



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203



Human Journals

Research Article

April 2017 Vol.:9, Issue:1

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Pharmacological Study of *Symplocos racemosa* Roxb



IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals



ISSN 2349-7203

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Submission: 7 April 2017
Accepted: 12 April 2017
Published: 25 April 2017



HUMAN JOURNALS

www.ijppr.humanjournals.com

Keywords: *Symplocos racemosa*, antioxidant, DPPH, Anti-inflammatory, radical scavenging

ABSTRACT

Objective: To investigate phytochemical screening, antioxidant, anti-inflammatory and cytotoxic activity of *Symplocos racemosa*. **Methods:** For phytochemical screening of *Symplocos racemosa* qualitative by some common methods with standards test were done. Detection of antioxidant activity was measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in colorimeter. Anti-inflammatory assay was done at Clinico lab, Mulund (E), Mumbai using whole human blood. **Results:** Phytochemical screening showed qualitatively presence of flavonoid and phenolic, saponin, tannins and glycosides qualitatively. The promising antioxidant activity as absorption of DPPH radicals decreased in DPPH free radical scavenging assay. Anti-inflammatory activity revealed moderate protection at 18% at 1000ug/ml concentration. **Conclusion:** The *Symplocos racemosa* extract revealed the presence of potent phytochemicals and anti-inflammatory bioactive constituents which are known to exhibit medicinal as well as physiological activity.

INTRODUCTION

Symplocos racemosa Roxb. (Family: Symplocaceae) is a widely used ayurvedic remedy for various ailments. It is also known as lodhra and is used in Indian System of Medicine (ISM) as single drug or in multicomponent preparations (viz. lodhrasava). Its bark is acrid, digestible, and astringent to bowels. It is useful in treatment of fever, eye diseases, for spongy gums and bleeding (Ambasta SP,1986, Chopra *et al*,1956). It cures 'Kapha', diseases of the blood, leprosy, dropsy and liver complaints. It is also useful in abortions and miscarriages and for ulcers of vagina. Traditionally bark is given in menorrhagia and other uterine disorders. Unani medicine uses it as emmenagogue, aphrodisiac. It is a potent remedy for inflammation and cleaning uterus. (Kirtikar and Basu, 1987)

Symplocos racemosa possesses cardiogenic, antipyretic, anti helminthics and laxative properties. It is beneficial in billow fever, urinary discharge, blood troubles, burning sensations, leucoderma and jaundice. In Indian traditional medicine, the bark is also useful in bowel complaints such as diarrhea, dysentery, liver complaints, fever; ulcer etc. The bark of this plant also possesses anticancer activity. The study indicates that the ethanol extract of *Symplocos racemosa* exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC-bearing mice. In addition, researchers have evaluated the antibacterial effect of *S. racemosa* extracts against acne inducing bacteria. *Symplocos racemosa* is used in Indian System of Medicine for various female disorders. In vivo effect of aqueous extracts of *Symplocos racemosa* on serum FSH and LH levels in immature female Sprague–Dawley rats under basal conditions has been observed. There are also lots of scientific literature data proving the different pharmacological activity of *Symplocos racemosa* extract, e.g. gonadotropin releasing, antioxidant, antiarthritic and antibacterial.(Devmurari (2010), Choudhary *et al.* (20 04), Vijayabaskaran *et al.*(2010c), Gopala Krishna *et al.* (2013), Sharma *et al.* (2013), Shailajan *et al.* (2011).

A number of psychopharmacological studies are being reported on this plant *S. racemosa* in order to provide scientific support to various traditional uses. Majority of the reports indicated usefulness of the stem bark for the treatment of gynecological disorders, fever, inflammation and liver disorders. Several flavonoid glucosides like symplocoside, symposide, leucopelargonidine-3 glucoside, ellagic acid, rhamnetin 3-digalactoside, triterpenoids like 19 α -hydroxy acetic acid-3, 28-O-bis- β -glucopyranosides, betulin, lino-leic acid, β -sitosterol and α -amyrin (Badoni *et al.*, 2010 ; Nagore *et al.*, 2012) and alkaloids like

loturine, loturidine, colloturine and harmine (De Silva *et al.*, 1979; Ishida *et al.*, 2001) have been described as chief bio actives from the plant.

In the present study, effort is made to study phytochemical, antioxidant and anti-inflammatory activity of *Symplocos racemosa* Roxb.

MATERIALS AND METHODS

Plant material (Bark) was collected from an ayurvedic shop from Mulund, Mumbai. Plant materials of *Symplocos racemosa* Roxb. were extracted using soxhlet extractor using ethanol as solvent for extraction. After extraction process was completed, the solvent was removed or recovered by means of rotary evaporator.

Phytochemical Analysis:

Extracts were tested for the presence of active principles such as Terpenoids, Saponins, Alkaloids, Flavonoids, Tannins, Phenol, and Lipids. Analysis of phytochemicals is done by standard methods (Harborne, 1973).

Antioxidant Assay:

Mandal *et al.* (2009) method was used for antioxidant determination. Methanolic extract of plant material was used with varying concentration ranging from (1-100ug/ml) for *Symplocos racemosa* roxb. Stock solution was added according to varying concentration in TEST & BLANK tube. After adding stock and diluents, DPPH reagent was added in dark in TEST and incubated for half an hour. Absorbance was measured at 517nm spectrophotometrically. Ascorbic acid has been used as a standard.

The DPPH radical scavenging activity was calculated from the absorption value by the following equation

$$\% \text{ radical Scavenging} = \frac{\text{Absorbance of Control} - (\text{Absorbance of Test} - \text{Absorbance of Blank})}{\text{Absorbance of Control}} * 100$$

Anti-inflammatory assay:

Materials used for Anti-inflammatory assay was whole human blood obtained from Clinico Laboratory, Mulund (E). The blood sample was diluted by adding into approximately 50ml of phosphate buffer saline. From the diluted sample, approximately 12-15 ml of blood was drawn in centrifuge tube and it was subjected to centrifugation. After centrifugation, the supernatant was discarded and the pellet was again mixed with some amount of blood to get more concentrated RBC. Then absorbance was measured at 560 nm to check 8×10^9 cells/ml. This RBC sample was then used for the experimental purpose.

The method originally devised by N. Sampath Kumar *et al.* (2011) was used with slight modification. Test sample for plant under study was prepared in (0.25 %) hypotonic saline to produce a range of concentration (25-100 μ g/ml). Aliquot was taken from the stock solution and various concentrations were prepared. After adding test sample, total volume was made up to 1 ml by adding hypotonic saline. From this 25 μ l of mixture was discarded. Rapidly 25 μ l of RBC suspension was added in each tube containing 8×10^9 cells/ml and was shaken gently, incubated at 45°C for 30 minutes in water bath. After incubation, the reaction was terminated by a rapid high speed centrifugation at 3500rpm for 1minute. After centrifugation, supernatant was transferred to another eppendorf tube and absorbance was measured at 560 nm. From the measured absorbance % protection for membrane, stabilization was determined.

RESULTS

Preliminary phytochemical analysis:

Phytochemical screening of plant extract of *Symplocos racemosa* showed the presence of various constituents which are shown in table 1. The preliminary phytochemical tests result indicates the presence of phenols, flavonoids, saponins, tannins, glycosides, steroids while terpenoids and alkaloids were found to be absent.

Table-1. Test for Phytochemicals

Phytochemical constituents	Method Followed	Result
Phenols	Ferric Chloride Test	+
Flavonoids	Alkaline Reagent Test	+
Saponins	Foam Test	+
Tannins	Braemer's Test	+
Glycosides	Legal's Test	+
Steroids	LiebermannBurchrad's Test	+
Alkaloids	Wagner's Test	-
Terpenoids	Salkowski Test	-

Keys:

+ Presence of the compounds

- Absence of the compounds



Table- 2. Antioxidant assay of Alcoholic extract of *Symplocos racemosa* by DPPH method

Concentration (µg/ml) Standard Ascorbic acid	% radical scavenging	Concentration (µg/ml) Test extract of <i>Symplocos racemosa</i>	% radical scavenging
5	19.61	5	40.32
10	22.33	10	59.57
15	35.03	25	69.70
25	56.45	50	78.59
50	96.26	75	84.18
		100	87.60

Testing the antioxidant activity, *Symplocos racemosa* gave highest % radical scavenging at 100µg/ml tested by DPPH methods. By DPPH method, the % radical scavenging at 100µg/ml was found to be 87.60 when compared it with standard

Table- 3 Alcoholic extract of *Symplocos racemosa* for Anti-inflammatory assay

Concentration (ug/ml) of Standard	%protection	Concentration <i>Symplocos racemosa</i> (µg/ml)	%protection
100	12.4	100	7.11
200	16.3	500	14.17
400	22.8	700	17.90
600	26.98	1000	18.98
800	30.15		
1000	30.29		

By anti-inflammatory testing, it was found that at 1000µg/ml, *S.racemosa* provided 18.98% protection, which is expected a decent value.

DISCUSSION

Traditional literature indicated that total 32 formulations contain *S. racemosa* bark as one of the major ingredients as mentioned in Ayurvedic Pharmacopoeia of India (2001) for the treatment of various ailments. Phytochemical investigations on this plant in present study have shown presence of many phytoconstituents. Similarly, various researchers have isolated active constituents from this plant and systematically evaluated for the several biological activities. Majority of the flavonoids and related compounds have been isolated from aerial parts of the plant while glycosides of different types have been extracted from the polar fractions of the bark of the plant (De Silva *et al.* 1979).

The ethanolic extract of *S. racemosa* bark showed good antioxidant activity in present study which can be compared with Vijayabaskaran *et al.*, 2010 which showed (10 and 30mg/kg) leaves and flowering tops showed significant antioxidant activity by reducing the extent of lipid peroxidation, superoxide dismutase and catalase activity in Swiss Albino mice. The ethanolic extract of *S. racemosa* bark showed potent ABTS radical scavenging activity,

moderate DPPH, nitric oxide and hydroxyl radical scavenging activity compared with the standard drugs ascorbic acid. Naik *et al.*(2014) have evaluated the antioxidant activity of the ethanolic extract of *S. racemosa* bark by scavenging DPPH free radicals with IC 50 value of 120µg/mL.

CONCLUSION

The present study was carried out for “Evaluation of phytochemicals, antioxidant and anti-inflammatory activity screening of *S. racemosa*.”

The present study reveals that the extract of *S. racemosa* was good source of phytochemicals which include flavonoids, phenols, tannins, saponins and glycosides. The study also indicated that the extract of plant material possibly prevents the effect of oxidative stress exhibiting % radical scavenging activity. This may be due to present of antioxidants such as flavonoids and other phenolic compounds and can be used as natural antioxidant and as a possible food supplement in pharmaceutical industries. *In-vitro* study indicates the importance of plant extracts as a source of preventing the progress of various oxidative stresses.

Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract along with other bioactive components with sophisticated methods.

For bark extract of *Symplocos racemosa*, 700µg/mL concentration appears to be the apt concentration, as it exhibits significant Antioxidants which is around 78.59% radical scavenging activity. At the same range of concentration, it showed very low irritation potential and low cytotoxicity to the chick embryo fibroblast cells. At this concentration, the extract provided 17.9% protection.

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