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Phytochemical Screening, Quantification of Total Phenols, Total Flavonoids and Antimicrobial Activity of Stem Extracts of Salacia Oblonga

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Abstract

Salacia oblonga, an endangered medicinal plant widely used for the traditional ayurvedic medicine, belonging to the family Clastraceae. Our present investigation revealed the preliminary phytochemical analysis, total phenols, total flavonoids and antimicrobial activities of aqueous stem extract of *salacia oblonga*. The stem extracts were prepared by using the solvents like ethanol, acetone, petroleum ether, chloroform and water. Preliminary screening of the extracts was carried out by standard procedures. Among the five different solvents, the aqueous stem extract shown more positive for the availability of natural chemical components. Total phenols and total flavonoids were quantified as 35.63 mg GAE/g and 19.82mg QE/g respectively. The aqueous stem extract was tested against the micro-organisms (*Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Staphylococousaureus and Escherichia coli*). Among the micro-organisms *Bacillus subtilis* shown maximum zone of inhibition (13.2mm) at a concentration of 30mg/ml of aqueous stem extract of *salaciaoblonga*. But *Escherichia coli* inactive against the stem extract.

Keywords: Antimicrobial Activity, Salaciaoblonga, Total Flavonoid, Total Phenol

1. Introduction

Biodiversity enclosed variety of floral species and fauna species. It provides food, fuels, shelter, medicines etc. It plays vital role for the production of wide variety of herbal drugs. Nowadays infectious diseases were major life threatening concern. Microorganisms are responsible for such infections[1]. Antibiotics can be able to cure the disease produced by microbes. Synthetic antibiotics were available in the pharmacy. But it can produce many side effects[2]. Due to low cost and no side effects, people are going towards natural herbal medicines. These herbal drugs were derived from plant extracts[3]. Plants have rich in secondary metabolites such as phenols, flavonoids, tannins, alkaloids, terpenoids, saponins and glycosides[4]. These are not important for the survival of plants, but they can have certain bio activity against bacteria, fungi, virus etc. This potential property may be induced the researchers to discover variety of herbal medicines[5]. Our environment composed several thousand species of plants. Among this most of the species were present in hot spots[6]. In India the two important hot spots are Eastern Himalayas and Western Ghats. A very few plant species were identified and submitted to the pharmacology.

Plant derived phenols and flavonoids were potential natural chemical constituents. Phenols are having

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hydroxyl group along with aromatic ring. They are very good free radical scavengers. It prevents the activity of free radicals and may protect us from tissue damage. Phenols are also active against microbial diseases, inflammation, cardiovascular, neurological diseases and cancer[7]. Flavonoids are another important plant metabolite which may be responsible for the colour of flowers. Flavonoids were potentially active against inflammation, microorganisms, cardio vascular diseases[8]. Both phenols and flavonoids were potential antimicrobial activities[9].

Salaciaoblonga comes under the family Celastraceae. It is a woody climber, commonly known as saptrangi, ponkoranti and ekanayaka. It is a strangling shrub, mostly present in Srilanka and India[10]. In India, it is concentrated in Western Ghats which is one of the hot spots. This herbal plant is familiar forthe treatment of diabetes[11]. It is rich in nutrients like iron, calcium, phosphorus, fibre etc. It is also used for curing rheumatism, fever, gonorrhoea, asthma, itchness, obesity and mensural problems[12]. The availability of potentially active agents like phenols, flavonoids, tannins, alkaloids, terpenoids, saponins and glycosides are responsible for its antioxidant, anti-inflammatory, antidiabetic activities[13]. Our present study revealed the quantification of important metabolites like phenols, flavonoids and its potential against microorganisms.

2. Materials and Methods

2.1 Materials

Ethanol, acetone, petroleum ether, chloroform, sodium carbonate, aluminium chloride, Folin-Ciocalteau reagent and other chemicals used for the qualitative and quantitative phytochemical analysis were bought 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.

2.2 Collection of Salaciaoblonga

The wild plant, salaciaoblonga was collected from Western Ghats, Karnataka, India. Then it was authenticated by Dr. Vijaya Kumar, Associate professor, Hindu college. The stem parts were separated and washed with clean water to remove the impurities. Then it was dried under shadow for four weeks. The dried stems were powdered by ballmills and stored.

2.3 Extract Preparation

Each 100g of dried stem powder was taken in a conical flask separately and mixed with 250ml of water, ethanol, acetone, chloroform and petroleum ether respectively. It was soaked for three days. Then it was filtered by using what man number 1 filter paper in Buchner funnel with suction. The filtrates were concentrated by using rotaevator under vaccum at 450C. Then it was stored in refrigerator under 100C for further analysis.

2.4 Phytochemical Screening

Screening of phytochemicals is an important tool to find out the natural chemical groups such as phenols, flavonoids, tannins, alkaloids, terpenoids, saponins and glycosides. General reactions like discoloration revealed the presence or absence of secondary metabolites.

2.4.1 Test for Flavonoids

About 0.5 ml of aqueous stem extract of Salaciaoblonga was shaken with pet ether to remove the fatty materials. The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. 3 ml of the filtrate was mixed with 4 ml of 1% KOH. A dark yellow colour was observed, which indicates the presence of flavonoids.

2.4.2 Test for Saponins

About 0.5 ml of stem extract was dissolved in 2ml of boiling water in a test tube, allowed to cool and shaken to mix thoroughly. Foam appears indicating the presence of saponins.

2.4.3 Test for Alkaloids

About 0.5 ml of stem extract was mixed with about 8 ml of 1% HCl, warmed and filtered. 2 ml of filtrate were treated separately with Mayer's reagent. Turbidity was observed to indicate the presence of alkaloids.

2.4.4 Test for Tannins

About 0.5 ml of extract was boiled with 20 ml of distilled water in a test tube and then filtered. 0.1% FeCl3 was added to the filtrate. Appearance of brownish green coloration showed the presence of tannins.

2.4.5 Test for Coumarins

About 0.5 ml of aqueous stem extract was taken in a small test tube and covered with filter paper moistened with 1 N NaOH. The test tube was placed for few minutes in boiling water. Then the filter paper was removed and examined in UV light for yellow florescence to indicate the presence of coumarins.

2.4.6 Test for Anthocyanin and Betacyanin

To 2ml of the stem extract, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 1000C. Formation of yellow colour indicates the presence of betacyanin.

2.4.7 Test for Glycosides

About 2ml of stem extract is mixed with 3ml of chloroform and 10% ammonium solution was added. Formation of pink colour was not identified, which indicates the absence of glycosides.

2.4.8 Test for Cardiac Glycosides

To 0.5 ml of the stem extract, 2 ml of glacial acetic acid and few drops of 5 % ferric chloride were added. This under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides.

2.4.9 Test for Terpenoids

To 0.5 ml of the stem extract, 2 ml of chloroform was added and concentrated Sulphuric acid was added carefully. Formation of red brown colour at the interface indicates the presence of terpenoids.

2.4.10 Test for Phenols

To 1ml of the stem extract, 2ml of distilled water followed by few drops of 10 % ferric chloride was added. Formation of blue colour indicates the presence of phenols.

2.4.11 Test for Quinones

To 1ml of the stem extract, 1ml of concentrated sulphuric acid was added. Formation of red colour indicates the presence of quinones.

2.4.12 Test for Steriods

To 0.5 ml of the stem extract, 2 ml of chloroform and 1 ml of Sulphuric acid were added. Formation of reddish brown ring at interface indicates the presence of steroids.

2.5 Quantitative Analysis of Phytochemicals

2.5.1 Quantification of Phenols in Salaciaoblonga

Total phenolic content was determined by the Folin-Ciocalteau colorimetric method of[14]. For the analysis, 100µl of dry powdered aqueous stem extract were added to 0.5ml of Folin-Ciocalteau reagent (1/10) dilution and 1.5ml Sodium carbonate (Na2CO3). The mixture was incubated for 15minutes under dark at normal room temperature. The absorbance of blue coloured solution was measured at 765nm using UV-visible spectrophotometer. The standard calibration curve was drawn using gallicacid. The results were expressed in mg of Gallic Acid Equivalent (GAE) per gram dry weight of plant powder.

2.5.2 Quantification of Flavonoids in Salaciaoblonga

Total flavonoids content of aqueous stem extracts was determined by the aluminium chloride colorimetric method[15]. 0.5 ml of stem extracts of Salaciaoblonga at a concentration of 1mg/ ml were taken and the volume was made up to 3ml with methanol. Then 0.1ml $AlCl_3$

(10%), 0.1ml of potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated at 415nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

2.6 Antimicrobial Activity (Disc Diffusion Assay)

The antibacterial study was done by Disc diffusion method[16]. Three different concentrations (10 mg/mL, 20 mg/mL and 30 mg/mL) of the concentrated aqueous stem extract of Salaciaoblonga were tested for its antimicrobial activity against the bacterial strains such as Bacillus subtilis, Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa. All the microbial strains were grown in Muller Hinton Broth medium (Himedia) for 24 hours at 37oC and plated on Muller Hinton Agar (Himedia) for agar diffusion experiments. Then 0.1 mL of each culture of bacteria spread on agar plate surfaces. Sterile disc (Himedia, 6mm in diameter) were placed on the agar medium to load 20 µL of different concentrations (10-30 mg/mL) of aqueous stem extracts of Salaciaoblonga were tested. Inhibition diameters were measured after incubation for 24hrs at 37oC. Blanks as negative control of solvent only were also tested for antibacterial activity in the same method.

3. Results

Phytochemicals are plant metabolites, produced by the metabolic pathway. Qualitative analysis revealed the pres-

Table 1. Phytochemical screening of stem extracts of *Salaciaoblonga*

Phytochemicals Tested	Stem extracts of *Salaciaoblonga*				
	Aqueous	Ethanol	chloroform	Petroleum ether	Acetone
Tannins	+	-	-	-	-
Saponins	++	++	-	+	+
Quinones	++	++	-	-	-
Terpenoids	+++	+	-	-	-
Steroids	+	+	-	+	-
Flavonoids	+++	+	+	+	-
Phenol	+++	+	+	+	+
Alkaloids	+	+	-	-	-
Glycosides	-	-	-	-	-
Cardiac glycosides	+	-	-	+	-
Coumarins	++	+	-	-	-
Anthocyanin	-	-	-	-	-
Beta cyanin	+	+	-	-	-

+++ very strong positive ++strong positive + positive -negative

ence or absence of secondary metabolites. Phytochemical screening was carried out by the general methods used by[17,18]. Screening of different solvent extracts of salaciaoblonga shown the presence of chemical constituents such as phenols, alkaloids, terpenoids, flavonoids, steroids, quinones, tannins, saponins and coumarins. The results were tabulated (Table 1). It shows that the aqueous extract of salaciaoblonga shown more positive on comparing

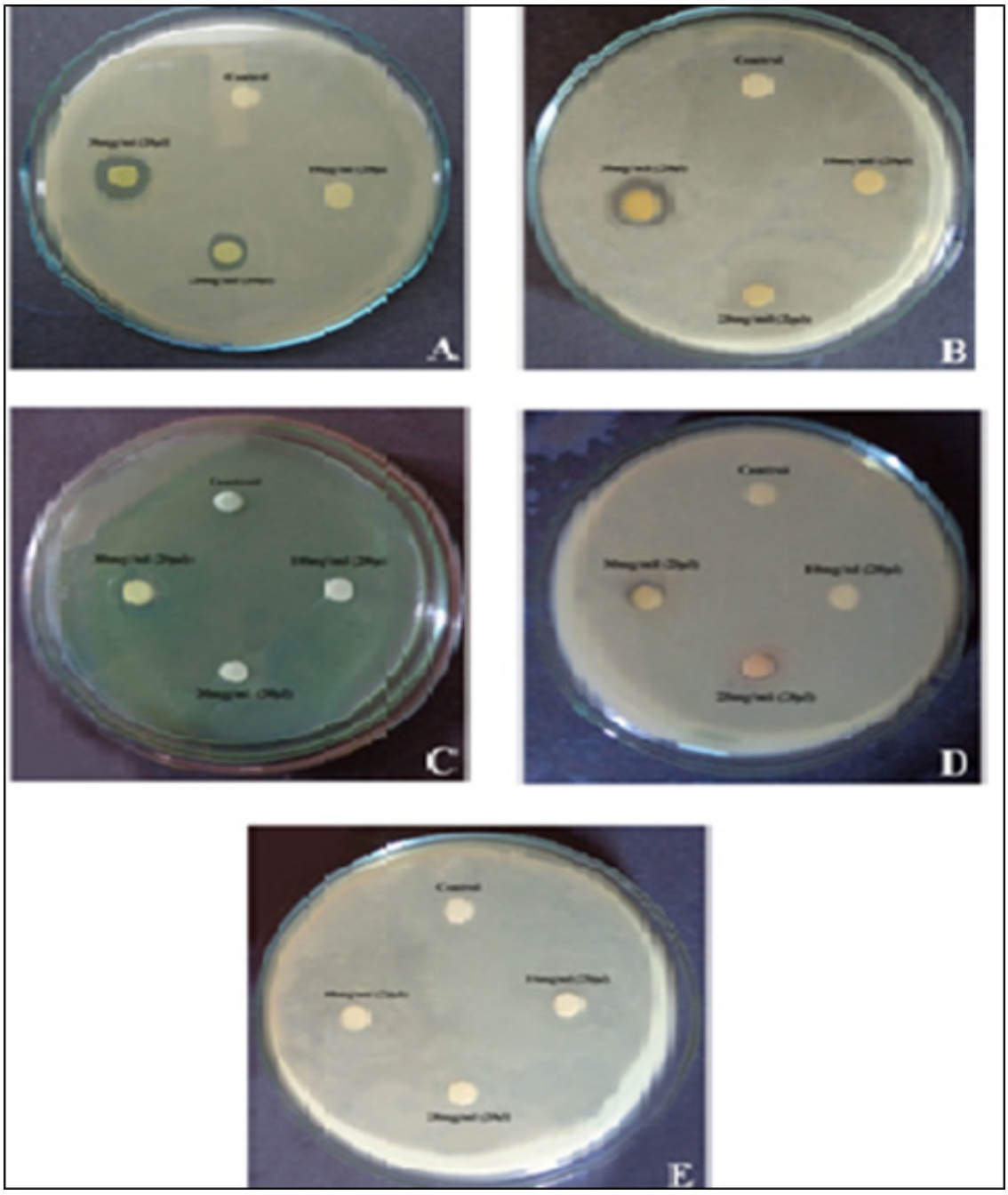

Figure 2. A,B,C,D,E represents the antimicrobial activity of aqueous stem extract of salaciaoblonga against *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus Escherichia coli* respectively. Antimicrobial activity of aqueous stem extract of *Salaciaoblonga*.

other solvent extracts. Thus the aqueous extraction was the best solvent for the salaciaoblonga stem powder. Phenols and flavonoids were shown very strong positive, which indicates the richness of antioxidant property and antimicrobial properties[19,20].

The aqueous stem extract of salaciaoblonga was utilized for the quantification of total phenol and flavonoid contents. Total phenolic content was determined by the Folin-Ciocalteau colorimetric method and the total flavonoid contents were estimated by aluminium chloride calorimetric method. Both plays vital role in the pharmacology. The total amount of phenols and flavonoids were measured as 35.63 mg GAE/g and 19.82mg QE/g respectively. The aqueous stem extracts were tested against five different microorganisms like Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. Disc diffusion assay was carried out to find the potential. Aqueous stem extract shown very good inhibitory zone against the microorganisms. It was measured as 13.2± 0.1 mm, 11.6 ± 0.23 mm, 9.1±0.2 mm and 8.7 ± 0.05mm (including the diameter of disc 6mm) for Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, and Staphylococcus aureus, at 30mg/ml concentration (Table 2). From our findings, the Bacillus subtilis shown maximum inhibitory zone and Escherichia coli was inactive against the extract (Figure 1). From the results, the anti- microbial potency of salaciaoblonga has been established.

4. Discussion

Disease produced by microorganisms plays global concern. Many antibiotics were available in the pharmaceutic field. Anyway, plant derived antibiotics placed in the top position. This is due the low cost and no side effects. Plants preparing phytochemicals such as: flavonoids, phenols, alkaloids, terpenoids, tannins, saponins etc. These secondary metabolites have potential activities against microorganisms, inflammation etc. Phytochemical screening plays vital role for the identification of natural chemical constituents. Phenols and flavonoids are very good antioxidants. This property influence the antimicrobial character of plant extracts. Many researchers proved the potency of phenolic compounds and flavonoids as antimicrobial agents[21,22]. Phenols and phenolic compounds are used for the treatment of skin diseases

Table 2. Inhibition zone of aqueous stem extract of *Salaciaoblonga*

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)	-	9.7±0.17	13.2± 0.1
Bacillus cereus (MTCC 10211)	-	-	11.6 ± 0.23
Pseudomonas aeruginosa (MTCC 14676)	-	-	9.1±0.2
Staphylococcus aureus (MTCC 9542)	-	-	8.7 ± 0.05
Escherichia coli (MTCC 1563)	-	-	-

Each value represents mean±SD of three replicated experiments.

*Includes the diameter of disc (6mm)

and other infections caused by microorganisms. Thus the plants are the natural source of antimicrobial potentials. The significant amount of phenols and flavonoids present in the Salaciaoblonga stem is responsible for the potent antimicrobial properties. Similar studies done by researchers in the plants like: R. arvensis, E. ravens, C.lanatusand F. critica given the evidence for the antimicrobial properties[23].

Many studies were carried out based on such efficacy on the higher plants[24,25]. Microorganisms are very small living species can produce variety of threatening diseases. They can damage the economic status also. They can cause serious problems like asthma, TB, itching, skin disease, infections etc. Phytomedicines can give remedy for the microbial diseases. Thousands of antibiotics are available in the market. But they can produce serious side effects. Thus traditional plant derived drugs placed the first position among the people. The search of novel drug is the challenging one among the researchers. Herbal extractions are very much important for the field of pharmacology. Our study revealed the herbal activity against certain microorganisms like Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Pseudomonasaeruginosa. Thus the plant salaciaoblonga can play vital role for the antimicrobial drug discovery. Similar studies were carried out on the root part of salaciaoblonga, against different microorganisms shown potent antimicrobial nature[26]. Our study supports the pharmacological action of salaciaoblonga stem.

5. Conclusion

From the present study, it was concluded that the herbal plant salaciaoblonga has vital potentially active phytochemical constituents such as phenols, alkaloids, terpenoids, flavonoids, steroids, quinones, tannins, saponins and coumarins. The significant quantity of phenols and flavonoids exhibits the potent antimicrobial properties against Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa and Staphylococcus aureus. This result motivates the researchers to isolate the antibiotic compounds in the stem of salaciaoblonga. Olden days the root part of salaciawas familiar for the herbal treatment. If that situation occurs, very quickly the plant will be vanished. Now the potential herb was under an endangered list. Protection of our natural environment with the biodiversity is the duty of each individual.

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