarly plant extract of different doses (100 mg and 300 mg/kg/mouse/day) were administered in fourth and fifth groups respectively, after the administration of last dose followed by 18 hrs, fasting three mice form each group were sacrificed for the study of antitumor activity, hematological and liver biochemical parameters. The remaining animals in each of the groups were kept to check the mean survival time (MST) and percent increase in life span of the tumor bearing hosts.[12-14]

Biochemical Assay

After the collection of blood samples the mice were sacrificed and their liver was excised. The isolated liver was rinsed in ice cold normal saline followed by cold phosphate buffer having pH 7.4, and blotted dry and weighed. A 10% w/v homogenate of liver was prepared in ice cold phosphate buffer (pH 7.4) and a portion were utilized for estimation of lipid peroxidation and other portion of the same after precipitating proteins with trichloroacetic acid (TCA) was used for estimation of glutathione remaining homogenate were centrifuged at 1500 rpm at 4^{o}C for 15 min. The supernatant thus obtained was used for the estimation of superoxide dismutase, catalase and protein content. [15]

Estimation of lipid peroxidation

Malondialdehyde (MDA), a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reactive substances (TBARS). Briefly, to 0.2 ml of tissue homogenate, 0.2 ml of 8.1% sodium lauryl sulfate (SLS), 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% thiobarbituric acid (TBA) were added in succession. The volume of mixture was made upto 4 ml with distilled water. The mixture was incubated for 60 min. at 95^{o}C in a temperature control water bath and cooled. Then, 5 ml of n-butanol: pyridine (15:1) mixture was added and the content was centrifuged thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min., the upper organic layer was separated and absorbance was read at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides were expressed as n moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of 1.56×10^5 cm^{-1}. [16-18]

Estimation of catalase

Catalase activity was measured. 0.1 ml of supernatant was added to cuvette containing 1.9 ml of 50 mm phosphate buffer solution (pH 7.4). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mm hydrogen peroxide. The role of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalane was expressed as unit / mg protein.[16-18]

Estimation of protein content

According to Alen H (1995) method, 10% w/v liver homogenate was prepared in phosphate buffer solution (pH 7.4) and used for the estimation of protein content. The prepared homogenate were centrifuged at 1500 rpm for 15 min. at 4^{o}C. The supernatant was used for estimation. Test solution was prepared by using 0.2 ml serum, 5 ml biuret reagent and 3 ml distilled water standard solution was prepared by using 3 ml solution of bovine albumin and 5 ml biuret reagent. The transmittance of the sample was read against the blank at 540 nm in UV visible, double beam spectrophotometer. The amount of protein was expressed in gm of protein in 100 ml[18] .

Estimation of reduced glutathione (GSH)

To measure the reduced glutathione (GSH) level, the tissue homogenate (in 0.1 m phosphate buffer (pH 7.4) was taken. The homogenate was added with equal volume of 20%, trichloroacetic acid (TCA) containing 1 mm EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 min, prior to centrifugation for 10 min at 200 rpm. The supernant (200 ml) was then transferred to a new set of test tubes and 1.8 ml of the Ellman's reagent (5,5, dithio-bis-2-nitrobenzoic acid)(0.1 mm) was prepared in 0.3 m phosphate buffer with 1% sodium citrate solution. Then all the test tubes make up to the volume of 2 ml. After completion of the total reaction, solution was measured at 412 nm against blank.[15, 16, 18]

Estimation of superoxide Dismutase (SOD)

SOD activity of the liver tissue was analyzed. Assay mixture contained 0.1ml of sample, 1.2ml of sodium pyrophosphate buffer (PH 8.3,0.052M), 0.1ml of phenazine methosulphate (186mm),0.3ml of 300mm nitro blue tetrazolium, 0.2 ml Nicotinamide adenine dinucleotide NADH (750mm). Reactions were started by addition of NADH. After incubation at 30˚ c for 90s, the reaction was stopped by the addition of 0.1ml of glacial acetic acid. Reaction mixture was stirred vigorously with 4.0 ml of n-butanol. Mixture was allowed to stand for 10min; centrifuged and n-butanol layer was separated. Color intensity of the chromogen in the butanol layer was measured at 560nm spectrophotometrically and concentration of SOD was expressed as units/mg protein.[15]

Statistical analysis

All the data were analyzed by using one way analysis of variance (ANOVA) and results are expressed as mean ± SEM.

RESULTS AND DISCUSSION

Phytochemical Screening of the plant material

Preliminary phytochemical screening showed that of *symplocos racemosa* contain higher amount of contains flavonoids, steroids, terpenoid, saponins, tannins, and protein. (Table 1)

Table 1. Preliminary phytochemical screening of the two extracts of *symplocos racemosa*.

Phytochemical test	Ethanol (95%v/v)
Carbohydrate	--
Glycoside	--
Alkaloid	--
Flavonoid	++
Protein and amino acid	++
Tannin	++
Saponin	++
Steroids	++
Terpenoid	++

++ = Present, -- = Absent

Antioxidant Activity

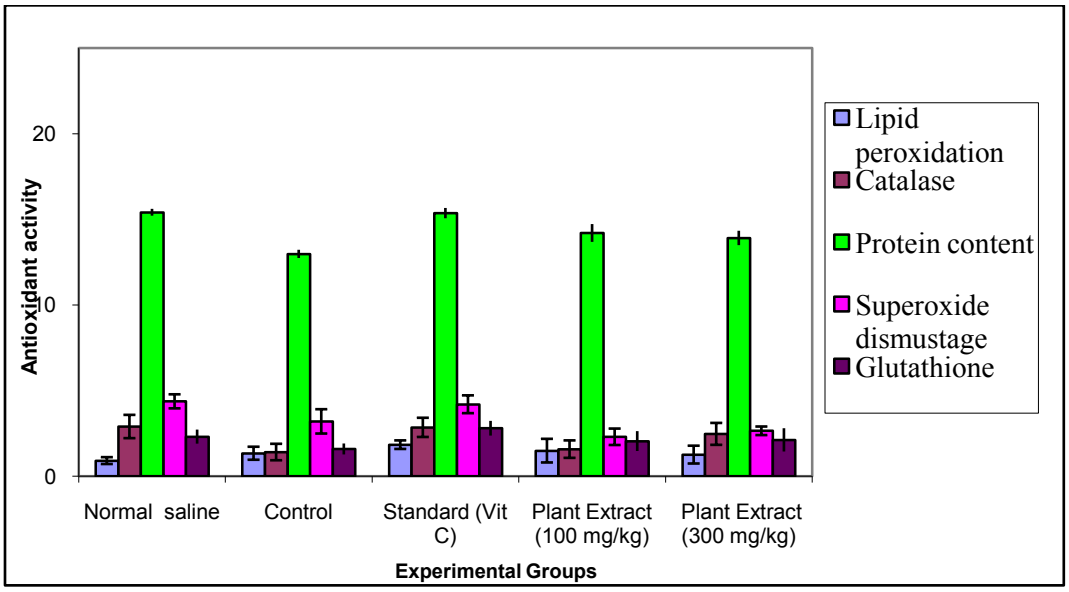
The level of lipid peroxidation, GSH, SOD, catalase and protein content were summarized in table 2. Lipid peroxidation mediated by free radicals considered being a primary mechanism of cell membrane destruction and cell damage. The oxidation of unsaturated fatty acids in biological membrane leads to a reduction in membrane fluidity and disruption of membrane structure and function. With reference to this, the active role of GSH against cellular lipid peroxidation has been well recognized and thereby reduces the reduced glutathione (GSH) can act either to detoxify activated oxygen species such as H_2O_2 or reduce lipid peroxides themselves. In the present study indicated that ethanolic extract of *Symplocos racemosa roxb* significantly reduced the elevated levels of lipid peroxidation and increased the level of glutathione content and thereby it may act as an antitumor agent. On the other hand, SOD is a ubiquitous chain breaking antioxidant and is found in all aerobic organisms. It is a metalloproteinase widely distributed in all cells and plays an important protective role against ROS induced oxidative damage. Catalase are present in all major organs in the body of animals and human being and is especially concentrated in liver and erythrocytes. Both enzymes play an important role in the elimination of ROS derived from the redox process of xenobiotics in liver tissues. It was suggested that catalase and SOD are easily inactivates by lipid peroxide or ROS. In correlation, it has been reported that EAC bearing mice showed decreased level of SOD activity and this may be due to loss of Mn^{++} SOD activity in liver. Inhibition of catalase activity in tumor cell lines was also reported. In this study, catalase and SOD were appreciably elevated by administration of plant extract as compared to EAC control mice, suggesting that it can restore the level of SOD and catalase enzymes.

Table 2: Effect of different doses of ethanolic extract of Symplocos racemosa roxb (Clarke) on different biochemical parameter in EAC bearing mice

GROUPS		Lipid peroxidation n mole MDA/gm of tissue	Catalase (units /mg tissues)	Protein content (gm / 100 ml)	Superoxide dismustage	Glutathione
1	Normal saline 0.5 ml/kg	0.91± 0.2	2.90 ± 0.68	15.54±0.2	4.37 ± 0.41	2.31 ± 0.4
2	control 2 X 10^6 cells / mice	1.34 ± 0.38	1.41 ± 0.48	12.98 ± 0.24	3.20 ± 0.71	1.59 ± 0.32
3	Standard (Vit C)	1.84 ±0.25	2.85±0.56	15.36±0.3	4.2±0.52	2.8±0.43
4	Plant Extract (100 mg/kg)	1.49±0.69	1.58 ± 0.51	14.20±0.52	2.30 ± 0.48	2.05 ± 0.58
5	Plant Extract (300 mg/kg)	1.26±0.52	2.47 ±0.64	13.91 ± 0.42	2.65 ± 0.25	2.12± 0.68

Values are mean ± SEM, No. of mice in each group (n = 6), EAC control group was compared with normal group, *P<0.5, experimental groups were compared with EAC control

Figure 1: Graphical Representation effect of plant extract on Different biochemical parameter counts in EAC bearing mice



CONCLUSION

From present investigation, it is clear that *s.racemosa* possess good anticancer potential by reducing lipid peroxidation , superoxide dismustage and catalase activity.

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