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STUDIES ON PHYTOCHEMICAL SCREENING, ANTIOXIDANT ACTIVITY AND ANTI-BACTERIAL ACTIVITY OF *SALACIA OBLONGA* STEM EXTRACT

C. GLADIS RAJA MALAR[a,b], C. CHELLARAM[b,c]*

[a]Sathyabama University, Chennai 600119, Tamilnadu, India, [b]Vel Tech Multitech Engineering College, Avadi. Chennai 600062, Tamilnadu, India, [c]Applied Biotechnology, Sur College of Applied Sciences, Sur 411, Sultanate of Oman
Email: chellaramc.sur@cas.edu.om

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ABSTRACT

Objective: The present study was performed to investigate the phytochemical screening, antioxidant activity and antibacterial activity from the stem extract of *Salacia oblonga* (*S. oblonga*).

Methods: The stem extracts were evaluated for antioxidant activities by DPPH (1, 1–Diphenyl-2-picryl-hydrazyl) radical scavenging assay. Different concentrations of aqueous stem extract were tested using the agar disc diffusion technique for the activity against *Bacillus subtilis*, *Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.*

Results: The phytochemical analysis revealed the presence of active ingredients such as steroids, saponins, phenols, flavonoids, terpenoids, alkaloids and quinones in the stem extract of *S. oblonga*. Among the extracts prepared from different solvents (ethanol, aqueous, acetone, petroleum ether and chloroform) the maximum antioxidant activity was found in the aqueous stem extract (84.3±0.0 %) of *S. oblonga* in Hubli accession followed by others accession like Udipi (72.9±0.25 %) and Jogimat (68.0±0.15%.) The antibacterial activity of aqueous stem extract of *S. oblonga* shown more active against *Bacillus subtilis.* It was found to be inactive against *Escherichia coli.*

Conclusion: It was concluded that the phytochemical screening of aqueous stem extract of *S. oblonga* in Hubli accession shown strong positive for the availability of natural chemical constituents and also an excellent antioxidant and antibacterial activity.

Keywords: *Salacia oblonga*, Phytochemicals, Antioxidant, Antibacterial activity, DPPH, Disc diffusion.

INTRODUCTION

Each and every human depends biodiversity not only for their survival and also to protect them from various diseases. It plays vital role for the production of potential drugs. The natural drugs are healthy to the human body comparing synthetic drugs [1]. The use of herbal plants well known in rural areas of many countries [2]. The presence of phytochemicals such as flavonoids, phenols, terpenoids, tannins, alkaloids and steroids are very much important for human health [3]. Such screening of plant extracts was already studied by many researchers [4]. Plants containing secondary metabolite such as phenolic compounds exhibit strong antioxidant property [5]. Active oxygen radicals are usually generated in our body by biological oxidation reactions [6]. They are highly unstable and reactive, thereby forming Reactive Oxygen Species (ROS), hydroxyl radicals. They have the ability to cause damage cells that may lead to cancer. It can damage the DNA, proteins and lipids [7]. It can start chain reactions. Antioxidants terminate these chain reactions by removing free radical intermediates. They are chemicals that block the action of free radicals. It is a molecule that stops the oxidation of other molecules. Thus, antioxidants are reducing agents such as thiols, ascorbic acid, polyphenols [8]. Plants producing natural antioxidants can reduce the stress [9].

In tropical countries, infectious death be a major cause for death. Antibiotic resistance is now a universal concern. Many infectious diseases are cured by using herbal plants. The antimicrobial compounds produced by plants are active against pathogens. The substances which can inhibit the activity of microorganisms are used in the treatment of infectious diseases. There are several reports regarding the antimicrobial activity of crude extracts of herbal plants [10]. India is the largest producer of herbal plants and called as botanical garden of the world [11, 12]. About 45,000 plant species are found in India [13]. Among the species, a very small percentage has been investigated phytochemically and submitted to pharmacological screening [14]. This is the origin for the finding of new drugs in the area of antibiotics [15, 16].

The present investigation shows the antioxidant and antibacterial medicinal property of the stem extract of *S. oblonga* commonly known as Ekanayaka, Saptrangi and Ponkoranti. It belongs to the family Celastraceae. It is a woody climber available in the forest of India and Sri Lanka. Nearly 18 species of Salacia grow in India [17]. It is very well known for its antidiabetic nature. It is also used for curing diarrhea, fever, arthritis, gonorrhea and skin diseases. Chemicals in Salacia seen to prevent sugars in food from being absorbed by the body. It inhibits the breakdown of sugars. It also prevents the storage of extra fat in our body. The roots were used in ayurvedic medicine for the treatment of diabetes [18]. This study showed the maximum antioxidant property of aqueous stem extract of *S. oblonga* than the extracts prepared from other solvents like ethanol, acetone, petroleum ether and chloroform. It also showed the highest antibacterial activity of aqueous stem extract of *S. oblonga* against the pathogen *Bacillus subtilis*.

MATERIALS AND METHODS

Materials

DPPH, BHT, ethanol, acetone, ethanol, methanol, petroleum ether, chloroform and other chemicals used for phytochemical screening were received from HIMEDIA laboratory, Mumbai, India. The microbial strains such as *Bacillus subtilis* (MTCC 10224), *Bacillus cereus* (MTCC 10211), *Pseudomonas aeruginosa* (MTCC 14676), *Staphylococcus aureus* (MTCC 9542), *Escherichia coli* (MTCC 1563) were collected from The Microbial Type Culture Collection and Gene Bank, Chandigarh, India.

Sample collection

The stems of *S. oblonga* were collected from the different regions of Karnataka like Hubli, Udipi and Jogimat. These plants are authenticated by Dr. N. Vijayakumar, Associate professor, Department of Botany, S. T. Hindu College, Nagercoil-629002.

Extract preparation

The healthy stems were dried in shadow, and the dried samples were powdered by ball mills. Stem extracts were prepared by the method given by [19]. About 20 g of air dried sample were mixed with 200 ml of acetone, ethanol, chloroform, petroleum ether and

water respectively for 1 min using an Ultra Turax mixer (13000 rpm) and soaked overnight at room temperature. Then it was filtered through Whatman No.1 filter paper in a buchner funnel. The filtered solution was evaporated under Rota evaporator at 40 °C. The concentrated extracts were stored in airtight container in the refrigerator below 10 °C.

Phytochemical screening of *S. oblonga*

The phytochemical screening of stem extracts of *S. oblonga* was assessed by the standard method as described by [20, 21]. Phytochemical screening was carried out on the stem extracts (Hubli, Udupi and Jogimat accessions) using different solvents (acetone, ethanol, chloroform, petroleum ether and water) to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, alkaloids, terpenoids, glycosides, cardiac glycosides, coumarins and steroids [22]. General reactions in these analyses revealed the presence or absence of these compounds in the stem extracts tested.

Qualitative analysis of antioxidant activity

The antioxidant activity of stem extracts of *S. oblonga* was determined by the method as described by [23]. 50µL of aqueous stem extracts of *S. oblonga* from different accessions were taken in the microtiter plate. 100µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 min in dark condition. The samples were then observed for a colour change from purple to yellow and pale pink. The yellow colour indicates strong positive and pale pink shows weak positive. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of antioxidant activity

The antioxidant activities were quantitatively determined by using 2,2-diphenyl-1-picryl hydrazyl (DPPH) as a free radical. 100µL of aqueous stem extract of *S. oblonga* was mixed with 2.7 ml of methanol, and then 200 µL of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 min under dark condition. Initially absorption of a blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control [24]. Subsequently at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517 nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% of Butylated Hydroxy Toluene (BHT). Antioxidant activity of stem extract was calculated by the following formula.

$$\% \text{ of antioxidant activity} = \frac{(\text{Absorbance of control-Absorbance of test Sample})}{(\text{Absorbance of control})} X\,100$$

Antibacterial activity

The antibacterial study was done by the method given by [25, 26]. Various concentrations (10 mg/ml, 20 mg/ml and 30 mg/ml) of the concentrated aqueous stem extract of *S. oblonga* were tested for its antimicrobial activity against pathogenic bacterial strains such as *Bacillus subtilis, Staphylococcus aureus, Bacillus cereus. Escherichia coli and Pseudomonas aeruginosa.* The bacterial culture was grown in Muller Hinton Agar and Muller Hinton Broth (Himedia) [27].

Disc diffusion assay

Antibacterial activity was measured by using the standard method of diffusion disc plates on agar [28, 29]. All the bacterial strains were grown in Muller Hinton Broth medium (Himedia) for 24 h at 37°C and plated on Muller Hinton Agar (Himedia) for agar diffusion experiments. Then 0.1 ml of each culture of bacteria was spread on agar plate surfaces. Sterile disc (Himedia, 6 mm in diameter) were placed on the agar medium to load 20 µL of different concentrations (10-30 mg/ml) of aqueous stem extracts of *S. oblonga* were tested. Inhibition diameters were measured after incubation for 24 h at 37°C. Blanks of solvent only were also tested for antibacterial activity in the same way, which is used as negative control.

RESULTS AND DISCUSSION

The phytochemical screening tests on the stem extracts of *S. oblonga* from different accessions using different solvents were carried out. The aqueous extract of Hubli accession shows more positive chemical constituents like flavonoids, phenol, alkaloids, steroids, terpenoids, quinones and saponins (table 1) followed by Udipi and Jogimat accessions (table 2, table 3). The presence of chemical constituents may have beneficial health effects [30]. Natural products provide an unlimited chance for the production different drugs due to the presence of chemical diversity [31]. The curative properties of herbal plants are due to the availability of secondary metabolites [32]. Tannic acid is responsible for antibacterial, antiseptic, antiulcer and antiviral properties. Flavonoids are potentially active free radical scavengers and also provide anti-inflammatory activity [33]. The presence of phenolic compounds exhibits antioxidant and antimicrobial potential [34-36]. Thus the screening test may be useful in the detection of bioactive compounds and may lead to the development of new drugs.

Free radical scavenging activity

The qualitative analysis of antioxidant property of *S. oblonga* collected from different accessions for different solvents (ethanol, aqueous, acetone, petroleum ether and chloroform) were shown in table 4. The aqueous stem extract of *S. oblonga* shows the more positive response for the free radical scavenging activity than other solvents. The quantitative response of radical scavenging activity given in table 5, shown the percentage of antioxidant activity of stem extract of *S. oblonga*. The data showed the aqueous stem extract of *S. oblonga* from Hubli accession (84.3±0.0 %) had more radical scavenging activity followed by others (Udipi-72.9±0.25 %, Jogimat-68.0±0.15 %). These results were compared with the antioxidant activity of standard BHT (94.8%). From our data, the aqueous stem extract of *S. oblonga* from Hubli accession (84.3±0.0 %) shown excellent antioxidant activity. The effect of antioxidant is its ability to trap free radicals. Many sources of antioxidants with different activities [37] were shown by researchers. The *in-vitro* assay of aqueous methanolic root extract of *S. oblonga* proved the antioxidant activity, which can prevent the oxidative stress in our body [38]. Many synthetic antioxidant components have shown toxic and mutagenic effects, which have shifted the vision towards the naturally occurring antioxidants [39]. In the present investigation the maximum scavenging activity enforcing the herbal usage of *S. oblonga* stems. Phenolic compounds are significantly exhibits more radical scavenging activity [40]. Thus, the cell damages were mainly prevented by the herbal drugs containing phenolic compounds.

Table 1: Phytochemicals screening of *S. oblonga* stems extract from Hubli accession

Phytochemicals tested	Extraction solvent				
	Aqueous	Ethanol	Chloroform	Petroleum ether	Acetone
Tannins	-	-	-	-	-
Saponins	+	-	-	-	+
Quinones	+	+	-	-	-
Terpenoids	+	+	-	+	-
Steroids	+	+	-	+	-
Flavonoids	++	+	+	+	-
Phenol	++	+	+	-	+
Alkaloids	+	-	-	-	-
Glycosides	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-
Coumarins	+	-	-	-	-
Antho cyanin	-	-	-	-	-
Beta cyanin	+	+	-	-	-

++strong positive, +positive,-negative

Table 2: Phytochemical screening of *S. oblonga* stem extract from Udipi accession

Phytochemicals tested	Extract solvent				
	Aqueous	Ethanol	chloroform	Petroleum ether	Acetone
Tannins	-	-	-	-	-
Saponins	+	-	-	-	+
Quinones	+	+	-	-	-
Terpenoids	+	+	-	+	-
Steroids	+	+	-	+	-
Flavonoids	+	+	+	+	-
Phenol	+	+	+	+	+
Alkaloids	-	-	-	-	-
Glycosides	-	-	-	-	-
Cardiac glycosides	+	-	-	-	-
Coumarins	-	-	-	-	-
Antho cyanin	-	-	-	-	-
Beta cyanin	+	+	-	+	-

+positive,-negative

Table 3: Phytochemical screening of *S. oblonga* stems extract from Jogimat accession

Phytochemicals tested	Extract solvent				
	Aqueous	Ethanol	Chloroform	Petroleum ether	Acetone
Tannins	-	-	-	-	-
Saponins	+	+	-	+	+
Quinones	+	-	-	-	-
Terpenoids	+	+	-	+	-
Steroids	+	+	-	+	-
Flavonoids	+	+	+	+	-
Phenol	+	+	+	+	+
Alkaloids	-	-	-	-	-
Glycosides	-	-	-	-	-
Cardiac glycosides	+	-	-	-	-
Coumarins	-	-	-	-	-
Antho cyanin	-	-	-	-	-
Beta cyanin	-	-	-	-	-

+positive,-negative

Table 4: Qualitative antioxidant potential of *S. oblonga* stem extracts

S. No.	Extraction solvent	Qualitative response of DPPH assay		
		Hubli	Udipi	Jogimat
1	Ethanol	++	+	+
2	Aqueous	+++	++	+
3	Acetone	+	+	-
4	Petroleum ether	+	+	-
5	Chloroform	-	-	-

+++very strong positive, ++strong positive,-negative.

Table 5: Quantitative antioxidant potential of *S. oblonga* stem extracts

S. No.	Extraction solvent	Quantitative response of DPPH assay (%)		
		Hubli	Udipi	Jogimat
1	Ethanol	70.2±0.3	62.0±0.16	61.5±0.31
2	Aqueous	84.3±0.0	72.9±0.2	68.0±0.15
3	Acetone	65.5±0.5	50.7±0.1	31.1±0.14
4	Petroleum ether	55.7±0.16	55.7±0.16	37.7±0.31
5	Chloroform	41.8±0.14	44.26±0.2	24.5±0.04

Each value represents mean±SD of three replicated experiments

Antibacterial activity

The antibacterial activities of stem extracts of S. oblonga were quantitatively measured by indicating the presence of zone and by measuring the diameter around the disc. The data presented in table 6, indicate that the stem extract of *S. oblonga* inhibit the growth of some microorganism to various concentration. The effect was tested against five different bacterial isolates such as *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Escherichia coli.* Different concentrations (10 mg/ml, 20 mg/ml and 30 mg/ml) of aqueous stem extract of *S. oblonga* were subjected for the assay. The pathogenic activities were more effective on 30 mg/ml concentration. The highest activity was shown by *Bacillus subtilis* (13.1±0.1 mm) followed by *Bacillus cereus* (11.6±0.23 mm),

Pseudomonas aeruginosa (9.0±0.02 mm), *Staphylococcus aureus* (8.7±0.05 mm) including the diameter of the disc (6 mm). There is no effect for the pathogen *Escherichia coli*. The minimum inhibitory zone of above pathogens was shown in fig. 1. Similar results were obtained on ethyl acetate extracts of the stem of *S. oblonga* with some other pathogens [41]. The development of new drug resistance strains be a great concern for global community. The effective treatment of infectious disease depends on the development novel drugs. In the present study the aqueous stem extract of *S. oblonga* shown effective activity against most of the bacterial strains. This activity exhibits the medicinal usage of *S. oblonga* stem, which protects the endangered species from extinction than the usage of the root. The tannin compounds can disintegrate the bacterial strains and also exhibit antidiabetic, anti-inflammatory activities [42].

Table 6 Average inhibition zone of aqueous stem extract of *S. oblonga*

Inhibition zone in diameter(mm)*			
Microorganisms tested	**Concentrations of aqueous stem extract**		
	10 mg/ml	**20 mg/ml**	**30 mg/ml**
Bacillus subtilis (MTCC 10224)	-	9.6±0.06	13.1±0.1
Bacillus cereus (MTCC 10211)	-	-	11.6±0.23
Pseudomonas aeruginosa (MTCC 14676)	-	-	9.0±0.02
Staphylococcus aureus (MTCC 9542)	-	-	8.7±0.05
Escherichia coli (MTCC 1563)	-	-	-

Each value represent mean±SD of three replicated experiments, *Includes diameter of disc (6 mm)

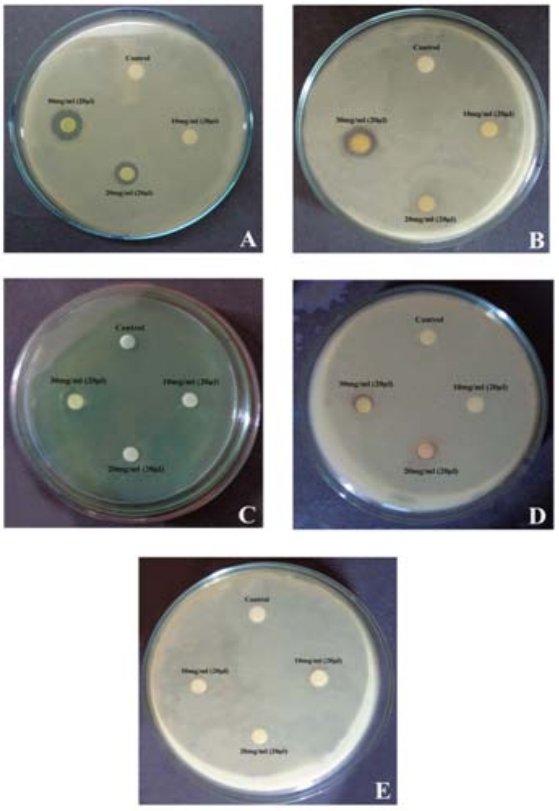

Fig. 1: Antibacterial activity of aqueous stem extract of *S. Oblonga*, A-Antibacterial activity of aqueous stem extract of *S. oblonga* against *Bacillus subtilis*, B-Antibacterial activity of aqueous stem extract of *S. oblonga* against *Bacillus cereus*, C-Antibacterial activity of aqueous stem extract of *S. oblonga* against *Pseudomonas aeruginosa*, D-Antibacterial activity of aqueous stem extract of *S. oblonga* against *Staphylococcus aureus*, E-Antibacterial activity of aqueous stem extract of *S. oblonga* against *Escherichia coli*

Statistical analysis

All experiments were repeated two or three times with two replicates for each condition tested, and similar results were obtained on all occasions. The results are expressed as the mean+SD and statistical analysis was carried out using Student's t-test and one-way analysis of variance was considered to be statistically significant.

CONCLUSION

From this study, it was concluded that the aqueous stem extract of *S. oblonga* collected from Hubli accession shown more positive for the presence of phytochemicals. The maximum antioxidant activity was shown by quantitatively. The damage caused by superoxide radicals, peroxy radicals; single oxygen can be treated by effective antioxidants due to their scavenging action. The availability of phenolic compounds and other phytoconstituents have proposed the usage of *S. oblonga* herbal drugs for clinical usage. The antibacterial activity of aqueous stem extracts shown more effective against *B. subtilis* than other pathogens. There was no effect against *E. coli.* The herbal activities for stem extracts of S. oblonga were shown by researchers. Nowadays many plants are red listed as endangered species. The herbal usage of stem extract of *S. oblonga* may protect the plant from extinction. Protection of our gifted herbal diversity may protect the universe.

CONFLICT OF INTERESTS

Declared none

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