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Antioxidant activites of *Xanthosoma sagittifolium* Schott using various *in vitro* assay models

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ABSTRACT

Objective: To evaluate the total phenolic, flavonoid contents and in vitro antioxidant activity of methanol extract of *Xanthosoma sagittifolium* corm. **Methods:** Total phenolic content was estimated using the Folin Ciocalteu method. The flavonoid content was determined using aluminium chloride. In vitro antioxidant activities were evaluated by studying DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity, ABTS radical cation scavenging activity and reducing power capacity were determined using standard procedure. **Results:** *Xanthosoma sagittifolium* corm exhibited 0.32g100g-1 total phenolic; 0.26g100g-1 flavonoid and better scavenging activity of DPPH (78.22±0.56%), hydroxyl radical (69.11±0.21%), superoxide radical (83.27±0.08%) and ABTS radical cations(76.11±0.07%).**Conclusions:** The present studies confirm the methanol extracts have potential in vitro antioxidant activity.

1. Introduction

Free radicals are chemically unstable atoms that cause damage to lipid cells, proteins and DNA as a result of imbalance between the generation of reactive oxygen species (ROS) and the antioxidant enzymes [1]. They are known to be the underlying cause of oxidative stress which is grossly implicated in the pathogenesis of various diseases such as cancer, diabetes, cardiovascular diseases, aging and metabolic syndrome [2,3]. Examples of these radicals include superoxide anions, hydroxyl, nitric oxide and hydrogen peroxide radicals. These radicals can be scavenged by the protective role of natural and synthetic antioxidant agents [4]. Several synthetic antioxidants such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and tertbutyl hydroquinone (TBHQ) are commercially available and currently in use. However, their use is now restricted due to their side effects [5]. The use of natural antioxidants has gained much attention from consumers because they are considered safer than synthetic antioxidants. Recently, there has been a worldwide trend towards the use and ingestion of natural antioxidants present in different parts of plants due to their phytochemical constituents [6,7].

The *Xanthosoma sagittifolium* Schott are plants of the tropical rain forest and, although in their natural habitat they grow under the forest canopy, under cultivation they are usually sown with full exposure to sunlight. They require well-drained soils and do not tolerate the permanent presence of water. The usable parts are the subterranean tuberous stems which have a nutritional value comparable to the potato and are probably easier to digest. *Xanthosoma sagittifolium* are medicinal species used as food and to prevent and treat bone diseases, such as osteoporosis, in traditional Brazilian medicine [8]. Hence, the present study has been made to investigate the *in vitro* antioxidant activity of *Xanthosoma sagittifolium* corm using different models.

2. Materials and Methods

The corm of *Xanthosoma sagittifolium* were collected from Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for

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the extraction of active constituents of the plant material.

2.1. Preparation of Extracts

Ten grams of powdered corm of *Xanthosoma sagittifolium* were extracted separately with methanol (100ml) in shaker for 24 h at room temperature. Extract was filtered through Whatman filter paper. The filtrates were subjected to analysis for total phenolic, flavonoid contents and *in vitro* antioxidant activities.

2.2. Estimation of Total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu method [9]. Samples (100 μl) were mixed thoroughly with 2 ml of 2% Na_2CO_3. After 2 min. 100 μl of Folin-Ciocalteu reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 743 nm against a blank. Total phenolic content was expressed as gram of gallic equivalents per 100 gram of dry weight (g100g-1DW) of the plant samples.

2.3. Estimation of Flavonoids

The flavonoids content was determined according to Eom et al [10]. An aliquot of 0.5ml of sample (1mg/ml) was mixed with 0.1ml of 10% aluminium chloride and 0.1ml of potassium acetate (1M). In this mixture, 4.3ml of 80% methanol was added to make 5ml volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

2.4. DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H [11].

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method [11]. Briefly, an 0.1mm solution of DPPH in methanol was prepared, and 1ml of this solution was added to 3 ml of the solution of all extracts in methanol at different concentration (125,250,500 &1000 μg/ml).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance were measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation).Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

$$\text{DPPH scavenging effect (\% inhibition)} = \{(A0 - A1)/A0) \times 100\}$$

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

2.5. Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of [12]. Stock solutions of EDTA (1mM), FeCl3 (10mM), Ascorbic Acid (1mM), H2O2 (10mM) and Deoxyribose (10 mM), were prepared in distilled deionized water.

The assay was performed by adding 0.1ml EDTA , 0.01ml of FeCl3,0.1ml H2O2, 0.36ml of deoxyribose, 1.0ml of the extract of different concentration (125,250,500 &1000 μg/ml)dissolved in distilled water,0.33ml of phosphate buffer (50mM , pH 7.9), 0.1ml of ascorbic acid in sequence . The mixture was then incubated at 370c for 1 hour. 1.0ml portion of the incubated mixture was mixed with 1.0ml of 10%TCA and 1.0ml of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation is calculated by using the following equation

$$\text{Hydroxyl radical scavenging activity} = \{(A0 - A1)/A0) \times 100\}$$

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

2.6. Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Robak and Gryglewski [13]. The superoxide anion radicals were generated in 3.0 ml of Tris - HCL buffer (16 mM, pH 8.0), containing 0.5 ml of NBT (0.3mM), 0.5 ml NADH (0.936mM) solution, 1.0 ml extract of different concentration (125,250,500 &1000 μg/ml), and 0.5 ml Tris - HCl buffer (16mM, PH 8.0). The reaction was started by adding 0.5 ml PMS solution (0.12mM) to the mixture, incubated at 25oC for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by using the following equation

$$\text{Superoxide radical scavenging activity} = \{(A0 - A1)/A0) \times 100\}$$

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference. All the test were performed in triplicates and

the results were averaged

2.7. Antioxidant Activity by Radical Cation ($ABTs^{\cdot+}$)

ABTs assay was based on the slightly modified method of Re et al [14]. ABTS radical cation ($ABTS_+$) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + Solution was diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of 100 μL of sample or trolox standard to 3.9 mL of diluted $ABTS_+$ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

ABTS radical cation activity = {(A0 −A1)/A0)×100}

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

2.8. Reducing Power

The reducing power of the extract was determined by the method of Singh et al [15] with minor modification to Oyaizu [16]. 1.0ml of solution containing 125,250,500 &1000 μg/ml of extract was mixed with sodium phosphate buffer (5.0 ml, 0.2 M, pH6.6) and potassium ferricyanide (5.0ml, 1.0%): The mixture was incubated at 500C for 20 minutes. Then 5ml of 10% trichloroacetic acid was added and centrifuged at 980g (10 minutes at 5℃) in a refrigerator centrifuge. The upper layer of the solution (5.0 ml) was diluted with 5.0ml of distilled water and ferric chloride and absorbance read at 700nm. The experiment was performed thrice and results were averaged.

2.9. Statistical analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

3. Result

3.1. Total phenolic content and flavonoid content

The total phenolic content and flavonoid content of the extract were found to be 0.32g100g-1 and 0.26g100g-1 respectively.

3.2. DPPH radical scavenging activity

The effect of *Xanthosoma sagittifolium* corm extract and standard ascorbic acid on DPPH radical was compared and shown in figure 1. The scavenging effect increases with the concentration of standard and samples. At 1000 μg/ml concentration *Xanthosoma sagittifolium* exhibited 78.22± 0.56% scavenging activity on DPPH. All the concentration of *Xanthosoma sagittifolium* showed higher activity than the standard ascorbic acid.

3.3. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined to be increase in the concentration of corm extract from 125 to 1000 μg/ml. The percentage of inhibition of the hydroxyl radical was varying from 38.27±0.05% in 125 μg/ml of extract to 69.11±0.21% in 1000 μg/ml of corm extract (Figure 2).

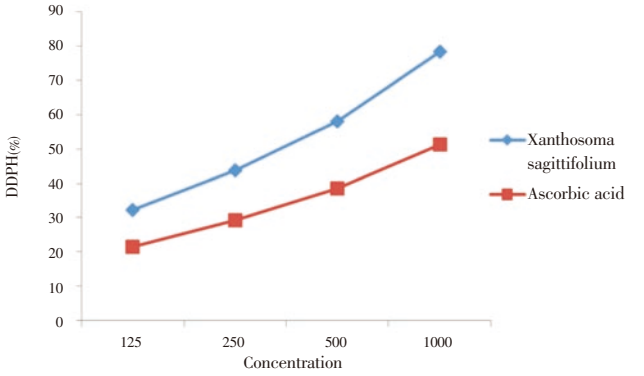

Figure 1. DPPH radical scavenging activity of methanol extract of *Xanthosoma sagittifolium*

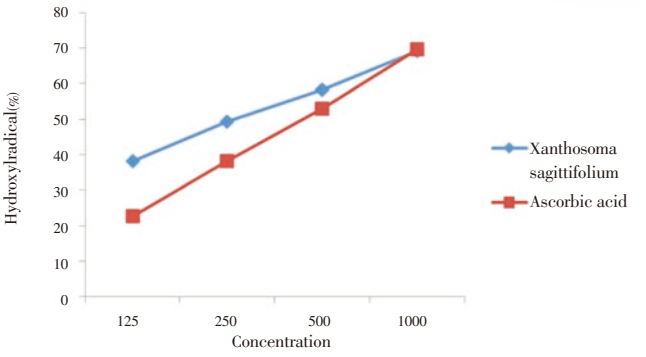

Figure 2. Hydroxyl radical scavenging activity of methanol extract of *Xanthosoma sagittifolium*

3.4. Superoxide radical scavenging activity

The *Xanthosoma sagittifolium* corm extract were subjected to be superoxide scavenging assay and the results were shown in Figure 3. It indicates that *Xanthosoma sagittifolium* (1000 μg/ml) exhibited the maximum superoxide radical

scavenging activity of 83.27±0.08%.

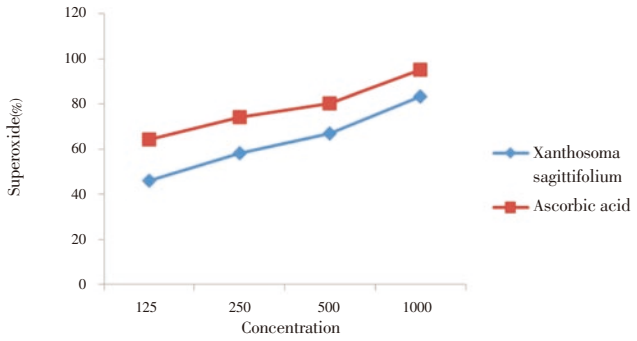

Figure 3. Superoxide radical scavenging activity of methanol extract of *Xanthosoma sagittifolium*

3.5. ABTs radical cation scavenging activity

The effect of *Xanthosoma sagittifolium* corm extract and standard trolox on ABTS radical cation was compared and shown in Figure 4.The scavenging effect increases with the concentration of standard and samples. At 1000 μg/ml concentration *Xanthosoma sagittifolium* exhibited 76.11± 0.07% scavenging activity on ABTS. All the concentration of *Xanthosoma sagittifolium* showed higher activity than the standard trolox.

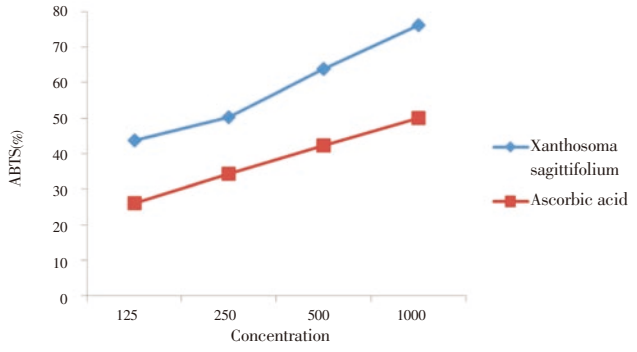

Figure 4. ABTs radical cation scavenging activity of methanol extract of *Xanthosoma sagittifolium*

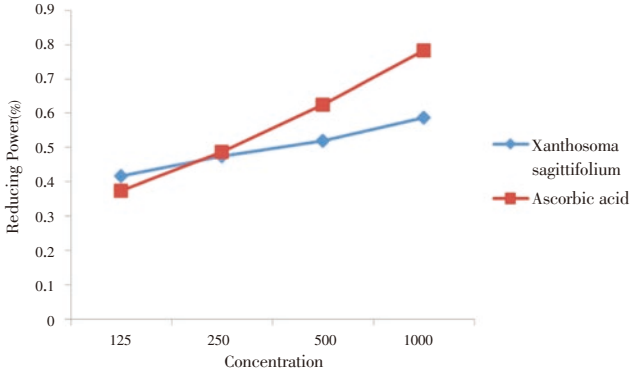

Figure 5. Reducing power ability of methanol extract of *Xanthosoma sagittifolium*

3.6. Reducing power

The reducing power *Xanthosoma sagittifolium* was compared with the standard ascorbic acid. The reducing power increases concentration. The reducing power of the samples was shown in Figure 5.

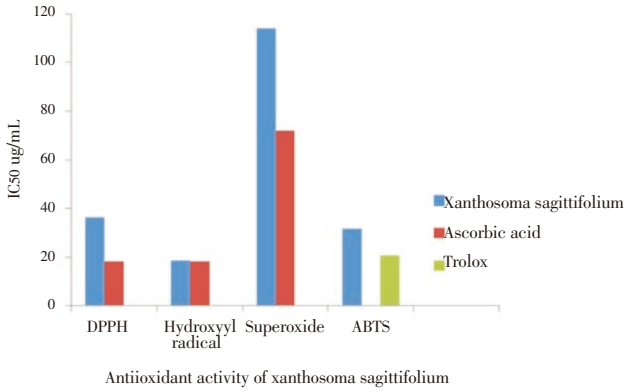

Figure 6. IC 50 values of methanol extract of *Xanthosoma sagittifolium*

3.7. IC50 value

IC50 values of *Xanthosoma sagittifolium* extract and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging activity and standard trolox for ABTs radical scavenging activity were found to the 36.22 μg/ml and 18.26 μg/ml; 18.86 μg/ml and 18.46 μg/ml; 114.16 μg/ml and 72.08 μg/ml; 31.93 μg/ml and 20.67 μg/ml respectively (Figure 6).

4. Discussion

Free radicals and other reactive species are thought to play an important role in many human diseases. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. Over production of oxidants in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA and proteins. Many synthetic drugs protect against oxidative damage but they have adverse side effects [17]. Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Natural antioxidants substances are presumed to be safe since they occur in plant foods and are seen as more desirable than their synthetic counter parts. Data from both scientific reports and laboratory studies show that plants contain a large variety of substance called "Plant Chemicals" or "Phytochemicals" that posses antioxidants activity [18,19].

Phenolic compounds have attracted much interest recently because *in vitro* and in vivo studies suggest that they have a variety of beneficial biological properties like anti- inflammatory, antitumor and antimicrobial activities [20,21]. Studies have attributed that antioxidant properties are due to the presence of phenols and flavonoids[22].

Therefore, it is necessary to determine the total amount of a phenols and flavonoids in the plant extract chosen for the study. Flavonoids are the most diverse and widespread group of natural compounds are likely to be the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging activity. Phenolic compounds are considered to be the most important antioxidants of plant materials. They constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. Antioxidant activity of phenolic compounds is based on their ability to donate hydrogen atom to free radicals [23].

DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. From the present results it may be postulated that *Xanthosoma sagittifolium* reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles. In the present study, methanol extracts exhibited comparable DDPH radical scavenging activity.

Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and cause damage to cell [24]. The present study shows the ability of the corm extracts to inhibit hydroxyl radical mediated deoxyribose degradation in a concentration dependent manner. The extract had significant scavenging effects on the hydroxyl radical, which increased with the increase in the concentrations from 125-1000 μg/ml. Superoxide anion is an oxygen-centered radical with selective reactivity. This species is produced by a number of enzyme systems in auto-oxidation reactions and by non enzymatic electron transfers that univalently reduce molecular oxygen. It can also reduce certain iron complexes such as cytochrome [25]. The present study showed potent superoxide radical scavenging activity for *Xanthosoma sagittifolium* corm extracts. Methanol extracts showed potent superoxide radical scavenging activity with IC50 value 114.16 μg/ml compared to ascorbic acid 72.08 μg/ml.

The reaction of the 2,2′-azinobis (3-ethylbenzothiazoline sulphonate) radical cation (ABTS) has been widely used to measure the antioxidant capacity of natural extracts [26,27]. The reductions of ABTS with free radical scavengers present in the tested sample occur rapidly and can be assessed by following the decrease in the sample absorbance at 734nm. The reaction time of the improved ABTS assay is only 6 min, much shorter than that of DPPH assay. Thus, ABTS assay can be considered for systematically assessing total antioxidant capacity of the crude extract from plant materials on a large scale. In the present study, the methanol extract of corm of *Xanthosoma sagittifolium* were fast and effective scavengers of the ABTS radical and this activity was comparable to that of trolox. At 1000 μg/ml, the extract exhibited higher activity than trolox. Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 734nm which decreases with the scavenging of the proton radicals [28].

Several reports indicated that the reducing power of bioactive compounds was associated with antioxidant activity [29,30]. Therefore, it is necessary to determine the reducing power of phenolic constituents contained in the plant extracts to elucidate the relationship between their antioxidant effect and their reducing power. In the present study, increase in absorbance of the reaction mixture indicates the reductive capabilities of *Xanthosoma sagittifolium* extract in concentration dependent manner when compared to the standard ascorbic acid.

The present study reveals that the corm of *Xanthosoma sagittifolium* exhibits satisfactory scavenging effect in all the radical scavenging assays. This is the first report on the antioxidant property of this plant. It is reported that total phenolics and flavonoids are natural products which have been shown to possess various biological properties related to antioxidant mechanisms [31]. Thus in the present study, the antioxidant potential of *Xanthosoma sagittifolium* may be attributed to the presence of flavonoids, phenolics and other constituents present them.

Conflict of interest statement

We declare that we have no conflict of interest.

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