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Phytochemical Screening, Total Flavonoid, Total Terpenoid and Anti-Inflammatory Activity of Aqueous Stem Extract of *Salacia oblonga*

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ABSTRACT

The present investigation was carried out to quantify the phytochemical constituents such as total flavonoid, total terpenoid and anti-inflammatory activity of aqueous stem extract of *Salacia oblonga.* Preliminary screening involved the qualitative methods to detect the availability of phenol, tannin, saponin, alkaloids, terpenoids, quinones, flavonoids, coumarins, steroids, glycosides etc. Total flavonoid and total terpenoids were quantitatively estimated. Since flavonoids and terpenoids have potential anti-inflammatory activity. Total flavonoid and total terpenoid contents of aqueous stem extract were found to be 19.82 mg quercetin equivalents per gram and 96.2 mg per gram respectively. *In vitro* anti-inflammatory activity of aqueous stem extract of *S.oblonga* was evaluated by albumin denaturation membrane stabilization tests. The percentage inhibition of denaturation at a concentration of 50 mg/ml of extract was 97.5% and the percentage inhibition of haemolysis shown 89.14%. From the result it was concluded that the availability of secondary metabolites in the stem extract of *S.oblonga* may be responsible for the anti-inflammatory activity. Therefore our study supports the isolation and herbal usage of *S.oblonga* stem for treating inflammation.

KEY WORDS: albumin denaturatio, anti-inflammatory, membrane stabilization, *Salacia oblonga*, terpenoids.

1. INTRODUCTION

Herbal plants are believed to be an important source of secondary metabolites with potential therapeutic effects. The plant metabolites such as alkaloids, flavonoids, terpenoids, saponins, tannins, glycosides, coumarinsand phenolic compounds are important natural chemical constituents (Edeoga, 2005; Tiwari, 2011; Maluventhan Viji, 2010). In India thousands of plant species are available. India is also called as botanical garden, due to the availability of several thousand species of plants (Ahmedulla, 1999). Most of the herbal plants are used as ayurvedic medicine. According to World Health Organisation (WHO) till 80% of people mainly depends on herbal medicines. This is due to the availability, low cost and no side effects (Deepa Murugesan, 2014). Plants have the ability to prepare wide variety of secondary metabolites. It motivates the researchers for finding new potential drugs with variety of activities (Igbinosa, 2009). Natural products like flavonoids and terpenoidsplays vital role with their bioactivity. Flavonoids are an important constituent distributed in plant kingdom (Asha Kale, 2010). Several thousands of flavonoids were identified sofar and many flavonoids have potential anti-inflammatory activity (Ram, 1970; Bonta, 1969). Terpenoids are aromatic compounds found in plant species, which is responsible for flavour and fragrance. Plant terpenoids play vital role in the herbal remedies (Anne, 2016). Terpenoids are secondary metabolites present in plants, and have bioactivities like antibacterial, antiparasite, antiviral, anticancer and anti-inflammatory (De Las Heras, 2009).

Inflammation is a localized physical problem in which a part of the body gets reddened or swollen (Pervical, 2009; Kumar, 2013). The purpose of inflammation is to eliminate the injured cells, removal of the necrotic cells and damaged tissues. It initiates tissue repair. There are two types of inflammation. Acute inflammation is the initial response of the body. Chronic inflammation is the prolonged one (Coussens, 2002). When inflammation starts, white blood cells released some chemicals in to the affected tissue to protect our body from other substances. The main reason for inflammation is the enzyme cyclooxygenasem (Anoop, 2015). It synthesizes prostaglandins which creates inflammation (Chellaram, 2012). It involves the raising of protein denaturation and membrane alteration. To protect us from inflammation, now anti-inflammatory drugs (NSAID's) are available (Chandrappa, 2013; Chellaram, 2009). These non-steroidal anti-inflammatory drugs preventing the formation of prostaglandins and reduce pain. But these drugs may produce many side effects like gastric erosions, stomach ulcer, asthma, kidney damage etc. (Peskar, 1977; Siju, 2014). In many countries medicinal plants are used to cure inflammation with low toxicity and higher therapeutic values.

Salacia oblonga (family: Clasteracea) is a herbal plant widely available in Srilanka, tropical regions of Africa and southern regions of India (Yoshikawa, 2002). It is a woody climber, called as saptrangi (Gladis Raja Malar, 2016). Nearly 18 species of salacia grow in India (Ramamoorthy, 2010). It is rich in antioxidants, secondary metabolites such as alkaloids, terpenoids, flavonoids, steroids, tannins, saponins and phenolic compounds (Gladis, 2015). The root part is used for the curing of diabetes (Jayaram Prakash Rao, 2010). It is also used for curing

rheumatism, skin disease and ghonorrheia (Chopra, 1956). Because of the richness of nutrients it may be used as a liver tonic. The root part of *S.oblonga* has rich in antioxidants and secondary metabolites, thus it has very good anti-microbial, anti-inflammatory and anti-diabetic activities.

Our present study investigates the screening of phytochemicals, quantification of flavonoids, terpenoids and the anti-inflammatory activity of aqueous stem extract of *S.oblonga*. For *in vitro* anti-inflammatory activity, albumin denaturation assay and membrane stabilization assay were performed. These assays proved the maximum percentage inhibition of *S.oblonga* stem extract and its herbal activity.

2. MATERIALS AND METHODS

Collection of *Salacia oblonga:* The healthy plants of *Salacia oblonga* were collected from Western Ghats, Karnataka, India. The collected plants were authenticated by Dr.Vijaya Kumar, Associate professor, Department of Botany, S.T.Hindu College, Nagercoil, 629002. Then the stem was washed thoroughly by tap water to remove the impurities. Then it was dried under shadow for four weeks. The dried stems were powdered by ball mills and maintained at Sathyabama 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 (Lee, 2003). About 30g of powdered sample were extracted with 300 ml five different solvents such as acetone, ethanol (75%), chloroform, petroleum ether and water. Then it was kept overnight at room temperature. The extraction samples were filtered by using whatman no.1 filter paper in a Buchner funnel through suction. The filtrate was concentrated under vacuum in a rotary evaporator at 40^0C.The concentrated extracts were stored in sample vials and refrigerated below 10^oC.

Phytochemical screening of *S.oblonga:* Preliminary phytochemical screening of stem extracts prepared from five different solvents (acetone, ethanol, chloroform, petroleum ether and water) of *S.oblonga* were performed by standard method described by (Brindha, 1981; Priya, 2014). General reactions used to identify the natural potential groups such as flavonoids, phenols, alkaloids, terpenoids, tannins, saponins, glycosides, cardiac glycosides, coumarins and steroids. Appearance and disappearance of coloration revealed the presence or absence of such potential groups.

Estimation of Total Flavonoid Content of *Salacia oblonga:* Total flavonoids content in the aqueous stem extracts was determined by the aluminium chloride colorimetric method (Mervat, 2009). 0.5 ml of aqueous stem extract of *S.oblonga* at a concentration of 1mg/ ml was taken and the volume was made up to 3ml with methanol. Then 0.1ml of 10% $AlCl_3$, 0.1ml of potassium acetate and 2.8 ml distilled water were added. The whole mixture was agitated constantly. Then it was incubated for 30 minutes. Absorbance was taken at 415 nm. Known concentration of quercetin was prepared and absorbance measured. A calibration plot was drawn. Total flavonoid content of test samples calculated from the calibration plot. Then it was expressed as mg quercetin equivalent /g of sample.

Estimation of Total Terpenoid Content of *Salacia oblonga:* Total terpenoid content in the aqueous stem extracts of *S.oblonga* were determined by the method as described by Ferguson, (1956). 1g of *S.oblonga* stem powder was taken in a conical flask and soaked in ethyl alcohol for one day. Then it was filtered and the filtrate was extracted with petroleum ether. The ether extract was taken as the measure of total terpenoid.

$$\text{Total terpenoid content} = \frac{(\text{Final weight of the sample} - \text{Initial weight of the extract}) \times 100}{\text{Weight of the Sample}}$$

Anti-inflammatory activity- Albumin denaturation assay: 0.2 ml of egg albumin (fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of the aqueous stem extract of *s.oblonga* were considered as reaction mixture, so that final concentrations become 1.56, 3.125, 6.25, 12.5, 25.0, 50.0 mg/ml. Similar volume of double-distilled water served as control. The test solutions were incubated at 37±2ºC in an incubator for 15 minutes. Then it was heated at 50ºC for 5 minutes. The reaction mixture was cooled to room temperature. Then absorbance was measured at 660 nm. Similarly absorbance was measured for the reference drug diclofinac sodium prepared as different concentrations (0.078, 0.156, 0.312, 0.625, 1.250 mg/mL). The experiment was performed in triplicates. The inhibition percentage of protein denaturation was determined as follows,

$$\% \text{ Inhibition of Protein Denaturation} = 100 \times [Vc - Vt / Vc]$$

Where, Vt = absorbance of test sample, Vc = absorbance of control.

The aqueous stem extract of *S.oblonga* and the reference drug concentration for 50% inhibition (IC_{50}) was determined form the dose response curve by plotting inhibition percentage with respect to the control against treatment concentration.

Anti-inflammatory activity-Membrane stabilisation assay: Drug used as Standard: Acetylsalicylic acid available in the commercial name of Ecosprinʀ -75 marketed by USV Limited, Mumbai, Maharashtra. It was used as a source of acetylsalicylic acid. The blood was taken from a healthy person. The person should not take any non-steroidal anti-inflammatory drug, 15 days before to the experiment. The blood was collected in a heparinzed vacutainer and washed three times with 0.9% of saline. Then it was centrifuged for ten minutes at 3000 rpm. The stock erythrocyte

suspension was prepared by using isotonic phosphate buffer, which contains 154mM sodium chloride in 10mM sodium phosphate buffer (pH 7.4).

Hypotonic solution –induced haemolysis or membrane stabilizing activity: This test was done according to the method described (Shinde, 1999) with slight modifications. The test sample consisted of stock erythrocyte (RBC) suspension 0.030ml mixed with 5ml of hypotonic solution (154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4) containing *S.oblonga* stem extract ranging from concentration 1.56 - 50 mg/ml. The control sample consisted of 0.030ml RBC suspension mixed with hypotonic buffered solution alone. The standard acetylsalicylic acid was treated similar to test at 0.078, 0.156, 0.312, 0.625, 1.250mg/mL concentrations. The experiment was repeated three times. The mixtures were incubated for ten minutes. Then it was centrifuged at 3000rpm for 10 minutes, and absorbance of the supernatant was measured spectrophotometrically at 540 nm. The inhibition percentage of haemolysis was determined as follows,

$$\% \text{ Inhibition of haemolysis} = 100 \times [A\ 1 - A\ 2 / A\ 1]$$

Where, A 1 = Absorbance of hypotonic buffered solution alone, A 2 = Absorbance of test /standard sample in hypotonic solution.

3. RESULTS AND DISCUSSION

Screening of phytochemicals on the stem extract of *S.oblonga* shown the availability of natural compounds such as phenols, flavonoids, alkaloids, terpenoids, saponins, coumarins, cardiac glucosides and tannins. The stem extracts were prepared by using ethanol, acetone, petroleum ether, chloroform and water. The aqueous stem extract of *S.oblonga* was shown (Table.1) more positive for the presence of natural chemical constituents.

Table.1. Phytochemical Screening of Stem Extracts of *Salacia oblonga*

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

+positive ++strong positive -negative

The medicinal value of plants based on the availability of secondary metabolites (Savithramma, 2011). If the natural constituents are present the herbal plant is used for curing of skin disease, cough, bronchitis, diarrhoea, painful swellings, leprosy, fever, ulcer, wounds, vomiting and cardiac disorders (Bedi, 2010). The natural chemical constituents in the aqueous stem extract of *S.oblonga* were shown very good antioxidant, antibacterial activities. The root extract of *S.oblonga* have antioxidant, antibacterial, anti-diabetic and anti-inflammatory activities due to the availability of natural products (Ismail, 1997).

Quantification of flavonoids and terpenoids: Flavonoids are the plant pigments. It is giving colour to flowers. They are ketone containing compounds. The antioxidant component flavonoid induces anti-inflammatory activity. It inhibits the reactive oxygen compounds and the pro inflammatory activity of enzyme cyclooxygenase. Flavonoids have potent anti-inflammatory activity by inhibiting prostaglandin synthesis (Lee, 2007). Terpenoids are natural secondary metabolite found in plant species which is providing flavour and fragrance. It prevents the development of chronic joint swelling (Agnihotri, 2010). Quantification of flavonoids and terpenoids in the aqueous stem extract of *S.oblonga* shown in Table.2. The total flavonoid and total terpenoid contents of *S.oblonga* shown 19.82 milligram quercetin equivalent per gram, 96.2 mg/g respectively.

Table.2. Quantification of TerpenoidsAnd Flavonoids of Aqueous Stem Extract of *S.Oblonga*

Sample	Flavonoids content (mg QE/ g)	Terpenoids content (mg/g)
Salacia oblonga–Stem Extract	19.82	96.2

Anti-inflammatory activity: The anti-inflammatory activity of aqueous stem extract of *S.oblonga* was estimated by protein denaturation assay and membrane stabilization method. By protein denaturation assay the aqueous extract of *S.oblonga* was subjected to different concentrations. It was shown maximum percentage inhibition (97.5%) at 50 mg/ml concentration (Table III) which was very close to the standard, diclofinac sodium (98.75%) at 1.250mg/ml concentration, was represented in Table 4.The IC_{50} values of *S.oblonga* stem and Diclofenac Sodium against protein denaturation were given Table.5. Protein denaturation means disintegration of protein structure by the external application of stress, heat, acid or base. This leads toloss of its originality which leads to inflammation. Plants play vital role for inhibiting the denaturation of proteins (Leelaprakash, 2011). In the present study, the result indicates that the aqueous stem extract of *S.oblonga* shown maximum inhibition percentage of protein denaturation. Similarly the root extract of *S.oblonga*, shown very good potential for the prevention of protein denaturation which was reported by Ismail (1997). But there is no report on the aqueous stem of *S.oblonga.* This supports the herbal usage of stem for curing inflammation.

Table.3. Influence of *Salacia oblonga* Stem against Protein Denaturation

S.No	Concentration (mg / mL)	% Inhibition of Protein Denaturation
1	1.56	25
2	3.125	62.5
3	6.25	80
4	12.5	88.75
5	25	96.25
6	50	97.5

Table.4. InfluenceofDiclofenac Sodium against Protein Denaturation

S.No	Concentration (mg / mL)	% Inhibition of Protein Denaturation
1	0.078	84.37
2	0.156	92.5
3	0.312	96.25
4	0.625	97.5
5	1.250	98.75

Table.5. IC_{50} Values of *Salaciaoblonga*stemAndDiclofenac Sodium against Protein Denaturation

S.No	Treatments	IC_{50} values (mg / mL)
1	*Salacia stem extract*	2.5
2	Diclofenac Sodium	0.046

HRBC membrane stabilization method is well known for anti-inflammatory activity (Gandhidasan, 1991). Anti-inflammatory activity of *S.oblonga* was also proved by HRBC (Human Red Blood Cells) membrane stabilization assay. In this assay acetyl salicylic acid was considered as standard. The aqueous stem extracts were used to find the inhibition percentage of hemolysis. It was shown that the potential inhibition percentage of hemolysis 89.14% at 50mg/ml concentration (Table.6). When the concentration increases, the inhibition percentage of hemolysis also increases. Similar correlations were shown by researchers (Siju, 2014). The result is comparable with the reference drug. The standard drug also subjected to the same assay. It was shown maximum inhibition tendency of hemolysis 93.14% at a concentration of 1.250 mg/ml (Table.7). These results were shown the effective bioactivity of *S.oblonga* stem for the pharmaceutical usage as anti-inflammatory drugs. The IC_{50} values were calculated and found to be 6.752mg/ml and 0.0455mg/ml for plant sample and standard respectively (Table VIII). During inflammation lysosomal hydrolytic enzymes were released, which causes damage of tissues (Sadique, 1989). Since the HRBC membranes are similar to lysosomal membrane, the inhibition of hypo tonicity induced HRBC lysis was considered as a measure of anti-inflammatory activity (Chou, 1997). Similar studies were examined for the potential anti-inflammatory activity of many herbal plants (Vinay Gupta, 2013). The root extract of*S.oblonga* had effective anti-inflammatory activity. But there is no report for the prevention of inflammation of *S.oblonga* stem.

Table.6. Influence of *Salacia oblonga* Stem extract against Membrane Stabilization

Concentration (mg/mL)	% Inhibition of Hemolysis	Concentration (mg/mL)	% Inhibition of Hemolysis
1.56	41.71	12.5	62.85
3.125	42.29	25	83.42
6.25	46.28	50	89.14

Table.7. Influence of Acetylsalicylic Acid against Membrane Stabilization

S.No	Concentration (mg / mL)	% Inhibition of Hemolysis
1	0.078	85.71
2	0.156	88.57
3	0.312	90.28
4	0.625	91.42
5	1.250	93.14

Table.8. IC_{50} Values of *Salacia oblonga* and Acetylsalicylic Acid against Membrane Stabilization

Treatments	**IC_{50} values (mg / mL)**
Salacia oblonga stem extract	6.752
Acetylsalicylic acid	0.0455

4. CONCLUSION

Our present investigation concluded that, *S.oblonga* is a medicinal plant with enormous biological activities. This is due to the potential constituents like flavonoids, terpenoids, phenols, alkaloids, saponins, quinones, steroids etc. Phytochemical analysis proved the availability of natural chemical constituents. Flavonoids and terpenoids were the major constituents for the anti-inflammatory activity. The assays carried out on the stem extracts indicate that the plant is an excellent source of anti-inflammatory drug which may helpful for the pharmaceutical companies. *S.oblonga* is now considered as an endangered plant because the root extract is rich in natural constituents and is helpful for the herbal purpose. This may extinct the wonderful multipotential plant from the universe. Our study motivates the protection and herbal usage of *Salacia oblonga* stem for long years. Further investigation is needed for the isolation of anti-inflammatory drugs.

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