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Research Article

# Phytochemical Screening and Free Radical Scavenging Activity of Chloroform Extract of *Sida acuta* Burm. F.

Perumalsamy Muneeswari[1], Subburaj Deepika[1], Palanisamy Chella Perumal[2], Velliyur Kanniappan Gopalakrishnan[1,2], Kannappan Poornima[1*]

[1]*Department of Biochemistry, Karpagam University, Coimbatore, Tamil Nadu, India 641 021*
[2]*Department of Bioinformatics, Karpagam University, Coimbatore, Tamil Nadu, India 641 021*

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**ABSTRACT**

*Sida acuta* is one of the Indian medicinal plant which belongs to the family Malvaceae. The whole plant is reported to have many biological activities such as abortifacient, anthelmintic, antiemetic, demulcent, diuretic, aphrodisiac, stomachic, diaphoretic, antipyretic and wound healing properties. Therefore main aim of the present study is to evaluate the phytochemical constituents and the free radical scavenging properties of the chloroform extract of s*ida acuta*. Screening of phytochemical constituents and free radical scavenging potential were analyzed by DPPH radical scavenging assay, Nitric oxide radical scavenging assay, Hydroxyl radical scavenging assay, Reducing power assay and FRAP assay. The preliminary phytochemical screening has shown the presence of Steroids, flavonoids, tannins and Glycosides. The chloroform extract of *Sida acuta* hold restrained free radical scavenging activities. Based on the results this study can be concluded that, *sida acuta* has rich free radical scavenging activities, may be which the presence these secondary metabolites in it. In future by isolating and identifying these compounds, if may be used to treat various diseases.

**Keywords:** *Sida acuta*, Chloroform extract, Phytochemical screening and Free radical scavenging activity.

## INTRODUCTION

Medicinal plants are the backbone of traditional medicines and variety of bioactive substances present in medicinal plants are widely used against various diseases[1]. About 80% of the population in various developing countries depends on traditional medicine for human alleviation due to its fewer side effects[2]. It is the property of most of the plant-based drugs to be simple, effective and offering a broad spectrum of activity with greater emphasis on preventive action[3]. In addition to that large numbers of secondary metabolites are also produced by some of the higher plants. The demand for natural food constituents has resulted in broad research on naturally occurring antioxidants which are able to deactivate highly reactive free radicals[4]. As a base for further pharmacological studies, there is a need to screen medicinal plants for their secondary metabolites and bioactive compounds. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources[5]. *Sida acuta* Burm.f. (*Sida acuta*) (Family of Malvaceae) is an erect perennial shrub found throughout the hotter parts of India and Nepal[6]. The bark is smooth, greenish, the root is thin, long, cylindrical and very rough; leaves are lance late, nearly glabrous, peduncles equal to the petioles, the flowers are yellow, solitary or in pairs; seeds are smooth and black. In Indian traditional medicine, the root of *sida acuta* is extensively used as a stomachic, diaphoretic and antipyretic[7]. It is regarded as cooling, astringent, tonic and useful in treating nervous and urinary diseases and also disorders of the blood, bile and liver[8]. The whole plant is used to treat snake bite and it lessened the hemorrhagic effect of Bothrops atrox venom. *sida acuta* has significant antiplasmodial activity due to its alkaloid content[9]. The paste of leaves is mixed with coconut oil and applied on head regularly for killing dandruffs and also for strengthening hair. It is naturally used in the treatment of malaria, diarrhea and many other diseases[10]. Therefore the present study is aimed to analyze the presence of phytochemical and evaluate the free radical scavenging activity of chloroform extract of *sida acuta*.

## MATERIALS AND METHODS

*Plant collection*

The whole plant of *sida acuta was* collected in and around area of K. Vengadeshwarapuram, Kalugumalai, Tuticorin District, Tamil Nadu and it was authenticated by Dr. GVS Moorthy, Scientist G, Botanical Survey of India, TNAU Campus, Coimbatore, Tamil Nadu India. The voucher number is BSI/SRC/5/23/2016/Tech./348. Collected whole plant material was washed under running tap water, air dried and powdered and stored in air tight container for further studies.

*Preparation of extract*

The powder was continuously soaked with petroleum ether, chloroform, ethyl acetate, ethanol and water. The extracts were collected and concentrated at 40$^{o}$C under

**Author for Correspondence*

Table 1: Qualitative analysis of secondary metabolites.

| Phytochemical constituents | Solvents | | | | |
|---|---|---|---|---|---|
| | Petroleum ether | Chloroform | Ethyl cetate | Ethanol | Water |
| Alkaloids | - | - | - | + | - |
| Steroids | + | + | - | + | - |
| Flavonoids | + | + | - | ++ | + |
| Tannins/phenols | + | ++ | + | ++ | - |
| Aminoacids and Proteins | + | ++ | + | - | - |
| Sugars | - | - | - | - | - |
| Glycosides | + | +++ | ++ | ++ | ++ |
| Saponins | + | ++ | + | - | – |
| Terpenoids | + | - | - | - | - |

'+'- Presence of secondary metabolites '-' Absence of secondary metabolites

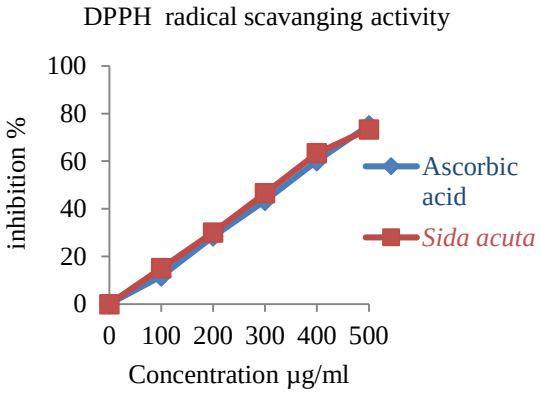


Figure 1: DPPH radical scavenging activity assay

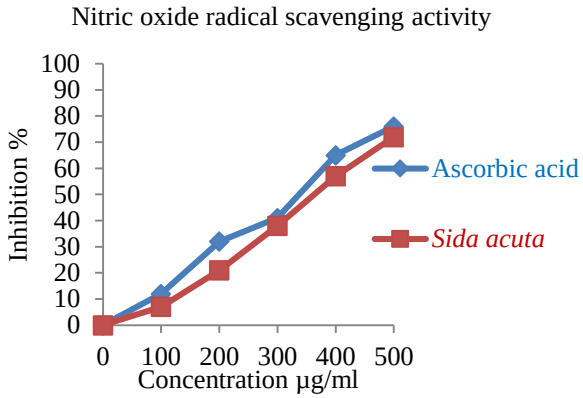


Figure 2: Nitric oxide radical scavenging activity assay

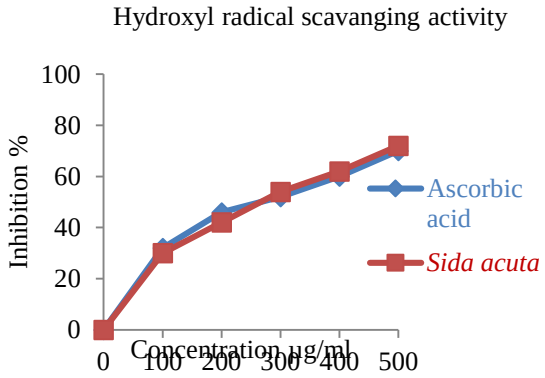


Figure 3: Hydroxyl radical scavenging activity assay

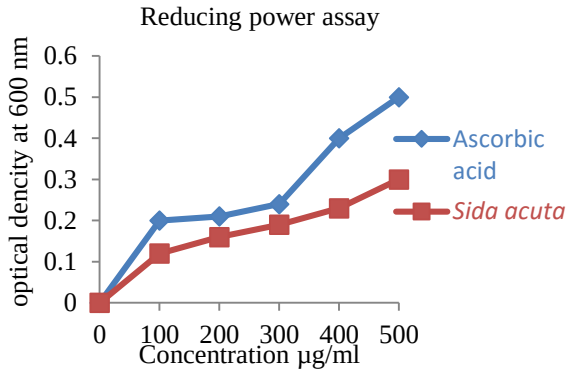


Figure 4: Reducing power assay

reduced pressure using rotary evaporator. The dried extracts were stored at 4°C until further use in the increasing order of polarity 50g of the plant powder is mixed with 250 ml of the respective solvents & kept in the shaker for 72hrs at room temperature.

*Phytochemical screening*

The preliminary phytochemical screening[11,12] was done on petroleum ether, Chloroform, ethyl acetate, ethanol and aqueous extract of *sida acuta*.

*Free radical scavenging activities*

The free radical scavenging activities of the chloroform extract of *Sida acuta* was determined by using various *in vitro* assays like DPPH radical scavenging assay, Nitric oxide radical scavenging assay, Hydroxyl radical scavenging assay, Reducing power assay and FRAP reducing scavenging assay.

*DPPH radical scavenging assay*

The DPPH radical scavenging activity of the chloroform extract of *Sida acuta* was analyzed by the standard method[13]. Briefly, the reaction mixture contained 100 µM DPPH in methanol, various concentrations (100-500 µg/ml) of the extracts and incubated for 30 minutes at room temperature. The decrease in absorbance was measured at 517 nm. The scavenging activity was calculated as a percentage of the radical reduction. All tests were performed in triplicates. Ascorbic acid was used as a reference compound.

*Nitric oxide radical scavenging assay*

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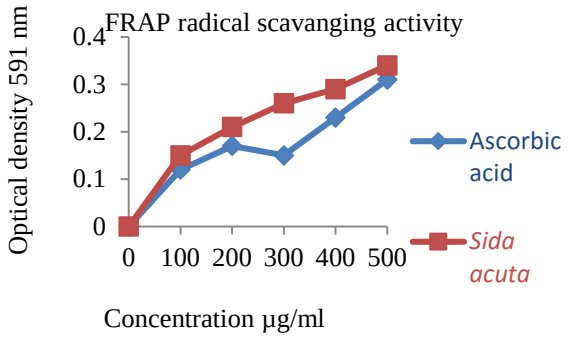


Figure 5: FRAP radical scavenging assay

The Nitric oxide was generated by sodium nitroprusside and measured[14]. The reaction mixture contained 10 mM sodium nitroprusside (SNP), phosphate buffered saline (pH 7.4) and various doses (100–500 µg/ml) of the test solution in a final volume of 3 ml. After incubation for 150 minutes at 25°C, 1 ml sulfanilamide (0.33% in 20% glacial acetic acid) was added to 0.5 ml of the incubated solution and allowed to stand for 5 minutes. Then 1 ml of napthylethylenediamine dihydrochloride (NED) (0.1% w/v) was added and the mixture was incubated for 30 minutes at 25°C. The pink chromospheres generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with NED was measured spectrophotometrically at 540 nm against a blank sample. All tests were performed in triplicates. Ascorbic acid was used as a standard drug.

*Hydroxyl radical scavenging assay*

The hydroxyl radical scavenging activity of the chloroform extract of *sida acuta* was measured[15]. All the solutions were freshly prepared. To 1ml of the reaction mixture contained, 2-deoxy-2-ribose (2.8 mM); $KH_2PO_4$-KOH buffer (20 mM, pH 7.4); $FeCl_3$ (100 µM); EDTA (100 µM); $H_2O_2$ (1.0 mM); ascorbic acid (100 µM) and various concentrations (100–500 µg/ml) of the test sample. After incubation for 1 hour at 37°C, 0.5 ml of the reaction mixture was added to 1 ml of 2.8% TCA, then 1 ml 1% aqueous TBA was added and the mixture was incubated at 90°C for 15 minutes to develop the color. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All tests were performed three times. Ascorbic acid was used as a positive control. Percentage of inhibition was evaluated.

*Reducing power assay*

The reducing power capacity of the plant was assessed[16]. Various concentrations (100-500 ug/ml) of the extract were mixed with 0.5 ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml potassium hexacyanoferrate (0.1%) and incubated at 50°C for 20 minutes. After incubation, 0.5 ml of TCA (10%) was added to the complete reaction. The upper portion of the solution (1 ml) was mixed with 1 ml distilled water, and 0.1 ml $FeCl_3$ solution (0.01%) was added. The reaction mixture was left for 10 minutes at room temperature and the absorbance was measured at 700 nm against a suitable blank solution. All tests were performed in triplicates. A higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as a positive control.

*FRAP antioxidant assay*

Ferric reducing power of chloroform extract of *sida acuta* was determined using FRAP assay[17]. Briefly, the stock solution of 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 20 mM $FeCl_3$, $6H_2O$ and 0.3 M acetate buffer (pH 3.6) were prepared. The FRAP reagent contained 2.5 ml TPTZ solution, 2.5 ml ferric chloride solution and 25 ml acetate buffer. It was prepared freshly and warmed to 37°C. 900 µl FRAP reagent was mixed with 90 µl water and 30 µl test sample/ethanol/distilled water/standard antioxidant solution. The reaction mixture was then incubated at 37°C for 30 mins and the absorbance was recorded at 595 nm. An intense blue color complex was formed when ferric tripyridyl triazine ($Fe^{3+}$-TPTZ) complex were reduced to ferrous ($Fe^{2+}$) form. The absorption at 540 nm was recorded. The calibration curve was plotted with absorbance at 595 nm vs concentration of ferrous sulphate in the range 0.1 mM ethanol solutions. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidants.

## RESULTS AND DISCUSSION

Medicinal herbs are moving from fringe to mainstream uses with a great number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. An imposing group of natural pharmacy- A gift provided by the rich floral Biodiversity of India to the herbal health practitioners[18]. Plants have shaped the basis of sophisticated traditional medicine practices that have been used for thousands of years by people in the world. Within the last few decades, many plants have been screened for their biological and pharmacological properties by researchers. These efforts are continually being taken to examine the merits of traditional medicine in the light of modern science with a view aimed at adopting effectively beneficial medical practices and discouraging harmful ones[19].

*Phytochemical screening*

The presence of secondary metabolites in the *sida acuta* were qualitatively analyzed in various solvent extracts. The results of the qualitative analysis revealed the presence of more number of phytochemical in the chloroform extract of *sida acuta* compared them with other solvents. Based on these results chloroform extract of *sida acuta* was selected for the analysis of free radical scavenging activity.

*Free radical scavenging activities*

Free radicals are the foremost cause of various chronic and degenerative diseases, in the living systems. The huge amount of synthetic molecules has been offered for free radical scavenging activity. But undesirable side effects are coupled with these compounds. An alternative solution for this problem is to consume the naturally available antioxidants from the medicinal plants because of lower side effects and comparatively safe[20].

*DPPH radical scavenging assay*

DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity

of plant extract[21]. The DPPH approach revealed scavenging capacity of chloroform extract *of sida acuta* and the $IC_{50}$ value was 325 µg/ml which was compared with standard Ascorbic acid (350 µg/ml) (Figure 1). The percentage inhibition of plant extract and standard drug are more are less similar at 500µg/ml (73% and 76% respectively, Figure 1). In this assay, these results confirmed that, the chloroform extract has high inhibition at low concentration compared with standard reference.

*Nitric oxide radical scavenging assay*

This assay revealed the abilities to inhibit the free radicals of chloroform extract of *sida acuta* and standard Ascorbic acid as illustrated in figure 2 and their $IC_{50}$ values were 300 µg/ml and 325 µg/ml respectively. The $IC_{50}$ value of plant extract is less than the standard. The percentage of inhibition at 500µg/ml of chloroform extract (high) and Ascorbic acid (less) were 76% and 72% respectively.

*Hydroxyl radical scavenging activity*

In the $Fe^{3+}$-EDTA-ascorbic acid and $H_2O_2$ reaction mixture, the plant extract and standard reference shows their inhibition power of hydroxyl radical-mediated deoxyribose as illustrated in Figure 3. The $IC_{50}$ values of chloroform extract of *Sida acuta* and standard Ascorbic acid were 220µg/ml and 250µg/ml respectively. The $IC_{50}$ value of extract is less than the standard. The percentage of inhibition at 500µg/ml of chloroform extract and Ascorbic acid were 70% and 72% respectively.

*Reducing power assay*

The reducing power capability was measured by transformation of $Fe^{3+}$ to $Fe^{2+}$ in the presence of chloroform extract of *sida acuta* and the reference Ascorbic acid. These results were illustrated in Figure 4. At 500µg/ml, the absorbance of plant extract and Ascorbic acid were 0.47 and 0.50 respectively. These results indicate that the plant extract has higher activity than Ascorbic acid.

*FRAP reducing power assay*

Antioxidants can be explained as reductions, and inactivates of oxidants. Some previous studies have also reported that the reducing power may serve as a significant indicator of potential antioxidant activity. Antioxidative activity has been proposed to be related to reducing power[22]. FRAP assay showed that, at 500 µg/ml the absorbance (Figure 5) of plant extract and Ascorbic acid were 0.34 and 0.31 respectively. These results indicate that the plant extract has higher activity than Ascorbic acid. The chloroform extract had significantly moderate FRAP activity when compared with standard drug. FRAP assay was used by several authors for the assessment of antioxidant activity of various food product samples and most of the secondary metabolites are redox-active compounds that will be picked up by the FRAP assay. Due to prooxidative enzyme systems, lipid oxidation, irradiation, inflammation, smoking, air pollutants and glycoxidation, reactive oxygen species or free radicals (superoxide, hydroxyl, hydrogen peroxide) are highly produced and exceeds the normal enzymatic (SOD, catalase, GPx) and non-enzymatic antioxidants (ascorbic acid, ∝ - tocopherol GSH, carotenoids and flavonoids) in the body[23]. Hyper physiological burden of free radicals causes the imbalance between free radicals and antioxidants and results in the oxidation of biomolecules (protein, amino acids, lipids and DNA) (i.e., oxidative stress) and lead to cell injury and death[24]. This has been implicated in a number of degenerative diseases like atherosclerosis, diabetes mellitus, ischemia / reperfusion (I/R) injury, Alzheimer's disease, inflammatory diseases, carcinogenesis, neurodegenerative diseases, hypertension, pulmonary diseases and hematological diseases[25]. Hence it is essential to supply exogenous supply of antioxidants. Antioxidants are compounds that protect cell against the damaging effects of reactive oxygen species which can neutralize free radicals before they can do harm and may help undo some damage already caused to specific cells. The antioxidants are important to human physical well-being because oxygen is a potentially toxic element since it can be transformed by metabolic activity into more reactive forms such as superoxide, hydrogen peroxide, singlet oxygen and hydroxyl radicals[26].

## CONCLUSION

In the present study the photochemical components of Alkaloid, steroids, flavonoids, tannins and Glycosides were present in the chloroform extract of *Sida acuta.* The *in vitro* free radical scavenging assays like DPPH radical scavenging assay, Nitric oxide radical scavenging, Hydroxyl radical Reducing power assay, FRAP reducing activity posses moderate antioxidants activities when compared with standard drug of Ascorbic acid. Therefore, based on the results it can be concluded that the chloroform extract of *Sida acuta* may hold enormous resource of pharmaceutical properties.

*Conflict of interest statement*

The authors declare that there is no conflict of interests regarding the publication of this paper.

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