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In vitro Antioxidant Potential of Methanolic Extract of *Symplocos racemosa* Roxb.

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Many hazardous diseases are caused by free radicals accumulation. A number of investigations are focusing to find out the plant oriented natural antioxidant moieties. The basic aim of this research was to investigate the antioxidant potential of different plant parts of the crude methanolic extract of *Symplocos racemosa. Symplocos racemosa* (Symplocaceae) commonly known as "Lodhra" and is useful in diarrhoea, dysentery, eye diseases, fever, ulcer, scorpion sting, diabetes and liver disorders. Here the bark, leaf and root were screened for *in vitro* antioxidant activity study using DPPH, nitric oxide, hydroxyl radical and ABTS assay methods. Both ascorbic acid and rutin are used as a standard. The results of the study indicated that the methanolic extract of *Symplocos racemosa* showed potent antioxidant activity in ABTS assay method than other methods. These research investigations revealed that *Symplocos racemosa* is a potent source of natural antioxidants. Hence the plant can be used for management of different stress and anxiety related ailments.

Keywords: *Symplocos racemosa*, Methanolic extract, Antioxidant activity.

INTRODUCTION

In all type of the living cells biochemical and physiological course of action taking place which result in the production of harmful free radicals and reactive oxygen species [1]. The biomolecular moieties such as DNA, proteins and lipids are damaged by these free radicals and reactive oxygen species, ultimately become leading source of different chronic serious ailments like cancer, aging, diabetes, atherosclerosis *etc*. [2]. A defense shield in the form of dietary antioxidants from plants is needed to overcome this hazard nature.

For the management of various ailments medicinal plants play an important role [3-8]. Strong antioxidants are present in plants because plants are richly supplied with vitamins, flavonoids coumarins, phenolics, terpenoids, tanins and alkaloids, *etc*. [9]. So, medicinal plants contain many key compounds that can be used for the management of oxidative stress induced diseases [10,11]. A number of investigational and epidemiological studies for the positive outcome by intake of antioxidant moieties of plant origin have been published [12,13].

Symplocos racemosa (Symplocaceae) commonly known as "Lodhra" in Sanskrit or "Rodhra". It is a small evergreen tree up to 6 m tall. It is found in the plains and lower hills through out North and East India [14]. The bark is dark grey and rough and is useful in diarrhea, dysentery, eye diseases, fever, ulcer, scorpion sting, diabetes and liver disorders [15]. It has been scientifically reported as an antimicrobial [16], anticancer [17] and has beneficial effects in gynaecological disorders [18]. As there is no published data for the antioxidant potential of different parts of *Symplocos racemosa*, so this study was designed to investigate the antioxidant potential of the plant.

EXPERIMENTAL

Extraction of plant: The plant *Symplocos racemosa* (Family: Symplocaceae) was collected from Patbil, Karanjia, Mayurbhanj, India. Further, it was authenticated by the taxonomist Dr. Pratap Chandra Panda, Senior Scientist, Regional Plant Resources Centre (RPRC), Bhubaneswar, India and its voucher specimen (Voucher No. 10122) was deposited in the Herbarium of RPRC for further reference. After due authentication the barks, leaves and roots were dried in shade and powdered to obtain coarse powder. The coarse powder material (250 g) was subjected to extraction with methanol (95 % v/v) by using Soxhlet apparatus. The methanol extract was concentrated *in vacuo* and kept in a vacuum desiccator for complete removal of solvent. The yield was 9.3 % w/w with respect to dried powder [19].

2,2-Diphenyl-1-picrylhydrazyl (DPPH) [Sigma-Aldrich, India], methanol, dimethyl sulphoxide, sodium nitroprusside, naphthyl ethylene diamine dihydrochloride, sulphanilic acid, potassium persulphate, ferric chloride, hydrogen peroxide, thiobarbituric acid, trichloro acetic acid, ascorbic acid and rutin were purchased from Merck India Ltd.

Pharmacological screening

Antioxidant assay: The antioxidant activity of plant extract was determined by different *in vitro* methods such as DPPH free radical scavenging assay, nitric oxide, hydroxyl radical and ABTS assay method. All the assays were carried out in triplicates and average values were considered.

Diphenyl-1-picrylhydrazyl (DPPH) assay: DPPH assay was performed according to the procedure of Brand-Willium *et al.* [20]. For this purpose 0.1 M DPPH (1,1-diphenyl-2-picrylhydrazyl) solution was made in methanol and absorbance of the solution was adjusted at 0.95 at 515 nm. Sample (100 µL) was mixed with 1 mL DPPH solution and incubated at 37 °C for 30 min. Methanol was used as control. After 30 min absorbance was noted at 515 nm. Ascorbic acid was used as standard.

DPPH scavenging activity was calculated according to following formula and IC_{50} was calculated.

Inhibition (%) = [(Absorbance of control – Absorbance of sample)/Absorbance of control] × 100

Nitric oxide scavenging activity: Nitric oxide scavenging activity was carried out using the alkaline dimethyl sulfoxide (DMSO) method [21]. Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use; the filtrate (200 µL) was added to 2.8 mL of an aqueous solution containing nitroblue tetrazolium (56 µM), EDTA (10 µM) and potassium phosphate buffer (10 µM, pH 7.4). Test solutions at different concentrations (5-100 µg/mL) were added and absorbances were recorded at 540 nm against the control.

Azino-*bis*(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) scavenging activity: Protocol of Re *et al.* [22] was adopted for evaluation of ABTS scavenging activity. ABTS solution (7 mM) was mixed with potassium oxidopersulphate (2.45 mM) solution and was placed in the dark for 12-16 h to get a dark coloured ABTS working solution. The solution was diluted with 50 % methanol and absorbance was adjusted at 0.7 (± 0.02) at 734 nm. Sample (100 µL) was mixed with 1 mL of ABTS working solution and decrease in absorbance was read 1 min after adding the sample and then up to 6 min. Percentage inhibition was calculated according to following formula:

Inhibition (%) = [(Absorbance of control – Absorbance of sample)/Absorbance of control] × 100

Hydroxyl radical scavenging assay: This activity was determined by following the protocol of Halliwell and Gutteridge [23]. Reaction solution consisted of 2-deoxyribose 500 mL (2.8 mM) in phosphate buffer (50 mM, pH 7.4), 200 mL premixed ferric chloride (100 mM) solution (1:1; v/v), 100 mL H_2O_2 (200 mM) and extract solution (100 mL). Ascorbate 100 mL (300 mM) was added to the reaction solution and incubated for 1 h at 37 °C. TBA solution 1 mL (1 %; w/v in 50 mM NaOH) and 1 mL TCA (2.8 %; w/v aqueous solution) were added to the reaction solution. Reaction solution was heated in boiling water bath for 15 min and then was allowed to cool. Absorbance was noted at 532 nm and scavenging activity of hydroxyl radical was calculated as follow:

Scavenging activity (%) = (1 – absorbance of sample/absorbance of control) × 100

Statistical analysis: Results were analyzed as mean ± SEM from triplicate observations. *In vitro* antioxidant assays were analyzed by ANOVA test followed by Tukey' test ($P < 0.05$) to find out the significant differences among IC_{50} of different fractions in each assay.

RESULTS AND DISCUSSION

***In vitro* antioxidant potential:** *In vitro* antioxidant potential of the plant was determined using different analytical assays. All antioxidant assays provide considerable support to antioxidant prospective of plant in comparison with standard ascorbic acid.

Crude methanolic extract may contain thousands of phytochemical constituents. Concentration of sample at which the inhibition percentage reaches 50 % is the IC_{50} value. IC_{50} value is negatively related to the antioxidant activity, as it expresses the amount of antioxidant needed to decrease the radical concentration by 50 %. The lower the IC_{50} value, the higher is the antioxidant activity of the tested sample. The IC_{50} values of MESRB, MESRL and MESRR for DPPH radical, nitric oxide radical, hydroxyl radical, ABTS radical scavenging activities are summarized in Table-1.

***In vitro* antioxidant potential of *Symplocos* bark:** MESRB exhibited potent ABTS radical scavenging activity with IC_{50} value 41.35 ± 0.49 µg/mL which is significantly lower than those of the standard ascorbic acid and rutin. The IC_{50} value of extract for DPPH, nitric oxide radical, hydroxyl radical was 130.76 ± 6.53, 573.8 ± 8.20 and 131.2 ± 6.20 µg/mL, respectively, which were significantly higher than those of the standard ascorbic acid and rutin. According to the results in Table-1, the MESRB possessed moderate antioxidant activity.

***In vitro* antioxidant potential of *Symplocos* leaf:** MESRL exhibited potent ABTS radical scavenging activity with IC_{50} value 38.35 ± 0.51 µg/mL which is significantly lower than those of the standard ascorbic acid and rutin. The IC_{50} value of extract for DPPH, nitric oxide radical, hydroxyl radical was

TABLE-1
FREE RADICAL SCAVENGING ACTIVITY OF METHANOLIC EXTRACT OF *Symplocos racemosa*

Extract/Standards	IC_{50} values ± SEM mg/mL* by methods			
	DPPH	Nitric oxide	ABTS	H_2O_2
Ascorbic acid	2.65 ± 0.03^{a}	–	11.15 ± 0.44^{a}	187.30 ± 1.85^{d}
Rutin	–	65.34 ± 2.50^{b}	0.50 ± 0.02^{a}	36.46 ± 0.24^{b}
MESRB	130.76 ± 6.53^{c}	573.8 ± 8.20^{d}	41.35 ± 0.49^{b}	131.2 ± 6.20^{c}
MESRL	127.43 ± 5.42^{b}	518.6 ± 8.10^{d}	38.35 ± 0.51^{b}	129.3 ± 6.02^{b}
MESRR	121.04 ± 6.01^{b}	497 ± 7.94^{d}	30.91 ± 0.44^{b}	115.5 ± 5.98^{b}

*Average of triplicate determinations. Values were mean ± SEM different letter (a-d) represent significance ($P < 0.05$).

127.43 ± 6.53, 518.6 ± 8.20 and 129.3 ± 6.20 µg/mL, respectively, which were significantly higher than those of the standard ascorbic acid and rutin. According to the results in Table-1, the MESRL possessed moderate antioxidant activity.

***In vitro* antioxidant potential of *Symplocos* root:** MESRR exhibited potent ABTS radical scavenging activity with IC_{50} value 30.91 ± 0.49 µg/mL, which is significantly lower than those of the standard ascorbic acid and rutin. The IC_{50} value of extract for DPPH, nitric oxide radical, hydroxyl radical was 121.04 ± 6.53, 497 ± 7.94 and 115.5 ± 5.98 µg/mL, respectively, which were significantly higher than those of the standard ascorbic acid and rutin. According to the results in Table-1, the MESRR possessed moderate antioxidant activity.

In pathological manifestations free radicals play a key role. Phyto-originated constituents perform their role by acting as a defense shield to protect the antioxidant defense mechanism [24]. The antioxidant potential of the plants have been evaluated by a number of techniques [25] and the plant constituents are more secure than their synthetic counterparts [26,27].

Preliminary phytochemical screening of *Symplocos racemosa* demonstrated the presence of nearly all active constituents of the plant such as alkaloid, carbohydrate, glycoside, steroid, protein, tannin, terpenoid, flavonoid and phenolic compounds [28]. All these constituents have been shown to exhibit strong antioxidant scavenging activity for the radicals that are involved in the lipid peroxidation [10,29]. These active constituents of plants play important role in the treatment of different diseases such as tanins possess anti-inflammatory and anticancer activity [30,31]; flavonoids are antioxidant, antiinflammatory and anticancer agents [32]; while alkaloids possess antileukemic and anticancer activity [33].

Extraction yield of different plant derived fractions varied widely; highest methanol extract yield proved that methanol behaves as a good solvent for compound extraction.

DPPH, a purple coloured bleaching solution, is an important source of free radical and is frequently used to measure the electron donating ability of the plant [34].

Results justify that the plant has ABTS radical scavenging activity and proved that the plant may be used for the treatment of radical related stress appreciable due to ABTS radical quenching ability [10].

Hydrogen peroxide is detrimental reactive oxygen radical that become toxic and damage the cell when converted into hydroxyl radical that may initiate lipid peroxidation and DNA mutations [35]. Present investigations suggested that all plant extracts were capable of quenching this radical, this may be due to their phenolic contents that converts H_2O_2 to water.

Consequently, it is claimed that potent antioxidant activity was present in plant leaves due to which it can be used as a potential source of antioxidant agents. Vijayabaskaran *et al.* [36] also reported *in vitro* antioxidant potential of ethanolic bark extract of *Symplocos racemosa* Roxb.

Conclusion

The results of the study indicated that the methanolic extract of *Symplocos racemosa* showed potent antioxidant activity in ABTS assay method than other methods and can be used as accessible source of natural antioxidants and a possible food supplement. Natural product antioxidants significantly contribute in preventions of pathological consequences caused by free radicals. Moreover, plant derived antioxidants are safer and cheaper than their synthetic counterparts. We concluded from this research that *Symplocos racemosa* possesses strong antioxidant potential and it would be advantageous to use the plant antioxidant in therapeutic drugs for the implications of human health.

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