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In vitro Studies on antioxidant activity of stem extract of *Salacia oblonga* from Karnataka regions, India

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ABSTRACT

The study was conducted to determine the antioxidant activity, total phenol and flavonoid content of the stem of Salacia oblonga, collected from five geographically distant regions of Western Ghat, Karnataka were examined using extracts of aqueous, ethanol, methanol, chloroform and petroleum ether. The five different solvent extracts of S. oblonga stem were evaluated for antioxidant activities by DPPH (1,1 – diphenyl -2- picryl-hydrazyl) radical scavenging activity using Butylated Hydroxy Toluene (BHT) as standard. Among five accessions with different solvents used, maximum antioxidant activity was found in aqueous stem extract (84.3 %) from Hubli(2) followed by others. Total phenol and flavonoid contents were quantitatively estimated. The aqueous stem extract of Salacia oblonga (Hubli(2) - Karnataka) was found maximum in total phenol and flavonoid contents were 34.63 mg GAE /g and 18.72 mg QE /g respectively. The aqueous stem extracts of S. oblonga had superior level of antioxidant activity. The powerful free radical scavenging effect is attributed to the greater amount of phenol and flavonoid compounds in the aqueous stem extract.

Key Words: *Salacia oblonga*, antioxidant activity, DPPH, phenol and flavonoid

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INTRODUCTION

Medicinal plants are great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, terpenoids, flavonoids and phenolic compounds [1-4]. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [5]. A number of studies have focused on the biological activities of phenolic compounds which are antioxidants and free radical scavengers [6-8]

Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and peroxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA [9-10].Oxidative stress, induced by oxygen radicals, is believed to be a primary factor in various degenerative diseases as well as in the normal process of ageing. Several biochemical reactions in our body generate reactive oxygen species (ROS) and these are capable of damaging crucial bio-molecules. If they are not effectively scavenged by cellular constituents, they lead to disease conditions [11-12]. Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells [13]. Phenols and flavonoids are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic activity etc. [14]. It has been established that oxidative stress is among the major causative factors in the induction of many chronic and

degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [15-17].

The genus *Salacia* (Family: Celastraceae) comprises of several medicinally important species (*S. oblonga*, *S. reticulata*, *S. chinensis*, *etc*.) and is known as 'Saptrangi' in Ayurvedic medicine [18]. This herb has been reported in Ayurveda as a treatment for Madhumeha (ancient name of Diabetes) and is distributed in Sri Lanka, India, China, Malaysia and other countries [19]. The root bark is used by either boiling in oil as a decoction or as powder for the treatment of Rheumatism, Itches, Asthma and Ear diseases along with Diabetes and Obesity [20]. Since 1960s, scientists have tried to explore the hypoglycemic potential of various extracts of roots, root bark, and stems of SO in diabetic models [21]. The present study aims to investigate the antioxidant activity, total phenol and total flavonoid content from stem extract of *Salacia oblonga*.

MATERIALS AND METHODS

Collection of *Salacia oblonga*

The healthy plants of *Salacia oblonga* were collected from five different regions of Western Ghats, Karnataka, India (a. Hubli1 ; b. Hubli 2; c, Udipi ; d, Jogimat and e. Bandal). The collected plants were brought to the laboratory and maintained at satyabama University, Chennai-600 119, Tamil Nadu, India.

Preparation of the plant extract

Extraction of the plant samples was done according to a combination of the methods used by [22] and [23] About 15g of dried powder sample were extracted with 150ml acetone, ethanol (75%), chloroform, petroleum ether and water for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No.1 filter paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rotaevator at 40^{o}C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in refrigerator below 10^{o}C.

Qualitative analysis of Antioxidant activity of *Salacia oblonga*

The antioxidant activity of the extracts was determined by following method as described by [24]. Aqueous stem extract of 50µL was taken in the microtiter plate. Methanolic DPPH (100µL of 0.1%) was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration from purple to yellow and pale pink were considered as strong and weak positive respectively.

Quantitative analysis of Free radical scavenging activity of *Salacia oblonga*

The antioxidant activity was determined using DPPH, (Sigma-Aldrich) as a free radical. Sample extracts of 100µl was mixed with 2.7ml of methanol and 200µl of 0.1 % methanolic DPPH. The suspension was incubated for 30 minutes in dark condition. Blank without the sample containing the same amount of methanol and DPPH solution was prepared and measured as control [25-26]. Subsequently, at every 5 min interval, the absorption of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicate, free radical scavenging activity was calculated by the following formula % DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance Of control)] x 100

Estimation of Total phenol content in *Salacia oblonga*

Total phenolic content in the aqueous stem extract was determined by the Folin-Ciocalteau colorimetric method of Lister and Wilson (2001). 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 (Na_2CO_3). The blend was incubated in the dark at room temperature for 15minutes. The absorbance of blue coloured solution of all samples was measured at 765nm using UV-visible spectrophotometer. The results were expressed in mg of gallic acid equivalent (GAE) per gram dry weight of plant powder.

Estimation of Total Flavonoid Content in *Salacia oblonga*

Total flavonoids content in the aqueous stem extracts was determined by the aluminium chloride colorimetric method [27]. 0.5 ml of stem extracts of *Salacia oblonga* 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.

RESULTS AND DISCUSSION

Stem extracts of *Salacia oblonga* are subjected for antioxidant activities by DPPH (1, 1- Diphenyl-2-picryl-hydrazyl) radical scavenging assay. Experiment conducted on the different extraction of acetone, ethanol (75%), petroleum ether, chloroform and aqueous extract showed the presence of antioxidants. 100µl of extracts were estimated for free radical scavenging activity using DPPH assay. The samples were observed for the colour change from purple to yellow and pale pink were considered as strong positive and positive respectively. (Table.1)

Table.1 Qualitative analysis of Antioxidant activity of *Salacia oblonga*

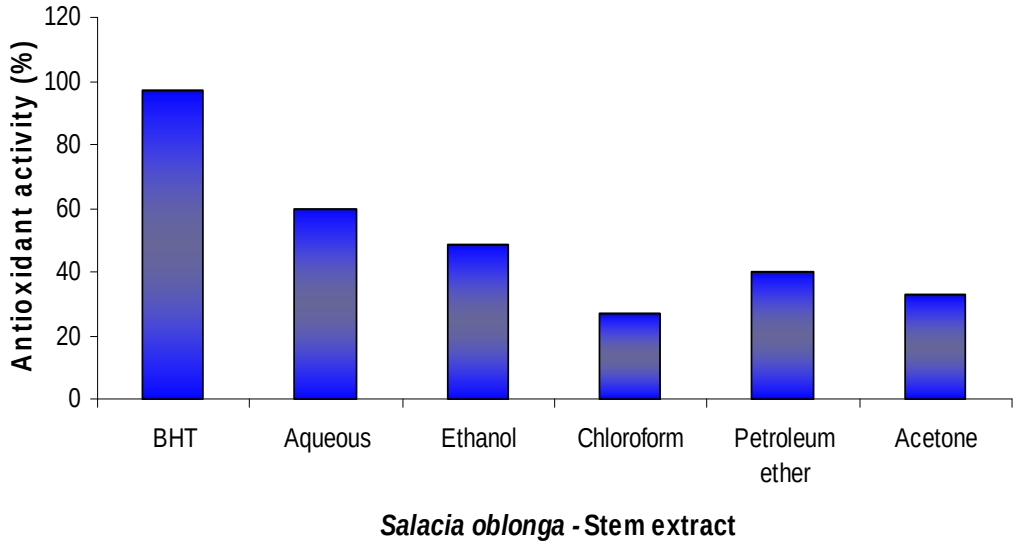
S.No	Solvents used	Qualitative Response of DPPH assay				
		Hubli1	Bandal	Hubli2	Udipi	Jogimat
1	Ethanol	+	+	++	+	+
2	Aqueous	++	+	+++	++	+
3	Acetone	+	-	+	+	-
4	Chloroform	-	-	-	-	-
5	Petroleum ether	-	-	+	+	-

+++ = Strong positive; + = positive; - = Negative

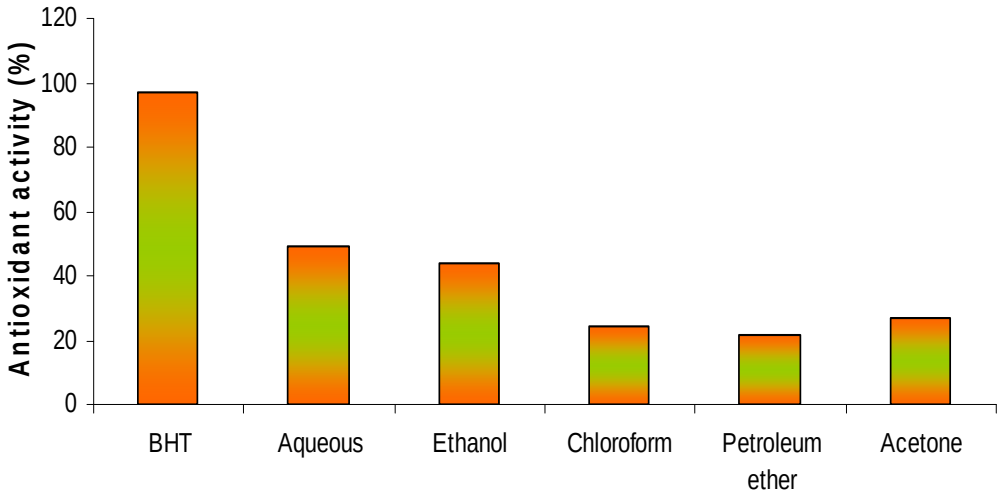
Among the five wild accessions and five different solvent extracts of *Salacia oblonga*, the aqueous stem extract collected from Hubli2 - Karnataka recorded the maximum antioxidant activity (84.3%) followed by Hubli -1 (81.4 %), Udipi (72.9 %), Jogimat (68.0 %) and Bandal (64.7%) accessions (Fig. 1). The positive control (BHT) recorded 98.4 %. Hubli - 2 accession values being close to synthetic antioxidant (BHT) as positive control (98.4%). In each case, aqueous stem extracts recorded higher percentage of free radical scavenging activity than ethanol extractions followed by acetone, petroleum ether and chloroform extract.

Figure 1. Quantitative analysis of Antioxidant activity of *Salacia oblonga stem extract*

1a. **Hubli - 1 accession**

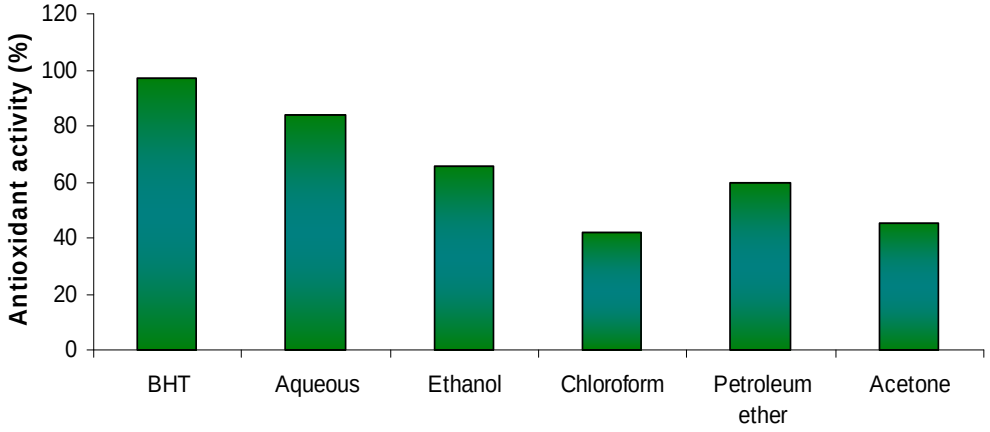


1b. **Bandal accession**



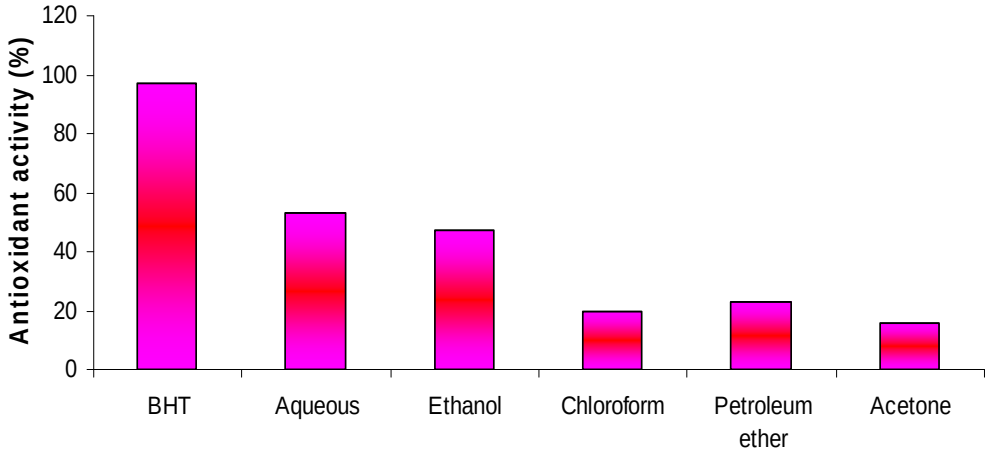
Salacia oblonga -Stem extract

1c. **Hubli - 2 accession**



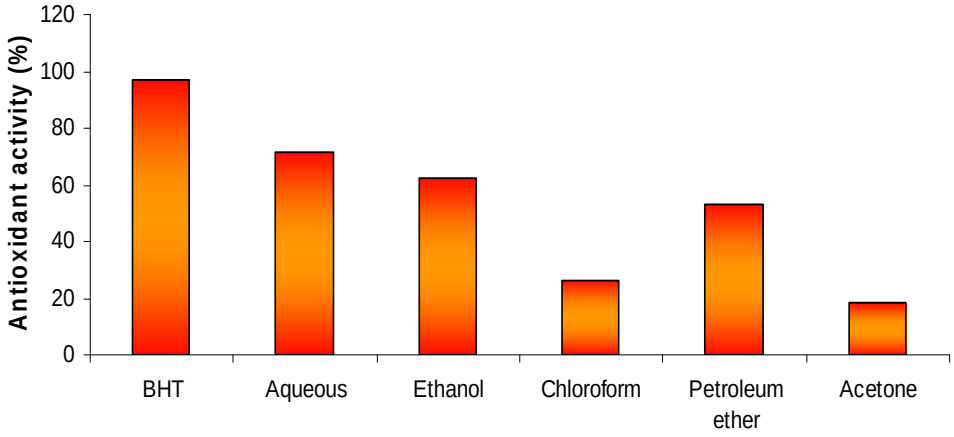
Salacia oblonga - Stem extract

1d. **Jogim**



***Salacia oblonga* - Stem extract**

1e. **Udipi accession**



Salacia oblonga - Stem extract

The antioxidant activity has been attributed to the phenolic compounds contained in the plants [28]. Scavenging activity for free radicals of DPPH (1,1-Diphenyl-2-picryl hydrazyl) has widely used to evaluate the antioxidant activity of natural products from plant and natural sources. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants [29-32].

The result of the present study showed that the phenol and flavonoid contents of the aqueous stem extracts of Salacia oblonga. The maximum amount of phenol (29.72 mg GAE/g) and flavonoid (18.72 mg QE /g) content was found in of *Salacia oblonga*(Hubili-2accession) followed by other accessions(Table.2&Table.3).Estimation of total phenol content shows the sufficient amount of phenol present in the test samples of this study.

Table. 2. Estimation of Total phenol content from aqueous stem extract of *S. Oblonga*

S.No	Plant sample location	Total phenol content (mg GAE/g)
1	Hubli -1	23.42
2	Hubili-2	34.63
3	Udipi	13.76
4	Jogimat	12.45
5	Bandal	10.24

Table.3 Estimation of Flavonoid content from aqueous stem extract of *S. Oblonga*

S.No	Plant sample location	Total flavonoid content (mg QE/g)
1	Hubli -1	13.43
2	Hubili-2	19.82
3	Udipi	10.49
4	Jogimat	9.23
5	Bandal	7.42

Phenolic compounds are a class of antioxidant agents which act as free radical terminators [33]. Phenolic compounds are important plant antioxidants which exhibited considerable scavenging activity against radicals. Thus, antioxidant capacity of a sample can be attributed mainly to its phenolic compounds [34-36]. Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. The mechanisms of action of flavonoids are through scavenging or chelating process [37-39].

CONCLUSION

In the conclusions, free radicals have been implicated in many disease conditions, the important ones being superoxide radicals, hydroxy radicals, peroxyl radicals, and single oxygen. Herbal drugs containing free radical scavengers are gaining importance in treating such diseases. Many plant extracts exhibit efficient antioxidant properties due to their phyto-constituents, including phenolics and flavonoids.

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