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In-vitro Antioxidant and Thrombolytic activity of Methanol extract of Sida acuta.

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The aim of this study was to investigate the antioxidant and Thrombolytic effects of the leaves of *Sida acuta* .The plant was extracted with methanol to yield the crude extract for investigating free radicals scavenging potentiality was subjected to this study with 1, 1-Diphenyl-1-picrylhydrazyl (DPPH) and reducing power capacity. The methanol extract of the plant exhibited the potential free radical scavenging activity (antioxidant activity) having IC_{50} value of 86.34µg/ml. The reducing power of the extract was linearly proportional to the concentration of the sample. Ascorbic acid was taken as reference standard. Measurements of platelet reactivity and assessment of the efficacy of antiplatelet drugs are widely recognized as pre-requisite for the diagnosis and treatment of stroke patients. A recently established shear-induced platelet reactivity test using non-anticoagulated blood (the Global Thrombosis Test) has facilitated measurements of physiologically relevant platelet function and thrombolytic activity. The thrombolytic effect of ***Sida acuta*** 24.786 % at 100µl of aqueous extract.

Keyword: *Sida acuta*, Antioxidant, DPPH, Reducing Power, Thrombolytic Effect.

1. Introduction

1.1.1 Medicinal Plant:

The definition of Medicinal Plant has been formulated by WHO (World Health Organization) as follows- "A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs." The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal Plants".

1.1.2 Free radicals

Free radicals play an important role in a number of biological processes, some of which are necessary

for life, such as the intracellular killing of bacteria by phagocytic cells such as granulocytes and macrophages. Free radicals have also been implicated in certain cell signaling processes. This is dubbed redox signaling. The two most important oxygen-centered free radicals are superoxide and hydroxyl radical. They are derived from molecular oxygen under reducing conditions. However, because of their reactivity, these same free radicals can participate in unwanted side reactions resulting in cell damage. Excessive amounts of these free radicals can lead to cell injury and death, which results in many diseases such as cancer, stroke, myocardial infarction, diabetes and major disorders. Many forms of cancer are thought to be the result of reactions between free radicals and DNA, resulting in mutations that can adversely affect the cell cycle and potentially lead to malignancy. Some of the symptoms of aging such as atherosclerosis are also attributed to free-radical induced oxidation of many of the chemicals making up the body. In addition free radicals contribute to alcohol-induced liver damage, perhaps more than alcohol itself. Radicals in cigarette smoke have been implicated in inactivation of alpha 1-antitrypsin in the lung. This process promotes the development of emphysema. Free radicals may also be involved in Parkinson's disease, senile and drug-induced deafness, schizophrenia, and Alzheimer's. The classic free-radical syndrome, the iron-storage disease hemochromatosis, is typically associated with a constellation of free-radical-related symptoms including movement disorder, psychosis, skin pigmentary melanin abnormalities, deafness, arthritis, and diabetes mellitus. The free radical theory of aging proposes that free radicals underlie the aging process itself, whereas the process of mitohormesis suggests that repeated exposure to free radicals may extend life span. Because free radicals are necessary for life, the body has a number of mechanisms to minimize free radical induced damage and to repair damage that occurs, such as the enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. In addition, antioxidants play a key role in these defense mechanisms. These are often the three vitamins, vitamin A, vitamin C and vitamin E and polyphenol antioxidants. Further, there is good evidence bilirubin and uric acid can act as antioxidants to help neutralize certain free radicals. Bilirubin comes from the breakdown of red blood cells' contents, while uric acid is a breakdown product of purines. Too much bilirubin, though, can lead to jaundice, which could eventually damage the central nervous system, while too much uric acid causes gout.

1.1.3 Thrombolysis:

Thrombolysis is the breakdown (*lysis*) of blood clots by pharmacological means. It is colloquially referred to as *elot busting* for this reason. It works by stimulating fibrinolysis by plasmin through infusion of analogs of tissue plasminogen activator (tPA), the protein that normally activates plasmin.

1.1.4 Thrombolytic therapy

Thrombolytic therapy is the use of drugs to break up or dissolve blood clots, which are the main cause of both heart attacks and stroke.Thrombolytic medications are approved for the immediate treatment of stroke and heart attack. The most commonly used drug for thrombolytic therapy is tissue plasminogen activator (tPA), but other drugs can do the same thing. According to the American Heart Association, you have a better chance of surviving and recovering from a heart attack if you receive a thrombolytic drug within 12 hours after the heart attack starts.Ideally, you should receive thrombolytic medications within the first 90 minutes after arriving at the hospital to treatment.

1.1.5 Introduction of the Plant Materials:

Common Wire weed (*Sida acuta*) is a species of flowering plant in them allow family, Malyaceae is believed to have

Originated in Central America, but today has a pan tropical distribution and is considered a weed in some areas.

2.1 Materials & Method:

2.1.1 *In vitro* Antioxidant Assays

A number of assays are used for measuring the antioxidant potential. Depending on the mechanism, methods for the evaluation of antioxidant activities

of the test samples can be divided into two categories:

1. Methods determining the ability of test samples to donate an electron to any electron acceptor.
2. Methods determining the ability of a sample to inhibit the enzymes, which produce reactive oxygen species.

2.2.1.1 DPPH Radical Scavenging Assay:

Free radical scavenging abilities of the test samples can be determined by measuring the change in absorbance of DPPH (1, 1-Diphenyl-2-picrylhydrazyl radical) at 517 nm by the spectrophotometric method described by Brand-Williams et al (1995).

Principle: DPPH$^{\cdot}$(1,1-Diphenyl-2-picrylhydrazyl radical) is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. The radical scavenging potential of the sample was determined by measuring the decrease in absorbance due to DPPH$^{\cdot}$ at 517 nm, representing the formation of its reduced form, 1, 1-Diphenyl-1-2- picrylhydrazine (DPPH), which was yellow in color. Because of the odd electron, the purple colored methanolic solution shows a strong absorption band at 517 nm. The mechanism of reaction was presented in Fig.-01

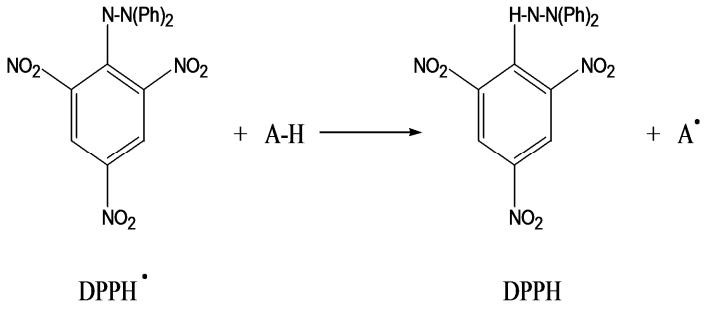

Fig 1: Mechanism of DPPH with an antioxidant having transferable hydrogen radical.

Reagents:

DPPH, Methanol, Ascorbic acid as standard

Procedure:

- 0.1ml of extract, at various concentrations (10, 50, 100 and 500 µg/ml) was added to 3ml of a 0.004% methanol solution of DPPH$^{\cdot}$.
- After 30min, absorbance of the resulting solution was measured against a blank at 517nm.
- The percentage DPPH radical scavenging activities (%SCV) were calculated by comparing the results of the test with the control (not treated with extract) using following formula:

$$\% \text{ SCV} = \frac{A_O - A_1}{A_O} \times 100$$

Where, SCV = Radical scavenging activity, A_0 = Absorbance of the control and A_1 = Absorbance of the test (extracts/ standard).

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted % SCV versus concentration curve. Test carried out in duplicate and ascorbic acid was used as standard.

2.2.1.2 Reducing power capacity assessment:

The reducing power of different extractives of Sida auta was evaluated by the method of Yen and Chen (1995).

Principle: In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe^{3+}-ferricyanide complex to the ferrous form by donating an electron. The amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

$$Fe^{3+}\text{-ferricyanide} + e \rightarrow Fe^{2+}\text{-ferricyanide}$$

Materials:

- Potassium ferricyanide [$K_3Fe(CN)_6$]
- Trichloro Acetic acid
- Ferric Chloride ($FeCl_3$)
- Phosphate buffer [$K_2HPO_4 + KH_2PO_4$]
- Ascorbic acid (Analytical or Reagent grade)
- Water bath

- Centrifuge machine
- Pipette (1-10 ml)
- UV spectrophotometer

Experimental procedure:

- ml of plant extract or standard of different concentration solution was taken in a test tube.
    - ml of potassium buffer (0.2 M) and 2.5 ml of Potassium ferricyanide [K_3Fe (CN) $_6$], (1%) solution were added into the test tube.
- The reaction mixture was incubated for 20 minutes at 50^0C to complete the reaction.
- 2.5 ml of trichloro acetic acid, (10%) solution was added into the test tube.
- The total mixture was centrifuged at 3000 rpm for 10 min.
- 2.5 ml supernatant solution was withdrawn from the mixture and mix with 2.5 ml of distilled water.
- 0.5 ml of ferric chloride ($FeCl_3$), (0.1%) solution was added to the diluted reaction mixture.
- Then the absorbance of the solution was measured at 700 nm using a spectrophotometer against blank.
- A typical blank solution contained the same solution mixture without plant extract or standard and it was incubated under the same conditions as the rest of the samples solution.
- Also the absorbance of the blank solution was measured at 700 nm against the solvent used in solution preparation. Increased absorbance of the reaction mixture indicated increase reducing power.

2.1.2 *In-vitro* Thrombolytic Activity Study:

Herbal Preparation for Individual Thrombolytic Activity Study of Extracts:

4 mg extract (***Sida acuta***) was suspended in 4 ml distilled water and make 500 µg and 1000 µg Conc. The suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight at room temperature to solubilize of water soluble part of the extract in aqueous medium and sediment the water insoluble part. After that, supernatant aqueous part was separated through a paper filter (Whatman No. 1). Then this solution is ready for in vitro thrombolytic activity study.

Specimen: With all aseptic condition 3ml of whole blood was drawn for healthy human volunteers without a history of oral contraceptive or anticoagulant therapy. 500 µl of blood was transferred to previously weighed eppendorf tube to form clots.

2.1.3 *In vitro* Thrombolytic Assay for Extracts:

0.5 ml of freshly collected blood was distributed in each of the different pre weighed and labeled sterile eppendorf tubes and incubated at 37^0C for 45 minutes. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight.

Clot weight = Weight of clot containing tube – Weight of tube alone.

To each eppendorf tube containing pre-weighed clot, 100µl of aqueous extract of Sida ***acuta*** added separately. As a negative non thrombolytic control, 100µl of distilled water were separately added to the control tubes. All the tubes were then incubated at 37^0C for 90 minutes and observed for clot lysis. After incubation, supernatant fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight take before and after clot lysis was expressed as percentage of clot lysis.The experiment was repeated several times with the blood samples of different volunteers.

% Clot lysis = (Weight of the lysis clot / Weight of clot before lysis) × 100 [Sweta Prasad and et al, 2007]

3.3 Result:

3.1.1 *In vitro* Antioxidant Assays

3.1.1.1 DPPH Radical Scavenging:

The results of DPPH radical scavenging assays on plant extracts and ascorbic acid were given in Table*s* and IC_{50} value of the samples were presented in Figure-3. IC_{50} of the standard and methanol extract *of sida acuta* are *15.99* µg/ml *and* 86.34 µg/ml respectively. The sample showed strong

radical scavenging activity with IC_{50} value *86.34* µg/ml.

Table 1: Percentage of DPPH radical scavenging activity of ascorbic acid at different concentration

Concentration (µg/ ml)	Absorbance	% SCV	IC_{50} (µg/ml)
25	*0.240*	**78.18**	
50	*0.193*	**82.45**	
100	*0.156*	**85.82**	*15.99*
200	*0.112*	**89.82**	

Table 2: Percentage of DPPH radical scavenging activity of Methanol extract of Sida acuta at different concentration

Concentration (µg/ ml)	Absorbance	% SCV	IC_{50} (µg/ml(
25	0.903	17.91	
50	0.812	26.18	
100	0.463	57.91	*86.34*
200	0.307	72.09	

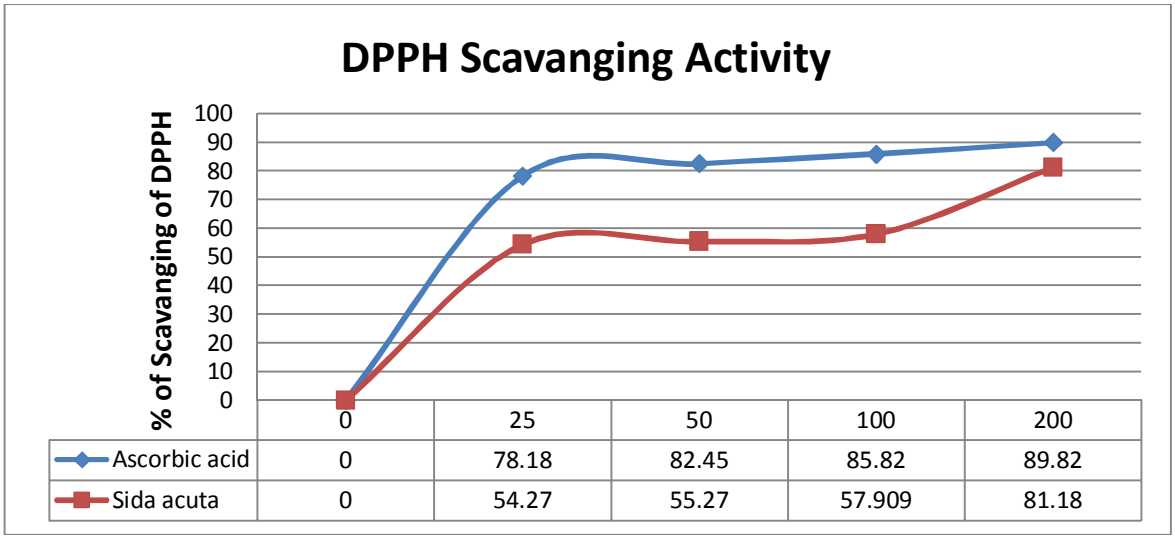

Fig 2: %-scavenging activity of ascorbic acid and methanol extract of Sida acuta at different concentration.

3.1.1.2 Reducing Power Capacity:

The reducing power of extract of these plants was found remarkable and the reducing power of the extract was observed to raise as the concentration of the extract gradually increases.

Table 3: Absorbance of ascorbic acid (Standard) at four concentrations

Concentration (µg/ml)	Absorbance
0	0
125	1
250	1.504
500	1.598
1000	1.878

Table 4: Absorbance of Methanol extract of *Sida acuta* at four concentrations

Concentration (µg/ml)	Absorbance
0	**0**
125	0.100
250	0.182
500	0.541
1000	0.986

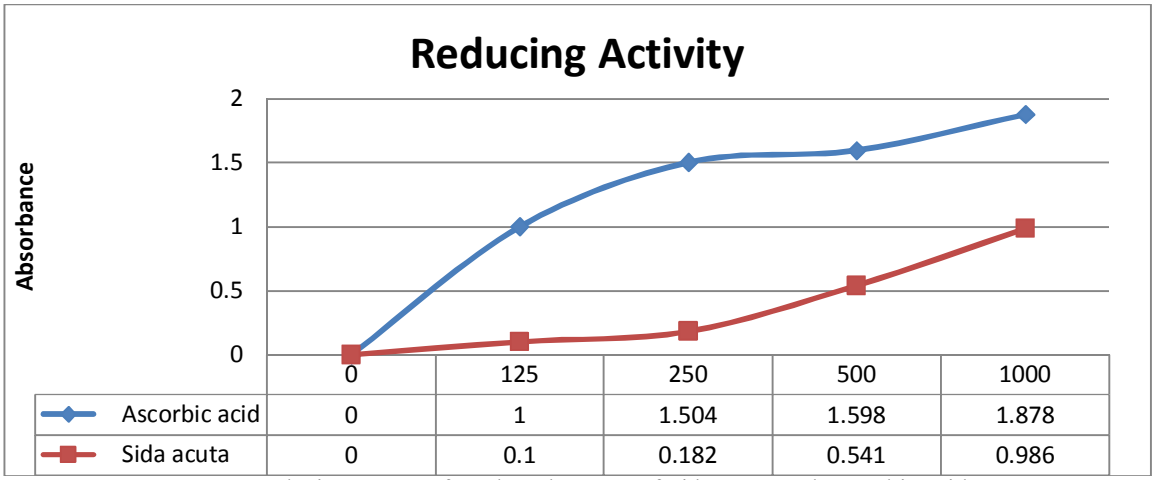

Fig 3: Reducing power of Methanol extract of Sida acuta and Ascorbic acid.

3.1.2 *Thrombolytic activity of Sida acuta*

Table 5: Thrombolytic activity of *Sida acuta*

No.	Empty Weight of tube (A)	Weight of clot with tube (B)	Weight of clot (C) W1	Average Weight of clot(C) W1	Weight of tube with Clot after(D) lysis(gm)	Weight of lysis clot(E) (D-A)	Weight of clot after lysis (X=C-E)	Average Weight of clot after lysis
A1	0.8172	1.3439	0.5267		1.2130	0.3958	0.1309	

A2	0.8385	1.3455	0.5070	0.5097	1.2416	0.4031	0.1039	0.1132
A3	0.8197	1.3152	0.4955		1.2104	0.3907	0.1048	
B1	0.8611	1.3560	0.4949		1.2466	0.3855	0.1094	
B2	0.8113	1.3205	0.5092	0.5087	1.2195	0.4082	0.1010	0.1061
B3	0.8197	1.3818	0.5221		1.2337	0.414	0.1081	
C1	0.8389	1.3273	0.4884		1.2064	0.3675	0.1209	
C2	0.8030	1.3014	0.4984	0.4896	1.1756	0.3726	0.1258	0.1252
C2	0.8109	1.2930	0.4821		1.1640	0.3531	0.129	
D1	0.8224	1.3393	0.5169		1.2061	0.3837	0.1332	
D2	0.8111	1.3314	0.5203	0.5192	1.1817	0.3706	0.1497	0.1691
D3	0.8299	1.3503	0.5204		1.1259	0.2960	0.2244	
E1	0.8068	1.2745	0.4677		1.1537	0.3469	0.1208	
E2	0.8363	1.2504	0.4141	0.4551	1.1428	0.3065	0.1076	0.114
E3	0.8113	1.2948	0.4835		1.802	0.3689	0.1146	
F1	0.8369	1.3492	0.5123		1.2367	0.3998	0.1125	
F2	0.8349	1.3304	0.4955	0.5018	1.2195	0.3846	0.1109	0.1127
F3	0.8500	1.3476	0.4976		1.2329	0.3829	0.1147	

A. % of clot lysis = (Weight of the lysis clot/ weight of clot before lysis)*100
= (0.1132/0.5097)*100
= 22.209

B. % of clot lysis = (Weight of the lysis clot/ weight of clot before lysis)*100
= (0.1061/0.5087)*100)
= 20.857

C. % of clot lysis = (Weight of the lysis clot/ weight of clot before lysis)*100
= (0.1252/0.4896)*100
= 25.572

D. % of clot lysis = (Weight of the lysis clot/ weight of clot before lysis)*100
= (0.1691/0.5192)*100
= 32.569

E. % of clot lysis = (Weight of the lysis clot/ weight of clot before lysis)*100
= (0.114/0.4551)*100
= 25.049

F. % of clot lysis = (Weight of the lysis clot/ weight of clot before lysis)*100
= (0.1127/0.5018)*100
= 22.459

Total % of clot lysis = 148.715 %

Average % of clot lysis = 24.786 %

Table 6: Thrombolytic activity of methanolic extract of Sida acuta,Control And Streptokinase (Standard)

Herb/Drug	% Clot lysis
Control	4.44
Streptokinase	85.25
Sida Acuta	24.786

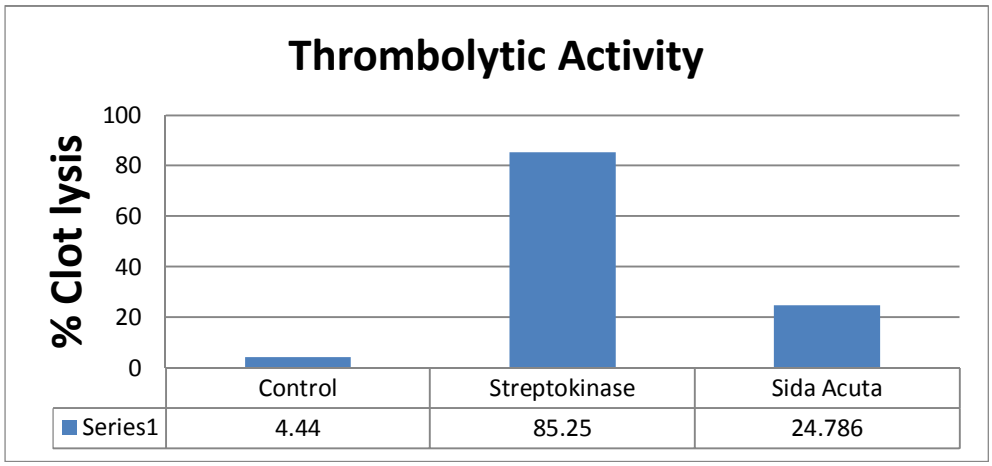

Fig 4: Thrombolytic activity of methanolic extract of Sida acuta

4.1 Discussion

The antioxidant activities of extracts have been evaluated by using a range of in vitro assays compared to standards. The extract of Sida acuta showed *Moderate* 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity *and* reducing power.The antioxidant property depends upon concentration and increased with increasing amount of the extract in all the models.The thrombolytic effect of ***Sida acuta*** 24.786 % at 100µl of aqueous extract. The average of percent clot lysis decrease with decrease in concentration. Here clot lysis varies for same concentration may be that is du e to hemoglobin level, Obesity, physiological condition of volunteer.

5.1 Acknowledgement

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6.1 Conclusion

In conclusion, the present study, using in vitro experiments established that methanol extract of sida acuta has moderate antioxidant effect as well as Thrombolytic activity.The Methanol extract of sida acuta showed mild 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and radical scavenging activity. The antioxidant property depends upon concentration and increased with increasing amount of the extract in all the models. IC50 value of the standard (ascorbic acid) and methanol extract of sida acuta are 15.99 □g/ml, 86.34 □g/ml respectively. The reducing power of the extract was linearly proportional to the concentration of the sample. Ascorbic acid was taken as reference standard. This is only a preliminary study but the plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug candidates. In conclusion the present study using in vitro experiments established that extract of Sida acuta has notable Thrombolytic activity.

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