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Antioxidant (*In-vitro*) and Thrombolytic (*In-Vitro*) activity of Petroleum ether 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 Petroleum ether 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 Petroleum ether extract of the plant exhibited the potential free radical scavenging activity (antioxidant activity) having IC_{50} value of 98.93µg/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*** 36.587 % at 100µl of aqueous extract.

Keyword: Sida acuta, Antioxidant, DPPH, Reducing power, Thrombolytic effect,Petroleum ether.

1. Introduction

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".

1.2 Free radical

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.

1.3 Reactive oxygen species

A collective term, "reactive oxygen species" (ROS), is used for oxygen-derived species including oxygen bearing free radicals, as well as certain non-radicals. Some non-oxygenated radicals are also generated in biological systems, such as carbon-centered free radicals (e.g. alkyl radical, R-H2C) and sulfur-centered radicals (e.g. thiol radical, R-S) which are produced by the attack of free radicals on hydrocarbons and the oxidation of glutathione, respectively. When the production of reactive oxygen species (ROS) exceeds the antioxidant capacity of the system, oxidative stress occurs in cellular system, including the superoxide anion radical, the hydroxyl radical, hydrogen peroxide and the peroxyl molecules, which consequently generate metabolic products that attack lipids in cel1 membrane or DNA. Improved antioxidant status helps to minimize the oxidative damage and thus can delay or decrease the risk for developing many chronic age related, free radical induced diseases. The interest in natural antioxidants, especially of plant origin, has greatly increased in recent years as

the possibility of toxicity of synthetic antioxidants has been criticized.

1.4 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.

1.5 Mechanisms of Thrombolysis:

Thrombolytic drugs dissolve blood clots by activating plasminogen, which forms a cleaved product called plasmin. Plasmin is a proteolytic enzyme that is capable of breaking cross-links between fibrin molecules, which provide the structural integrity of blood clots. Because of these actions, thrombolytic drugs are also called "plasminogen activators" and "fibrinolytic drugs".There are three major classes of fibrinolytic drugs: tissue plasminogen activator (tPA), streptokinase (SK), and urokinase (UK). While drugs in these three classes all have the ability to effectively dissolve blood clots, they differ in their detailed mechanisms in ways that alter their selectivity for fibrin clots.

1.6 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.

1.7 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.

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Malvales
Family:	Malvaceae
Subfamily:	Malvoideae
Tribe:	Malveae
Genus:	Sida
Species:	***S. acuta***

Binomial name

Sida acuta Sida carpinifolia

2.1 Materials & Method

2.1 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.

2.1.1 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˙(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˙ 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 Petroleum etheric solution shows a strong absorption band at 517 nm. The mechanism of reaction was presented in Fig.-02

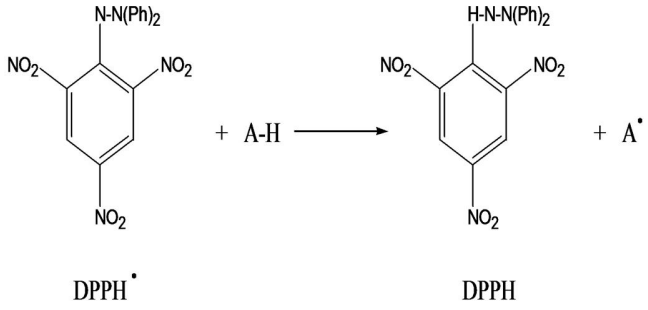

Fig.-02: Mechanism of DPPH˙ with an antioxidant having transferable hydrogen radical.

Reagents:

DPPH, Petroleum ether, 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% Petroleum ether solution of DPPH˙.
- 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.

2.1.2 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.

2.2 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.

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 In Vitro Antioxidant Assays

3.1.1DPPH Radical Scavenging:

The results of DPPH radical scavenging assays on plant extracts and ascorbic acid were given in Tables and IC_{50} value of the samples were presented in Figure-3. IC_{50} of the standard and Petroleum ether extract of sida acuta are 15.99 µg/ml and 98,93 µg/ml respectively. The sample showed strong radical scavenging activity with IC_{50} value 98,93 µg/ml.

Table 01: 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 02: Percentage of DPPH radical scavenging activity of Petroleum ether extract of Sida acuta at different concentration.

Concentration (µg/ ml)	Absorbance	% SCV	IC_{50} (µg/ml(
25	0.876	20.36	
50	0.798	27.45	
100	0.654	50.54	98.93
200	0.432	70.73	

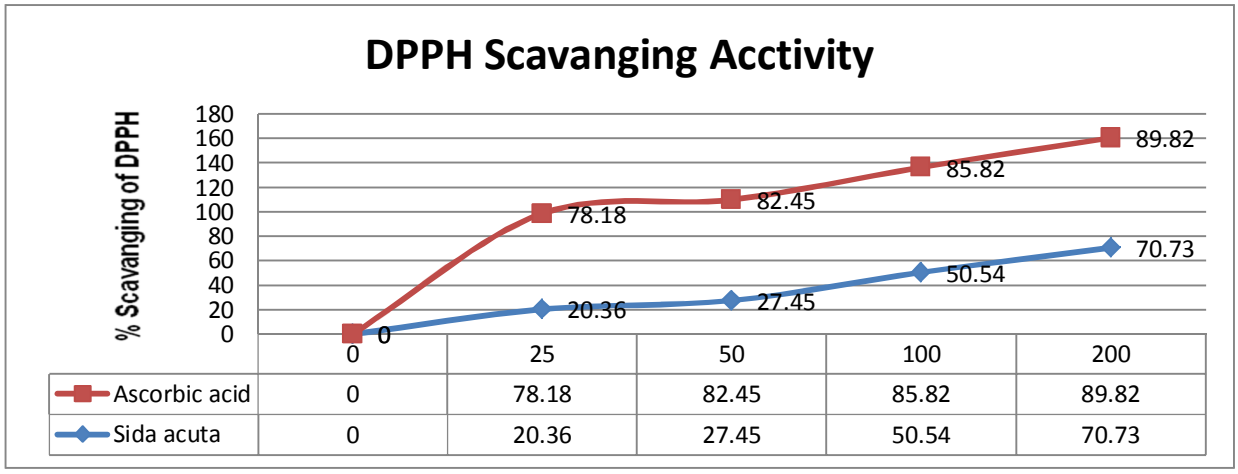

Fig.03: %-scavenging activity of ascorbic acid and Petroleum ether extract of Sida acuta at different concentration.

3.1.2 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 03: 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 04: Absorbance of Petroleum ether extract of *Soda acuta* at four concentrations

Concentration (µg/ml)	Absorbance
0	**0**
125	0.112
250	0.189
500	0.554
1000	0.879

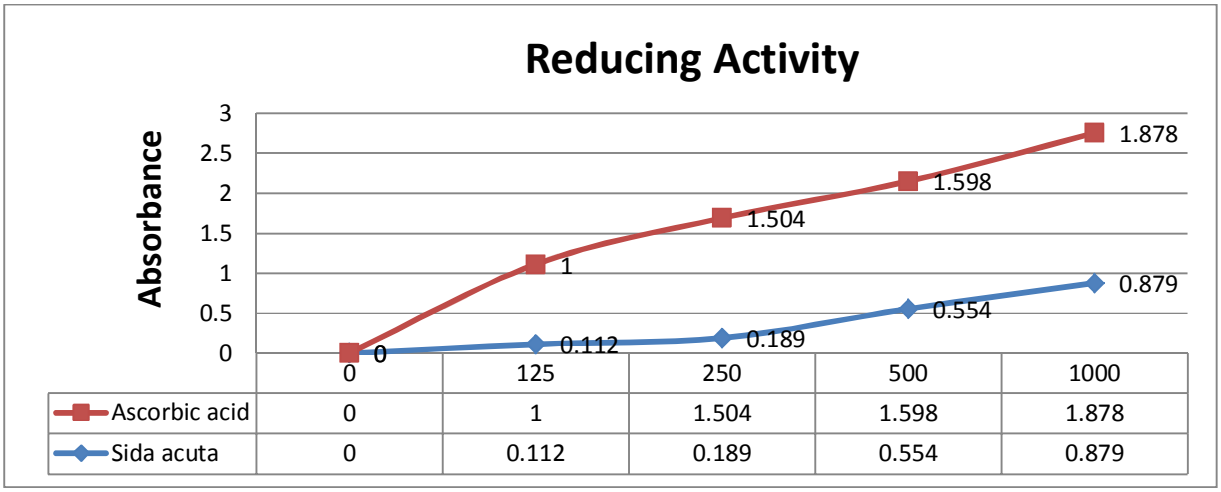

Fig. 04: Reducing power of Petroleum ether extract of Soda acuta and Ascorbic acid

3.2 Thrombolytic activity of Sida acuta

Table-05: 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	1.4538	0.6366		1.1230	0.3058	0.3308	
A2	0.8385	1.4555	0.6170	0.6167	1.2365	0.3980	0.2190	0.2519
A3	0.8197	1.4164	0.5967		1.3437	0.3907	0.2060	
B1	0.8611	1.4160	0.5549		1.2341	0.3245	0.2304	
B2	0.8113	1.4305	0.6192	0.6061	1.2234	0.3456	0.1535	0.1772
B3	0.8197	1.4639	0.6442		1.2431	0.3233	0.1478	
C1	0.8389	1.4500	0.6111		1.2134	0.3213	0.2897	
C2	0.8030	1.3894	0.5864	0.5858	1.2321	0.3726	0.2138	0.2471
C2	0.8109	1.3710	0.5601		1.3457	0.3223	0.2378	
D1	0.8224	1.3385	0.5161		1.2313	0.3865	0.1296	
D2	0.8111	1.3103	0.4992	0.5077	1.2341	0.3345	0.1647	0.1530
D3	0.8299	1.3369	0.5078		1.4321	0.3425	0.1653	
E1	0.8068	1.3635	0.4677		1.2341	0.3423	0.1254	
E2	0.8363	1.3987	0.5624	0.5396	1.1234	0.3645	0.1979	0.1637
E3	0.8113	1.3112	0.4999		1.3421	0.3321	0.1678	
F1	0.8369	1.4501	0.6132		1.2346	0.3223	0.2909	
F2	0.8349	1.4350	0.6001	0.6036	1.3457	0.3214	0.2787	0.2819
F3	0.8500	1.4476	0.5976		1.3421	0.3213	0.2763	

A. % of clot lysis = (Weight of the lysis clot/ weight of clot before lysis)*100
   i. = (0.2519/0.6167)*100
   ii. = 40.84
B. % of clot lysis = (Weight of the lysis clot/ weight of clot before lysis)*100
   i. = (0.1772/0.6061)*100)
      1. = 29.236
C. % of clot lysis = (Weight of the lysis clot/ weight of clot before lysis)*100
   i. = (0.2471/0.5858)*100
   ii. = 42.181
D. % of clot lysis = (Weight of the lysis clot/ weight of clot before lysis)*100
   i. = (0.1530/0.5077)*100
   ii. = 30.135
E. % of clot lysis = (Weight of the lysis clot/ weight of clot before lysis)*100
   i. = (0.1637/0.5396)*100
   ii. = 30.337
F. % of clot lysis = (Weight of the lysis clot/ weight of clot before lysis)*100
   = (0.2819/0.6036)*100
   = 46.793

Total % of clot lysis = 219.522 %
Average % of clot lysis = 36.587 %

Table 06: Thrombolytic activity of Petroleum etheric extract of Sida acuta,Control And Streptokinase (Standard)

Herb/Drug	% Clot lysis
Control	4.44
Streptokinase	85.25
Sida Acuta	36.587

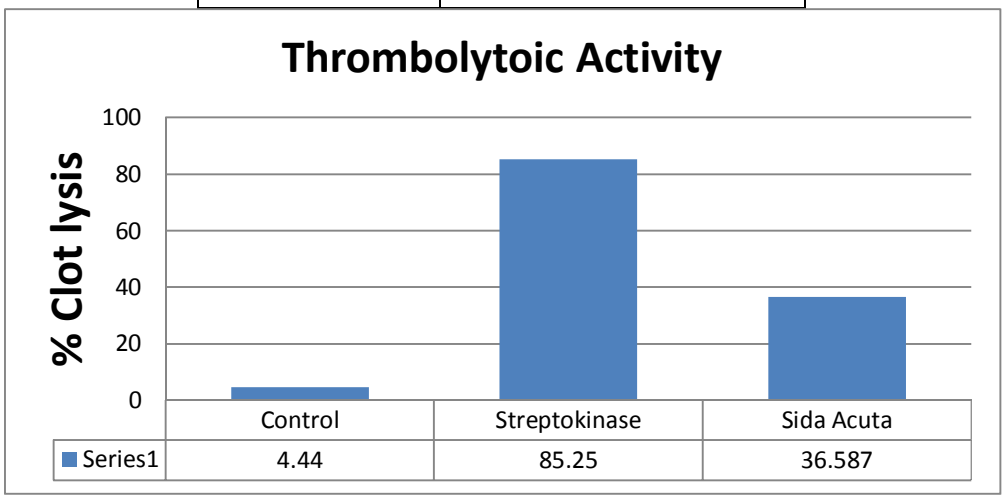

Fig.05: Thrombolytic activity of Petroleum etheric extract of Soda acuta

3. 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*** 36.587 % 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.

4. Conclusion

In conclusion, the present study, using in vitro that Petroleum ether experiments established extract of sida acuta has moderate antioxidant effect as well asThrombolytic activity.The Petroleum ether extract ofsida 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. IC_{50} valueof the standard (ascorbic acid) andextract of Petroleum ether sida acuta are 15.99 µg/ml, 98.93 µg/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 fficacy and can be a potential source of e .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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