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Evaluation of Antioxidant Properties of *Salacia Macrosperma* Leaf Extracts

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Abstract: *In the present study experiments were carried out to determine the total phenolic content and their comparative antioxidant activities in relation to phenol content employing Folin-ciocalteu, DPPH, and ABTS assays of the different solvent extracts of the plant S. macrosperma. It was found that on an average, the plant extract with higher levels of phenol content had higher antioxidant activity. The antioxidant activities were found to be highest and lowest at methenolic and hexane extracts, respectively in both the assays. At 1mg/ml concentration, DPPH and ABTS assays showed highest activity in the methanol extract of 84 and 99% respectively. The presence of phenolic compounds was confirmed by HPLC.*

Keywords: *Salacia macrosperma, Antioxidant activity, DPPH assay, ABTS assay, Total phenolic content*

1. INTRODUCTION

In Siddha and Ayurveda, plants and food extracts were used as preventive measures and rejuvenating agents for the diseases. Charaka *and Susruta* two of the earlier Indian authors had sufficient knowledge of properties of the Indian medicinal plants.

During the past decade there has been an ever increased demand especially from the developed countries for more and more drugs from the plant sources. This revival of interest in plant-derived drugs is mainly due to current widespread belief that green medicine is safe and more dependable than the costly synthetic drugs, many of which having the adverse side effects. In contrast to the traditional use of medicinal plants involves utilizing an entire portion of the plant like roots or leaf rather than extracting the single compound. Modern medicine again tracing such compounds of medicinal value associated with the plants and the exploitation of such compounds to the health benefits. Among those *Salacia* is one such, with huge medicinal properties like antidiabetic, antirheumatoid arthritis, antiobesity, etc. [1-2].

Salacia Linn. belonging to Hippocrateaceae family (under the class Magnoliopsida and order Celastrales) is a large genus of creeping or climbing shrubs or often small trees distributed mainly in the warmer parts of the world. The genus *Salacia* is morphologically and chemically distinct species distributed in Southern India and Western Ghats [3]. *Salacia* is an important medicinal plant, which considered as a source of secondary metabolites with a wide range of pharmaceutical attributes. Species of *Salacia* include *S. chinenesis, S. fructicosa, S. macrosperma, S. Reticulata, S. oblonga* and *S. madagascariensis* etc., extensively found in southern parts of India. Historically, the *Salacia* plants are used as part of the traditional Ayurvedic system of Indian medicine to treat diseases such as diabetes [3]. Currently, extracts of *Salacia* are consumed in commercial foods and food supplements in Japan for the treatment of diabetes and obesity. In indigenous system of medicine, Salacia are used for the treatment of diabetes, diarrhoea, liver problems etc, without the sufficient knowledge of its bioactivity [4]. Now it has been understood that it is mainly because of the bioactive compounds found with it are able to inhibit the α–glucosidase activity as a result of it, blood glucose level will be less. A most potent natural α-glucosidase inhibitor named salacinol has been isolated from an antidiabetic Ayurvedic traditional medicinal plant *Salacia*. Kotalanol and de-o-sulfonated-kotalanol are the most active principles in the aqueous extracts of *Salacia reticulata*, which are traditionally used in India, Sri Lanka, and Thailand for the treatment of diabetes. Along with these two, 13- MRT, were also found with antidiabetic activity of *Salacia*. [5-6].

Salacia oblonga is one of the major and essential components of anti-diabetic ayurvedic formulations available in the market. However only a few studies have looked at the effects of *S. oblonga* and other

species in humans and the early results are promising in terms of blood sugar control [7]. Many medicinal plants contain large amounts of antioxidants other than vitamin C, E and carotenoids. Antioxidants are molecules that can delay or prevent an oxidative reaction catalyzed by free radicals. This antioxidant effect is mainly due to the presence of phenolic components such as flavonoids, phenolic acids and phenolic diterpenes. Phenolic acids are involved in repair and adaptive systems, and can act preventively and sometimes therapeutically in various diseases: cardiovascular, respiratory, dermatological and digestive system diseases [8].

Flavonoids have the most potent antioxidant ability because of the chemical structure with O-diphenolic group, a 2, 3-double bond and hydroxyl group at position 3, 5. They can make complexes with metals and are effective hydroxyl radical and peroxyl radical scavengers. Antioxidant properties of polyphenols arise from their high reactivity as hydrogen or electron donor, chain breaking functions or ability to chelate metal ions. Phenolics may change the fluidity of membranes thus esterically hinder diffusion of radicals and restrict peroxidation reactions [6].

Less than 10% of the medicinal plants that are traded in the country are cultivated and about 90% are collected from the wild. These are often harvested in a destructive and unsustainable manner. Their great demand in Ayurveda and the pharmaceutical industry has imposed tremendous pressure on natural populations from the Western Ghats of India. The biodiversity hotspot of the Western Ghats, which is ranked fifth in the world in its biological resources, harbours about 4000 species that are used in herbal drug formulations. Besides, several plant species from the Western Ghats are gaining international importance due to their newly identified pharmacological and curing properties [9].

Not many studies have reported on some of the species of *Salacia* such as *S macrosperma* and no information is available on the phytochemical and genetic analysis of *Salacia* species of Western Ghats. Still there is lack of information on their phyto-chemical medicinal properties of various plant species. Hence the current work has been undertaken to evaluate the biological activities of the *S. macrosperma* plant extracts.

2. MATERIALS AND METHODS

2.1. Extract Preparation

Healthy plant leaves of *S. macrosperma* were collected, washed with deionised water, shade dried and powdered. Dry powder is suspended in methanol, incubated for an hour on a shaker, followed by ultra sonication for 30 mins. Sonicated samples were filtered and evaporated to dry. Dried samples were stored at -4C and used for the further studies.

2.2. Bioactivity Assays

2.2.1. Total Phenolic Content

Total phenolic content of different samples were determined by using FC (Folin–ciocalteau) reagent described previously by Singleton and Rossi (1965) [10]. Phenol reacts with the phosphomolybdate in alkaline condition to produce blue colour and was read at 765 nm using spectrophotometer. The results were expressed as gallic acid equivalence (GAE) in micrograms.

Requirements: FC reagent (1:10 dilution), gallic acid, distilled water**,** 7.5 % Na_2CO_3, spectrophotometer, test tubes.

Methodology: 2.5 ml of FC reagent (1:10 dilution) was incubated with 50µl of each sample for three mins. To this mixture 2ml of Na_2CO_3 was added and incubated for 15 mins at 45^{o}C. Absorbance of each sample was measured using spectrophotometer at 765nm using methanol with FC as blank. Assay was conducted with reference to the standard Gallic acid concentration. Results were expressed as gallic acid equivalence (GAE) in micrograms.

2.2.2. DPPH Assay

The DPPH assay was carried according to the method described by Brand-Williams et al. (1995) with some modifications [11]. DPPH (1, 1-Diphenyl, 2-picryl hydrazyl) contains an odd electron, gives purple colour to the compound. As the antioxidative compound gives it and hydrogen it gets paired with the hydrogen donated from the antioxidants and becomes decolorized.

Requirements: 300 mM DPPH, 96 well micro titre plate, ELISA reader, methanol.

Procedure: 95µl of DPPH was incubated with 5µl of the samples of different species of Salacia. DPPH with methanol was used as the positive control. The mixture was incubated in dark for 30 mins at room temperature. The obsorbance of the samples were measured at 517nm. Percentage of DPPH radical scavenging was calculated by using the following equation. This was compared with the percentage of radical scavenging activity of ascorbic acid as standard.

$$\% \text{ Antiradical Activity} = \frac{\text{OD of Control - OD of Sample}}{\text{Absorbance of Control}} \times 100 \quad (1)$$

2.2.3. ABTS Assay

ABTS assay was performed as per the procedure given from Arnao et al. (2001) [12]. 2, 2^1-azino-di-3 ethyl benzthiazoline sulphonate (ABTS) is purple compound formed due to the oxidation of $ABTS^{\cdot}$ To $ABTS^{\cdot\cdot+}$. Antioxidants inhibits the oxidation of $ABTS^{\cdot}$ To $ABTS^{\cdot\cdot+}$as a result, decoloration takes place. Highly potent antioxidants scavenge the $ABTS^{\cdot}$ potentially and decolorize it completely.

Requirements: ABTS, methanol, potassium per sulphate, distilled water, spectrophotometer.

Procedure: 7 mM ABTS reagent was prepared in 2.45 mM potassium per sulphate and it was diluted with methanol to get an OD of 0.7 at 734nm. 990 µl of this reagent was mixed with 10 µl of sample. 10 µl of methanol with 990 µl of ABTS was maintained as control. The mixture was incubated for about five mins and absorbance was measured at 734nm. *% of activity* was calculated from the resulted absorbance (OD) value with respect to positive control value and obtained results were compared with the standard ascorbic acid standards.

2.2.4. High Performance Liquid Chromatography

HPLC was performed using Thermosypersil C18 Column, with a solvent system of 0.1 % H_3PO_4 in water and Acetonitrile in the ratio of (65:35). The injection volume was 20 µl and the flowrate kept was 1 ml/min employing Photodiode detector (PDA).

All experiments were performed in triplicate and an average values was reported.

3. RESULTS AND DISCUSSION

3.1. Total Phenolic Content

In most of the cases, the amount of total phenolic content found in the plant is directly proportional to the amount of bioactivity. Phenolic compounds are the major contributors to the antioxidant properties of plants [13]. Total phenol content may vary in the different plants and also depends on solvent system used for the extraction. Further it also depends on the method of extraction. In the current sudy it was observed that the highest phenol content of GAE of 250µg/ml, was obtained in the sonicated extract while, it was 180µg/ml in the soxhlet extract from 1 mg/ml of *Salacia macrosperma* leaf powder. It was found that phenol content in the plant extracts appeared to be directly proportional to extract concentration (**Figure 1**). Previous studies also confirm the relation between total phenolic compounds to antioxidant property through many plant and food systems [13-15]. The highest concentration of the phenol content was obtained in methanol extract compared to other solvent systems at all concentrations studied**.**

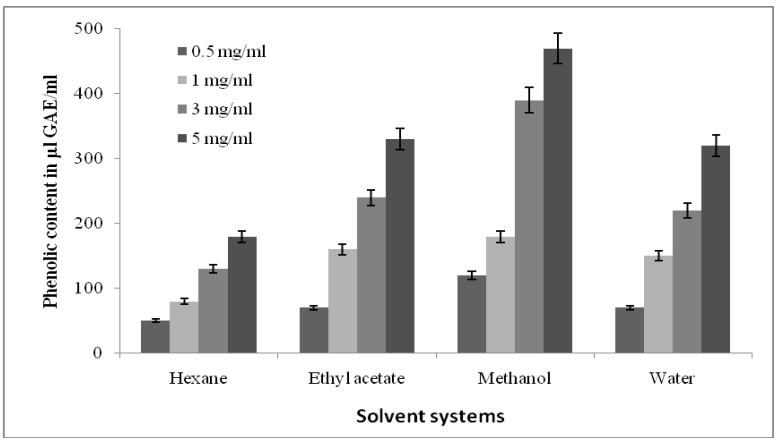

Fig1. *Total phenol estimation from the Salacia macrosperma at different solvents*

3.2. DPPH Radical Scavenging Activity

The antioxidant abilities of the various *Salacia* extracts were determined as the ability of the extract to scavange DPPH radicals according to the method of Bondet (1997) [16]. The decrease in absorbance was measured due to scavenging for DPPH radical by the plant extracts through spectrophotometry at 517nm after incubation period. There was significantly high levels of decolouration of the DPPH was observed with increased phenolic content. The reduction in DPPH radical is a measure of antioxidant activity. A significant decrease in concentration of DPPH radical was found with increase in concentration of plant phenolic content. Among all the solvent systems studied methanolic extract has shown significantly better scavenging activity even at 1 mg/ml concentration and it was very close to the standard values (**Figure 2)**. Other samples were shown lesser values compared to standard in almost all the cases.

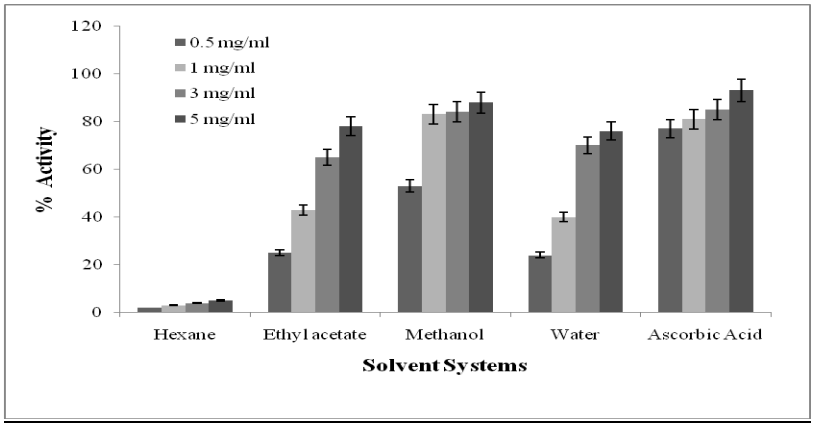

Fig2. *DPPH Assay for extracts of S. macrosperma*

3.3. ABTS Radical Scavenging Activity

ABTS is designed to measure the overall antioxidant capacity within a given sample to inhibit the oxidation of ABTS in comparison to Ascorbic acid. Here also significantly high levels of decolouration was observed with respect to increased phenolic content [17]. From the obtained results, as like DPPH assay the methenolic extract has resulted significantly better activity compared to other solvent systems studied. Methenolic extracts has shown closer value when compared to the ascorbic acid standard samples in almost all the concentrations studied. Hexane extract has shown lowest value for the assay. It is also confirms that the phenolic content is directly proportional to the antioxidant activity of the compound.

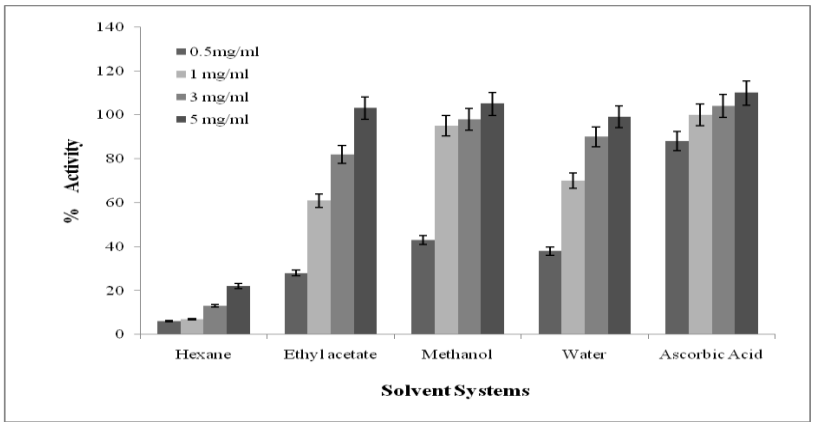

Fig3. *ABTS Assay done for Salacia macrosperma samples*

3.4. HPLC Profiles

HPLC profile of methanol extract has resulted in five major and eight minor peaks. The profile showed compounds separated at a retention time of 13.351, 16.442, 18.149, 20.544 and 22.305 for major, 9.002, 10.594, 14.611, 15.103, 24.178, 26.371, 28.427 and 34.057 minutes for minor peaks respectively. Similarly HPLC profile for Ethyl acetate extract showed five prominent peaks at 19.619, 21.936, 26.139, 31.560 retention time and one minor peak at 28.531 (Figure 5). This confirms the

presence of more compounds in methanol extract compared to the ethyl acetate. Aqueous extract was also found similar profile for compounds but in the lesser concentrations. The peaks identified may be due the presence of phenolic compound such as different forms of catechins, ferulic acid, gallic acid etc. Further studies will be required for the identification and characterisation of these compounds.

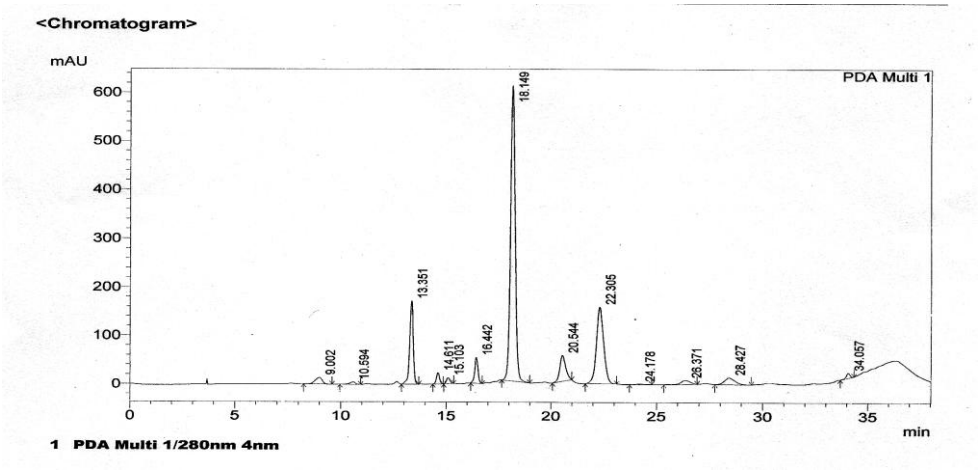

Fig4. *HPLC profile for Methanol fraction of Salacia macrosperma*

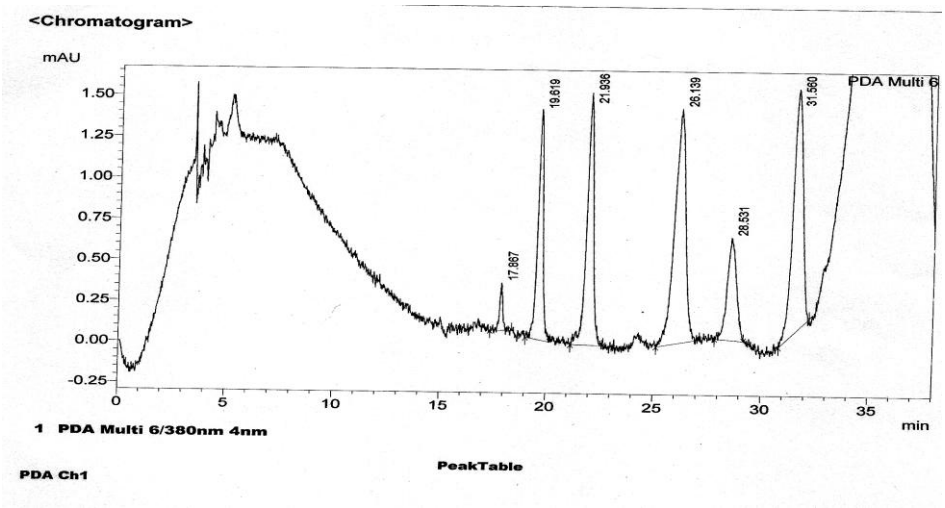

Fig5. *HPLC profile of Ethyl acetate extract of Salacia macrosperma*

4. CONCLUSION

The results of these assays justify the use of the investigated plants for medicinal purpose for its pharmacological properties. Several methods were used to measure the antioxidant activities of the natural compounds. The generally used free radicals for these assays are ABTS and DPPH specifically due to their reproducibility of the assays. The concentrations of the total phenolic content play a major role in exhibiting these properties. HPLC profiles confirmed the presence of phenolic comopounds.

The results obtained confirm the therapeutic potency of *Salacia* used in traditional medicine. In addition, these results form a good basis for selection of the plant for further phytochemical and pharmacological investigation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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