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Original Article

ANTIOXIDANT ACTIVITY AND CYTOTOXIC POTENTIAL OFHYDROALCOHOLIC EXTRACT OF *SALACIA FRUTICOSA* (ROOT)-*IN VITRO*

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ABSTRACT

Objective: To evaluate the antioxidant and cytotoxic potential of hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson-root by various *In-Vitro* methods.

Methods: The various concentration of hydroalcoholic extract was evaluated for antioxidant activity and cytotoxic potential using standard methods like ABTS, hydroxyl, DPPH, nitric oxide radical scavenging activity, reducing power assay and MTT assay respectively.

Results: Antioxidant activity showed that the IC_{50} values of extract was found to be 13.62 µg/ml, 134.64µg/ml, 35.53 µg/ml & 66.23 µg/ml in ABTS, hydroxyl, DPPH & nitric oxide radical scavenging activity respectively and reducing power gets increased with increasing the concentration of extract which was compared with the standards ascorbic acid and rutin. The cytotoxic potential showed that the IC_{50} values of extract was found to be 129.13 µl/ml, 151.33 µl/ml, 203.25 µl/ml and 302.33 µl/ml for Human Colon Cancer Cells (HT-29), Human Liver Cancer Cells (HepG2), Human Breast Cancer Cells (MCF-7) and Normal Human Dermal Fibroblast (NHDF) respectively. The extract showed dose dependent inhibition of viable cells & produced potent proliferative inhibitory action on cancer cell lines. The relative sequence of sensitivity to extract was observed to be HT-29>HepG2>MCF-7>NDHF cell lines.

Conclusion: Thus our results conclude that the hydroalcoholic extract of *Salacia fruticosa*-root was found to be potent antioxidant & anticancer agent. Further studies are undergoing in order to clarify their molecular mechanisms by isolating the active principle present in the root.

Keywords: *Salacia fruticosa,* ABTS assay, MTT assay, HT-29.

INTRODUCTION

Cancer is medically called as malignant neoplasm, in which the cells divide and grow uncontrolled, forming malignant tumors and invading nearby parts of the body through the lymphatic system and blood stream [1]. Chemoprevention involves the use of specific natural or synthetic chemical agents to reverse, suppress, or prevent carcinogenesis before the development of invasive malignancy [2]. Among FDA approved anticancer agents, 60% of drugs were obtained from natural origin. Since reactive oxygen radicals play an important role in carcinogenesis, plants with antioxidants property received considerable attention as cancer chemopreventive agents [3, 4].

Salacia fruticosa Heyne ex Lawson belongs to the family Hippocrateaceae/Celastraceae, commonly known as Ponkarandi in Malayalam, Korandi in Tamil. It is a woody climbing shrub, which is native to India (Karnataka, Tamilnadu & Kerala) and Srilanka. In Tamilnadu, it is mostly seen in Dindugul & Kanniyakumari [5-7]. *Salacia fruticosa* contains abundant amount of phytoconstituents in which, *Root* contains Friedelan-3-one-29al, friedelan-3-one-29ol, friedelin, friedel-1-en-3-one, amyrin, Sitosterol, Salacinol, kotalanol, kotalagenin-16 acetate, magniferin, epicatechin, glycosidal tannins, triterpines, hydroxy ferruginol, lambertic acid [5,8,9]. Root is used for treating gonorrhea, rheumatism, obesity and skin diseases, antidiabetic, antihypertensive, hepatoprotective, anticaries and anticancer potentials [7, 9-11]. There is no pharmacological & scientific data available to substantiate their activity. Thus we have undertaken this study to screen the preliminary phytochemical, *In-vitro* antioxidant and cytotoxic potential of Hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson–root.

MATERIALS AND METHODS

Collection and authentication of plant

The root of *Salacia fruticosa* Heyne ex Lawson was collected in the month of February, 2014 from Tirunelveli, Tamilnadu. The root was identified and authenticated by Retired Scientist Dr. V. Chelladurai, Research Officer–Botany, CCRAS & Govt. of India.

Preparation of extract [12]

The extract is prepared by the cold maceration method. The root of *Salacia fruticosa* was cut into pieces; shade dried and then coarsely powdered using mechanical grinder. Then it is passed through sieve no: 40 and powder was subjected to cold maceration using sufficient quantity of ethanol and water (70:30). This process was carried out with stirring the mass once daily for 10 days until the extraction was completed. On final day, it was strained and pressed. The expressed liquids were added to the strained liquids and the combined liquids were clarified by filtration and the filtrate was subjected to distillation at temperature 60 °C for removing the solvent. Then the solidified residue was collected the colour was noted & then stored in dessicator.

Fig. 1: Root-Cut pieces of *Salacia fruticosa* Heyne ex Lawson

Phytochemical screening [13, 14]

The hydroalcoholic extract of root of *Salacia fruticosa* was subjected to qualitative and quantitative analysis. The following chemical tests was performed to determine the presence of phytoconstituents like carbohydrates, glycosides, alkaloids, phenolic compounds, tannins, flavanoids, steroids, saponins & etc., The results were shown in table 1 & 2.

Evaluation of *in vitro* antioxidant activity

Based on the phytochemical constituents present in the extract of *Salacia fruticosa*, the antioxidant potentials was studied using methods: Scavenging of ABTS Radical Cation, Scavenging of Hydrogen peroxide, Scavenging of DPPH radical, Scavenging of Nitric oxide Radical, Reducing power assessment. The Final concentration of our plant extract and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25 and 15.625 μg/ml in different screening methods for screening antioxidant activity. The absorbance was measured spectrophotometrically against the corresponding blank solutions. The percentage inhibition was calculated by using the following formula

$$\text{Radical scavenging activity (\%)} = \frac{OD_{control} - OD_{sample}}{OD_{control}} X\ 100$$

IC_{50}, which is the concentration of the sample required to scavenge 50% of free radicals was calculated.

Scavenging of ABTS radical cation [15, 16]

Preparation of extract and standard solutions

Accurately weighed 13.5 mg of the extract and standards (ascorbic acid and rutin) were dissolved separately in 2 ml of freshly distilled DMSO. These solutions were serially diluted with freshly distilled DMSO to obtain the lower dilutions.

Procedure

ABTS (54.8 mg, 2 mM) was dissolved in 50 ml of distilled water and potassium per sulphate (0.3 ml, 17 mM,) was added. The reaction mixture was left to stand at room temperature overnight in dark before usage. To 0.2 ml of various concentrations of the extract/standards added 1.0 ml of freshly distilled DMSO and 0.16 ml of ABTS solution to make a final volume of 1.36 ml. After 20 min, absorbance was measured spectrophotometrically at 734 nm.

Scavenging of Hydrogen peroxide [17]

Preparation of extract and standard solutions

Accurately weighed 30 mg of the extract and standards (ascorbic acid and rutin) were dissolved separately in 10 ml of methanol. These solutions were serially diluted with methanol to obtain the lower dilutions.

Procedure

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1 ml of the extract or standard in methanol were added to 2 ml of hydrogen peroxide solution in PBS. After 10 min, the absorbance was measured at 230 nm.

Scavenging of DPPH radical [18]

Reagents

2, 2-Diphenly 1-picryl hydrazyl solution (DPPH, 100 μM)

Preparation of extract solutions

Accurately weighed 21 mg of the extract and dissolved in 1 ml of freshly distilled DMSO separately to obtain solutions of 21 mg/ml concentrations. These solutions were serially diluted separately to obtain the lower concentrations.

Procedure

The assay was carried out in a 96 well microtitre plate. To 200 μl of DPPH solution, 10 μl of the extract or standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37 °C for 30 min and the absorbance of each solution was measured at 490 nm using ELISA reader.

Scavenging of nitric oxide radical [19]

Reagents

Sodium nitroprusside solution (10 mM), Naphthyl ethylene diamine dihydrochloride (NEDD, 0.1%), Sulphanilic acid reagent (0.33% w/v)

Preparation of extract solutions

Accurately weighed 21 mg of the extract and dissolved in 1 ml of freshly distilled DMSO separately to obtain solutions of 21 mg/ml concentrations. These solutions were serially diluted separately to obtain the lower concentrations.

Procedure

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, pH 7.4, 1 ml) and extract or standard (1 ml) in DMSO at various concentrations and it was incubated at 25 °C for 150 minutes. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotisation. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink coloured chromophore was formed. The absorbance was measured at 540 nm.

Reducing power assessment [20]

Chemicals and reagents

Potassium ferricyanide ($K_3Fe(CN)_6$, 1%): Trichloracetic acid (TCA, 10%), Ferric chloride ($FeCl_3$) solution (0.1%), Phosphate buffer pH 6.6.

Preparation of test solutions

10 mg of the extract was weighed accurately and dissolved in 10 ml DMSO to get 1 mg/ml solution. The solution was diluted with DMSO to obtain lower concentrations.

Preparation of standard solutions

Ascorbic acid: 10 mg of ascorbic acid was weighed accurately and dissolved in 10 ml distilled water to get 1 mg/ml solution. This solution was diluted with water to obtain lower concentrations.

Rutin: 10 mg of rutin was weighed accurately and dissolved in 10 ml methanol to get 1 mg/ml solution. This solution was diluted with methanol to obtain lower concentrations.

Procedure

1 ml of extract, 2.5 ml of phosphate buffer, 2.5 ml of $K_3Fe(CN)_6$ were incubated at 50 °C for 20 min, 2.5 ml trichloroacetic acid (TCA) was added to the mixture and centrifuged for 10 min at 3000 rpm. From the upper part 2.5 ml was diluted with 2.5 ml of distilled water and shaken with 0.5 ml fresh $FeCl_3$. The absorbance was measured at 700 nm after 20 min. The blank solution contained distilled water instead of the samples.

Results of the *in vitro* antioxidant activity were shown in the table 3.

Screening of *in vitro* cytotoxic potential

Cell viability assay [21]

Principle

MTT [(3-(4,5-dimethylthiazol-2yl)-2,5-phenyltetrazoliumbromide] measures the metabolic activity of the viable cells. The reaction between MTT and 'mitochondrial dehydrogenase' produces water-insoluble formazan salt. This method involves culturing the cells in a 96-well microtiterplate, and then incubating them with MTT solution for approximately 2 hours. During an incubation period, viable cells convert MTT to a water-insoluble formazan dye. The formazan dye in the MTP is solubilized and quantified with an ELISA plate reader. The absorbance directly correlates with the cell number. This is applicable for adherent cells cultured in MTP.

Materials

RPMI-1640 media, penicillin-G, streptomycin, amphotericin-B, EDTA, trypan blue, SDS lysis buffer and MTT (3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide) were procured from Himedia, Mumbai, India. Fetal bovine serum was obtained from Gibco's, USA, Trypsin (Trypsin-EDTA [1x] in HBSS) from Gibco's UK and Dimethyl sulphoxide (DMSO) from Merck India Ltd, Mumbai, India.

Additional equipments required

CO_2 incubator, Laminar air flow cabin, Refrigerated centrifuge, ELISA-reader, Deep freezer, Ultrasonic bath, Vaccum pump, Pipettes, Culture plates, Centrifuge tubes, Eppendorf tubes, Aerosol resistant tips, Flat-bottomed 96-well MTP, tissue culture grade.

Cell lines

Normal Human Dermal Fibroblast (NHDF), Human Liver Cancer cells (HepG2), Human Breast Cancer cells (MCF-7) and Human Colon Cancer cells (HT-29) were obtained from the National Centre of Cell Sciences (Pune, India).

Procedure

0.1 ml of the cell suspension (containing $1x10^5$cells) and 0.1 ml of the extract (31.25-1000 µg/ml) in DMSO were added to the 96 well plates and kept in carbon dioxide incubator with 5% CO_2, at 37 °C for 72 hours. Blank contains only cell suspension and control wells contain 1% DMSO and cell suspension. After 72 hours, 20 µl of MTT was added and kept in carbon dioxide incubator for 2 hours followed by 80 µl of lysis buffer (15%SLS in 1:1 DMF and water). The plate was covered with aluminum foil to protect it from light. Then the 96 well plates are kept in the rotary shaker for 8 hours. After 8 hours, the 96 well plates were processed on ELISA reader for absorption at 562 nm. The readings were averaged and viability of the test samples was compared with DMSO control. The percentage growth inhibition was calculated using the following formula

$$\% \text{ growth inhibition} = 100 - \frac{\text{Mean OD of Individual Test Group}}{\text{Mean OD of Control group}} \text{ X } 100$$

Results of *in-vitro* cytotoxic potential on various human cancer and normal cell line were compared by IC_{50} values to produce the cytotoxic potential.

RESULTS

Preliminary phytochemical screening

Quantitative analysis

The dried and coarse powdered root of *Salacia fruticosa* Heyne ex Lawson was extracted by cold maceration method. The residue obtained was viscous brown and the percentage yield was found to be 14.39% w/w which is shown in table 1.

Qualitative analysis

The hydroalcoholic extract of *Salacia fruticosa*–Root showed the presence of various active components shown in table 2.

Table 1: Percentage yield of hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson–Root

Plant name & part used	Extract	Colour	Nature	Percentage yield (W/W)
Salacia fruticosa Heyne ex Lawson-root	Hydroalcoholic extract	Brown	Viscous	14.39%

Table 2: It shows the result of preliminary phytochemical screening of hydroalcoholic extract of *Salacia Fruticosa* Heyne ex lawson-root

S. No.	Phytoconstituents	Name of the test	Hydroalcoholic extract
1	Carbohydrates	Molisch's Test	+
		Fehling's test	+
		Barfoed's test	-
		Benedict's test	+
		Selliwanoff's test	-
2	Glycosides	Legal's test	+
		Borntrager's test	-
		Keller-killiani test	-
		Conc. H_2SO_4 test	+
3	Alkaloids	Dragendroff' reagent	+
		Mayer's reagent	-
		Wagner's reagent	-
		Hager's reagent	+
4	Protein and amino acid	Million's test	+
		Ninhydrin test	-
		Biuret test	-
		Xanthoproteic test	+
5	Phenolic compounds and tannins	$FeCl_3$ test	+
		Lead acetate test	+
		Ellagic acid test	+
6	Flavanoids	Alkaline reagent test	+
		Shinoda's test	+
		Ferric chloride test	+
		Fluorescence test	+
		Conc. H_2SO_4 test	+
7	Fixed oil and fats	Spot test	-
		Saponification test	-
8	Steroids and triterpenoids	Libermann-Burchard test	+
		Salkowski test	+
9	Saponins	Foam test	+
10	Mucilage and gum	With 90% alcohol	-

'+'indicates Present; '-' indicates absent

***In-vitro* antioxidant activity**

In ABTS radical scavenging activity of Hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson–Root was, compared with the standards ascorbic acid was shown in the table 3. The IC_{50} values of extract and ascorbic acid were found to be 13.62 µg/ml, and 8.73 µg/ml. The Hydrogen peroxide radical scavenging activity of Hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson–Root

was compared with the standards ascorbic acid and rutin was shown in the table 3. The IC_{50} values of extract, ascorbic acid and rutin were found to be 134.64 µg/ml, 158.3 µg/ml and 44.45 µg/ml. The DPPH free radical scavenging activity of Hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson–Root was compared with the standards ascorbic acid and rutin was shown in the table 3. The IC_{50} values of extract, ascorbic acid and rutin were found to be 35.53 µg/ml, 6 µg/ml and 5.72 µg/ml. The nitric oxide radical scavenging activity of Hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson–Root was compared with the standards rutin was shown in the table 3. The IC_{50} values of extract and rutin were found to be 66.23 µg/ml and 62.45 µg/ml.

Fig. 2 shows the reducing power of hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson-Root was compared with the standards ascorbic acid and rutin. Absorbance of the extracts was increased when the concentration increased. A higher absorbance indicates a higher reducing power.

Table 3: *In vitro* antioxidant activities of hydroalcoholic extract of *Salacia fruticosa* on various assay models

Hydroalcoholic Extract/Standards	IC_{50} (µg/ml)*			
	ABTS	DPPH	H_2O_2 radical scavenging	Nitric oxide scavenging assay
Hydroalcoholic extract	13.62±1.104	35.5±1.79	134.64±4.29	66.23±1.18
Rutin	-	5.72±0.39	44.45±0.66	62.45±1.48
Ascorbic acid	8.73±0.28	3.6±0.13	158.3±2.31	-

Values are expressed as mean±SEM, *Average of three independent determinations

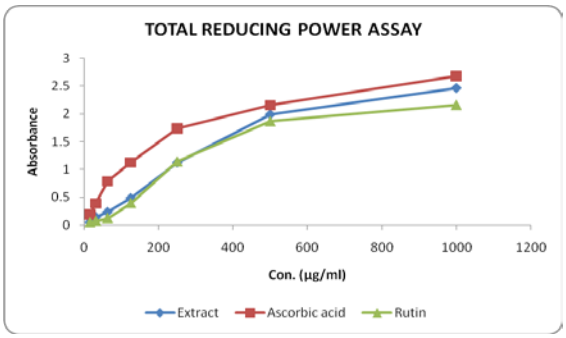

Fig. 2: Reducing power assay of Hydroalcoholic extract of *Salacia fruticosa*

***In-vitro* cytotoxic study**

Cell viability assay

Cytotoxicity of hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson-Root was evaluated using MTT assay. The IC_{50} values of extract on different human cancer cell lines was found to be 129.13 µl/ml, 151.33 µl/ml and 203.25 µl/ml for Human Colon Cancer Cells (HT-29), Human Liver Cancer Cells (HepG2) and Human Breast Cancer Cells (MCF-7) respectively.

And also the IC_{50} value of extract on Normal Human Dermal Fibroblast (NHDF) was found to be 302.33 µl/ml was shown in the table 4 & fig 3.

The relative sequence of sensitivity to extract was observed to be HT-29 cell lines>HepG2 cells lines>MCF-7 cell line>NDHF.

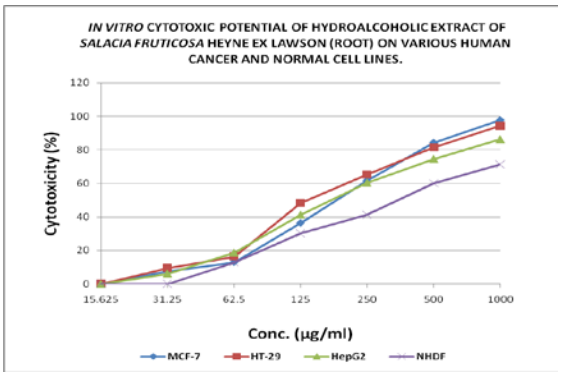

Fig. 3: *In vitro* cytotoxic potential of hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson (root) on various human cancer and normal cell lines.

Table 4: *In vitro* cytotoxic potential of hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson (root) on various human cancer and normal cell lines

Cell Lines studied	IC_{50} (µg/ml)*
Human Breast Cancer Cells (MCF-7)	203.25±0.25
Human Colon Cancer Cells (HT-29)	129.13±0.50
Human Liver Cancer Cells (HepG2)	151.33±0.40
Normal Human Dermal Fibroblast (NHDF)	302.33±1.23

*Average of three determinations, three replicates, IC_{50} indicates that the drug concentration inhibiting 50% cellular growth following 72 hours of drug exposure.

DISCUSSION

Phytochemical screening

The presence of different phytoconstituents of hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson–Root, was detected by qualitative phytochemical analysis, it showed the presence of carbohydrates, glycosides, alkaloids, phenolic compounds and tannins, flavanoids, triterpenoids, steroids and saponins. The phenolic compounds & tannins, steroid and triterpenoids and flavonoid found to be positive for all respective tests, so these phytoconstituents may be the major active principles. Due to these active principles, the *in vitro* antioxidant activity and *in vitro* cytotoxic potential of hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson–Root was studied.

***In vitro* antioxidant activity**

The antioxidant activity has been attributed by various mechanisms, like hydrogen donation, prevention of chain initiation, binding of transition metal ion catalyst, prevention of continued hydrogen abstraction, reductive capacity, radical scavenging and decomposition of peroxidase [22, 23]. The *in vitro* methods are based on inhibition of formed free radical. Important is that all the

methods developed have strength and limitations and a single measurement of antioxidant capacity usually not sufficient. Therefore in the present study, the antioxidant activity of hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson–Root was analyzed by various methods such as ABTS radical cation scavenging activity, hydroxyl radical scavenging activity, and DPPH free radical scavenging activity, nitric oxide scavenging activity and reducing power assessment.

Scavenging of ABTS radical cation

ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) assay is relatively recent one, which involves a more drastic radical, chemically produced & often used for screening complex antioxidant mixture such as plant extracts, beverages and biological fluids. The solubility in both the organic and aqueous media and the stability in a wide pH range raised the interest in the use of $ABTS^{\bullet+}$for the estimation of the antioxidant activity. [15].

The hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson–Root, showed potent antioxidant activity by ABTS method. Here the extract radical scavenging showed may be due to the direct role of its phenolic compounds. And also the extract showed higher scavenging activity by this method compared to other scavenging method in this study.

Scavenging of hydrogen peroxide [17]

Hydrogen peroxide is highly important reactive species (ROS) which is generated *in vivo* by several oxidase enzymes, because its ability to prevent the biological membrane. There is increasing evidence that it may be toxic if converted to hydroxyl radical ($OH^{\cdot}$**)** which cause severe damage to the biological system [24]. So the removing of hydrogen peroxide is very important for any antioxidant defense in cell system. Polyphenols have been proved in earlier to protect mammalian cells from damage induced by hydrogen peroxidase [25]. Thus, the phenolic compound of hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson–Root may be probably be involved in the scavenging hydrogen peroxide by donating an electron to H_2O_2 and thus neutralize it into water molecule.

Scavenging of DPPH radical [18]

DPPH free radical scavenging is one of the basic reproducible methods for antioxidant screening of various compounds. It is chemically stable radical. Free radical scavenging potentialities were tested against methanolic solution of DPPH. DPPH accept an electron of hydrogen radical to become a stable, diamagnetic molecule [25]. It can be oxidized only with difficulty and irreversibly. Because the odd electron 2, 2-Diphenly 1-picryl hydrazyl solution (DPPH) shown a good absorption band at 490 nm, its solution appearing a deep violet colour. As this electron becomes paired off, the absorption vanishes or reduces and resulting decolorization is stoichiometric with respect to the number of electron taken up. Antioxidants react with DPPH and convert it to 2, 2-Diphenly 1-picryl hydrazine.

The purple colour of DPPH changes to yellow (decolorization) absorbance at 490 nm, indicates the changes in scavenging efficacy based on the substance added. It has been found that the cysteine, glutathione, ascorbic acid, tocopherol, flavanoids, tannins and aromatic amines reduces & decolorize the DPPH by their hydrogen donating property/ability [23]. The scavenging activity in DPPH method may be due to the presence of flavonoid, amines and phenols in the hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson–Root.

Scavenging of nitric oxide radical [19]

Nitric oxide is a reactive oxygen species generated from sodium nitroprusside in an aqueous solution of physiological pH and react with the oxygen to form nitrite ions, which is estimated by modified Griess Ilosvay reaction. It is well known that nitric oxide plays an important role in various diseases such as carcinomas, juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis [26]. In this assay the nitrite ions react with the Griess reagent, which form a purple azodyes. The presence of test compounds, likely to be the scavenger, the amount of nitrite will decrease. The degree of decrease in formation of purple azo dyes will reflect the extent of scavenging. The absorbance of chromophore formed is measured at 540 nm.

In this study, results shows that the hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson-Root produced good nitric oxide scavenging activity and found to be equally potent when compared to the standard rutin.

Reducing power assessment

In the measurement of the reducing ability, it has been investigated the $Fe^{3+}$to Fe^{2+}transformation in the presence of the extract or standard. $Fe^{3+}$is often used as an indicator of electron donating activity and which is an important mechanism of antioxidant action (especially phenolic compound) and can be strongly correlated with the other antioxidant properties[27]. The reducing properties of the plant extract are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating the hydrogen atom [22].

The reductones are reported to react with certain precursors of peroxides, thus prevention of peroxidase formation [23]. In this present study, the hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson-Root, showed significant antioxidant activity in reducing power assay. The reducing power gets increased with increase in concentration of the extract.

***In vitro* cytotoxic study**

Anticancer properties of many natural compounds isolated from different Indian plant extracts have been reported. Research is being carried out throughout the world to find a lead compound which can block the development of cancer in humans. Nature has always been a great contributor towards this goal. Plant-derived natural products such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties, which include cytotoxic and chemopreventive effects [28]. The isolation of the vinca alkaloids, vinblastine and vincristine from the Madagascar periwinkle, *Catharanthus roseus* introduced a new era in the use of plant material as anticancer agents. They were the first agents to advance into clinical use for the treatment of cancer [29].

In the present study, Cytotoxicity of hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson-Root was evaluated using MTT assay at different concentration on various human cancer cell lines and normal cell lines.

Cell viability assay [21]

Proliferation assay are widely used in cell biology for the determination of growth expression factor, cytokinines and nutrients as well as cytotoxic and chemotherapeutic agents. Thus the present investigation was carried out by MTT [(3-(4, 5-dimethylthiazol-2yl)-2, 5-phenyltetrazoliumbromide] assay. The cytotoxic potential of hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson-Root on various Human cancer and normal cell lines was studied in this work. Cytotoxic was defined as the cell killing property of a chemical compound independent from the mechanism of death [30]. Cytotoxic assay is an appropriate assay method for screening new substances with in short time in order to determine the cytotoxicity on cancer cells [31].

MTT assay is based on the ability of cell to change the soluble tetrazolium bromide (MTT) in to an insoluble formazan precipitate [32]. The effectiveness of extract was measured on the basis of growth inhibition effect of extract on cancer cell line. Thus our results showed that the effect was dose dependent inhibition of cancer cells and act as good cytotoxic effect on viable cells of HepG2, HT-29 and MCF-7 cancer cell lines. The effect was more pronounced against HT-29 cell lines when compared to the others. This demonstrated the difference in sensitivity of cancer cell lines to phytochemical presents in the hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson-Root. This difference may also due to the difference in the characteristic of cell lines.

At the same time the IC_{50} value for normal cell lines was found to be very high indicates that the selectivity of extract towards cancer cells.

CONCLUSION

Thus the present study concluded that the hydroalcoholic extract of *Salacia fruticosa* Heyne ex Lawson-root showed significant antioxidant and anticancer activity by using various *in vitro* models. Further studies are undergoing in order to clarify their molecular mechanisms by isolating the active principle present in the root.

CONFLICT OF INTERESTS

The authors confirm this paper content has no conflict of interest.

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