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Research Article

IN VITRO ASSESSMENT OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *SALACIA* SPECIES – A COMPARATIVE STUDY

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ABSTRACT

Objective: The present work is to evaluate the antioxidant activity of hydroalcoholic extracts of *Salacia oblonga, Salacia reticulata* and *Salacia roxburghii* using different *in vitro* models e.g. radical scavenging activity using 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical assay, hydroxyl radical scavenging activity and Ferricthiocyante assay (FTC) and antibacterial activity against Escherichia coli,Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa and klebsiella pneumoniae.

Method: Different concentrations of hydroalcoholic species of three plants were prepared and tested for their antioxidant activity and antibacterial activity.

Results: It was seen that *Salacia reticulata* showed better antioxidant effect than the other two species and *Salacia oblonga* and *Salacia reticulata* showed better antibacterial activity.

Conclusion: The study concludes that the hydroalcoholic extract of three *Salacia* species have antioxidant activity and *Salacia oblonga* and *Salacia reticulata* have antibacterial activity against the tested microorganisms.

Keywords: *Salacia* species, Antioxidant activity, Antibacterial activity.

INTRODUCTION

Salacia species (e.g., *S. oblonga*, *S. prinoides*, *S. reticulata*), belongs to the family Hippocrasteceae, and is known as "Ponkoranti" in Ayurvedic medicine. These species are widely distributed in Sri Lanka, India, China, Vietnam, Malaysia, Indonesia and other countries. The roots and stems of these plants have been used in the treatment of diabetes and obesity in the Ayurvedic system of Indian traditional medicine. [1].

Millions of chemical reactions are occurring constantly in the body. Reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxynitrite radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases [2 and 3]. The ROS is responsible for the oxidation of lipid, DNA, protein, carbohydrate and other biological molecules and may cause DNA mutation or/and serve to damage target cells or tissues, which often results in cell senescence and death [4 and 5]. Due to this there is a need to reduce the oxidative stress and prevent the consequences of ROS. The knowledge and application of various potential antioxidant activities in reducing oxidative stresses in vivo has prompted many investigators to search for potent and cost-effective antioxidants from various plant sources [6-13]. The interaction of antioxidants with free radicals might prevent some of the damage caused by the free radicals [14]

The major cause of premature deaths of today is mainly due to infectious diseases and almost 50,000 people suffer every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world [15 and 16]. Due to the increasing failure of chemotherapeutics and antibiotic resistance by pathogenic microbial injections agents there has been an urge to screen medicinal plants for their anti-microbial properties [17]. The use of plant extracts and phytochemicals both with known antimicrobial properties is of great significance, in the past few years a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal plants [18 and 19].

The present work is to investigate the antioxidant and antimicrobial activity of extracts of three different species of *Salacia* i.e. *Salacia oblonga, Salacia reticulata* and *Salacia roxbhurgii*.

MATERIALS AND METHOD

Preparation of Extract

Salacia oblonga, Salacia reticulata and *Salacia roxbhurgii* was collected from the forests of Kerela and Madhyapradesh and were authenticated by Prof. N.K Dubey, Banaras Hindu University. The collected material was dried under shade and then powdered with mechanical grinder. The dried powder material was extracted with ethanol and water in a soxhlet apparatus. The extract was concentrated to dryness under reduced pressure and controlled temperature (40 to 50°C) using rotary evaporator. The obtained extract was taken and used for the antioxidant and antimicrobial assays.

Antioxidant activity

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured in this assay. To 1 ml of sample solution with different concentration (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 mg/ml) was added to 2ml of sodium phosphate buffer (150mM , pH 7.4) containing 10mM ferrous sulphate, 2mM sodium salicylate and 6mM hydrogen peroxide. This mixture was then incubated for 30 minutes at 37^0C. Then the absorbance of the solution was detected at 510nm [20] using UV spectrophotometer (UV-3200 from Labindia).

Hydroxyl radical scavenging activity (%) = $(A_0 - A_1 + A_2)/A_0 * 100$

Where A_0 –Absorbance of control sample

A_1 – Absorbance in the presence of tested sample

A_2 – Absorbance of test sample without sodium salicylate solution.

DPPH radical scavenging activity

The DPPH• assay was used to measure the free radical scavenging capacity of the plant extracts. Used as reagent, DPPH• obviously offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants [21]. 200 µL of 0.004% DPPH• methanolic solution was pipetted into each well of a 96-well plate followed by 20 µL of sample, or standard, or solvent for the blank. The mixture was incubated at 30^0 C for 1 h, and the absorbance at 515 nm was measured with a microplate reader. The

inhibition percentage of the radical scavenging activity was calculated using the equation. The experiment was repeated twice and the average was taken.

$$\text{Inhibition (\%)} = 100 - 100\ (A_S \div A_0)$$

Where, A_0 is absorbance of the blank and A_S is absorbance of the sample

Ferric thiocyanate assay

To 4 mg (4 mL) of a sample (final concentration, 0.02%) in 99.5% ethanol 4.1 mL of 2.5% linoleic acid in 99.5% ethanol, 8.0 mL of 0.05 M phosphate buffer (pH 7.0) was added, and 3.9 mL of water in a screw cap tube and placed in an oven at 40°C in the dark [22]. To 0.1 mL of this mixture, 9.7 mL of 75% (v/v) ethanol and 0.1 mL of 30% ammonium thiocyanate were added. Three minutes later, 0.1 mL of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture and the absorbance was measured at 500 nm each 24 h until one day after absorbance of the control reached its maximum value. Antioxidant activity was represented by the absorbance readings on the final day of the assay (7th day). The experiment was repeated thrice and the average value was taken.

Antibacterial activity

Antimicrobial testing was done by the modified agar well diffusion method as described by [23]. Bacterial strains were cultured overnight in nutrient agar at 37±2°C. Overnight grown culture of microorganisms was used for the assay. The turbidity of resulting suspension was compared to 0.5 McFarland turbidity standards. The Mueller Hinton Agar media was prepared and poured into petri dishes. Once the media solidifies it was then inoculated with microorganisms (*E.coli* MTCC 443, *P. aeruginosa* MTCC 2599, *B. subtilis* MTCC 441, *S. aureus* MTCC No: 7443, *K. pneumoniae* MTCC No: 3384) suspended in nutrient broth. The wells were made in the plates using the help of the borer (6mm) and filled with extract. The test was carried out in triplicates to eliminate any error. The petri dishes were incubated for 24 h at 37±2°C for bacteria. The antimicrobial activity was calculated by measuring the diameter of zone of inhibition in millimeters around the well.

RESULTS

Hydroxyl radical scavenging activity

The •OH radical is an extremely reactive in biological systems and has been implicated as highly damaging species in free radical pathology, capable of damaging biomolecules of the living cells. These radical combines with nucleotides in DNA and cause strand breakage leading to carcinogenesis, mutagenesis and cytotoxicity. Hydroxyl radical (•OH) scavenging capacity of an extract is directly related to its antioxidant activity.

The percentage of inhibition of *Salacia oblonga* was more effective (71%) for hydroxyl radical scavenging activity than *Salacia* roxbhurgii (68%) and *Salacia reticulata* (64%) (Fig 1)

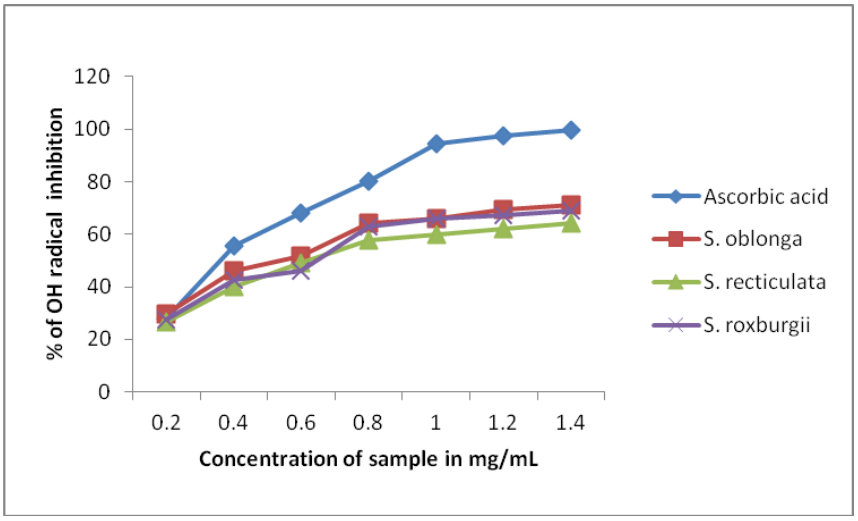

Fig. 1: Percentage inhibition of hydroxyl radical scavenging activity

(Su et al. 2009)

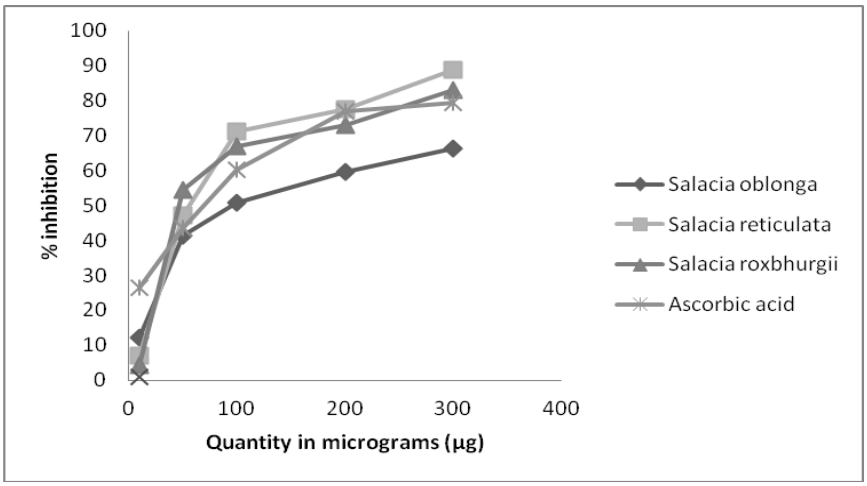

Fig. 2: DPPH radical scavenging activity of *Salacia* species

(Kim et al. 2003)

DPPH radical scavenging activity

In the present experiment, hydroalcoholic extracts of three species of *Salacia* were evaluated for the free radical scavenging activity using the DPPH radical assay.

DPPH radical scavenging activity of hydroalcoholic extracts of three species of *Salacia* was compared with that of Ascorbic acid. It was observed that the *Salacia reticulata* had higher scavenging activity compared to the other two plant extracts. At a concentration of 100ug/ml, the scavenging activity of *Salacia reticulata* was 71.31% while at the same concentration; the *Salacia oblonga* was 50.77% and *Salacia roxbhurgii* was 66.98%. The standard ascorbic acid exhibited 60.27%.

From the above table it has been observed that *Salacia reticulata* exhibited maximum percentage of inhibition when compared to other two species.

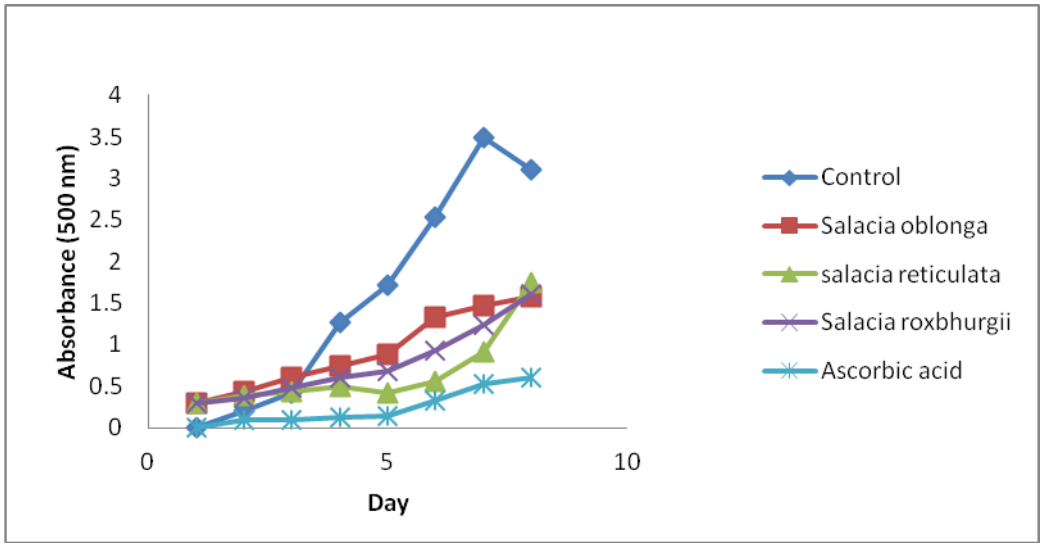

Fig. 3: Antioxidant activity of *Salacia* extracts by Ferric thiocyanate method.

(Kikuzaki and Nakatani 1993)

Table 1: Results of minimum inhibitory concentration *Salacia oblonga, Salacia reticulata* and *Salacia roxbhurgii* extracts.

Name of Plant Extract	Zone of inhibition (mm) for 100ug of sample				
	E.coli	***P.auruginosa***	***S.aureus***	***B.subtilis***	***K.pneumonia***
Salacia oblonga	17±0.3	15±0.2	14±0.5	19±0.4	11±0.2
Salacia reticulata	18±0.3	17±0.1	12±0.2	18±0.6	10±0.4
Salacia roxbhurgii	-	-	-	-	-

- No Inhibition

The ferric thiocyanate method was originally designed for measuring lipid peroxide content in an emulsion system, whereby the endpoint measure is the amount of Fe2+ that is oxidized to Fe3+ by lipid peroxides. The Fe3+-thiocyanate complex produces a deep red colour, which is detectable at 500 nm.

From the above graph it was observed that *Salacia reticulata* showed higher antioxidant activity in FTC method compared to other two species of *Salacia*.

Antibacterial assay

The result expressed that both the species of *Salacia oblonga* and *Salacia reticulata* showed antimicrobial activity except *Salacia roxbhugii* against the microorganism screened in this study.

DISCUSSION

Natural products, such as plant extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched chemical diversity [24] According to World Health Organization 80% of world's population relies on traditional medicine for their primary healthcare needs. The practice of using plant-based medicine is increasing day by day and researchers have now been working to use these in place of synthetic medicine for various medical disorders. This may be due to the various compounds like peptides, unsaturated long chain fatty acids, aldehydes, alkaloids, essential oils, phenols and water or ethanol soluble compounds present in these plants [25].

Nowadays it is thought that stress inherent in modern life style, which may cause increased incidence of disease such as cancer, diabetes, cardiovascular disorders and hypertension. To overcome these public health problems there is a need of effective therapeutic agents with low incidence of side effects and recently plenty of research has been done on plants to prove their therapeutic value.

Numerous plant products have been used traditionally used for the treatment of various diseases due to their free radical scavenging and antioxidant properties [26]. For the preparation of herbal- based drug in Indian traditional medicine, it is important to know the antioxidant activity due to the components present in the plants [27].

Free radicals cause decrease in membrane fluidity, loss of enzyme receptor activity and damage to membrane protein leading to death. These free radicals are involved in different disorders like ageing, cancer, cardiovascular disease, diabetes, rheumatoid arthritis, epilepsy & degradation of essential fatty acids. Antioxidant helps in treatment of above disorders. In our study all the three plant extract showed potential antioxidant activity.

It has been suggested that the hydroxyl radical, formed via the metal catalyzed Haber-Weiss reaction or Fenton reaction and are the major active oxygen species causing lipid peroxidation and enormous biological damage [3]. In this process, the ferric iron is reduced by superoxide, with subsequent oxidation of ferrous iron by hydrogen peroxide forming hydroxyl radical thereby initiating the series of oxidation reactions. The results obtained in the present study may be attributed to a number of reasons including, the scavenging of hydroxyl or superoxide radical by changing the ratio of fe3+/fe2+, reducing the rate of conversion of ferrous to ferric or by chelating iron [28]

The DPPH radical is a stable organic free radical with an absorption maximum band around 515-518. It is a useful reagent for evaluation

of antioxidant and free radical scavenging activity of compounds [29]. In the DPPH test, the antioxidants reduce the DPPH radical to a yellow-coloured compound, diphenylpicrylhydrazin, and the extent of the reaction depend on the hydrogen donating ability of the antioxidant [30]. In this study it was observed that *Salacia oblonga* shows better DPPH scavenging activity than the other two species (Figure 2).

Many organisms acquire several resistances mechanisms; making them multi-drug-resistant (MDR). The development of novel antimicrobial agents with activity against pathogens that have become resistant to currently available agents is one tactics for combating resistant organisms [31]. Therefore the rapid propagation in antibiotic resistance and the increasing interest in natural products have placed medicinal plants back in the front lights as a reliable source for the discovery of active antimicrobial agents and possibly even novel classes of antibiotics [32]. So in keeping view it is necessary and to screen plants for their antibacterial activity so that it can even act as an antibiotic.

This suggests that the physio-chemical nature of the individual compounds in the extract may be contributing to the antioxidant activity.

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that hydroalcoholic extract of *three species of Salacia* might contains large amounts of flavonoids and phenolic compounds exhibiting high antioxidant and free radical scavenging activities. We can conclude that the *Salacia reticulata* showed better results in ferric thiocyanate assay and DPPH assay compared to other two species. These *in vitro* assays indicate that these plant extracts are a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Moreover we may conclude that *Salacia oblonga* showed better antibacterial activity.

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