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Ethno pharmacological investigation in *Sterculia villosa* to determine anti-diabetic, anti-inflammatory, antioxidant, thrombolytic, and cytotoxic effect

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Abstract
Crude ethanolic extract of *Sterculia villosa Roxb*. were investigated to determine different medicinal activity to assure this plant as a new source of lead compounds. Preliminary phytochemical screenings with the crude extract revealed the presence of alkaloids, glycosides, tannins, flavonoids, aldehyde and reducing sugars. Based on phytochemical analysis, we performed in vitro anti-diabetic, anti-inflammatory, anti-oxidant, anti-cancer and thrombolytic investigation in our study. In case of anti-diabetic study, plant extract showed dose depended significant ($p < 0.001$) hypoglycemic action at 0.9 mg/ml,1.1mg/ml1.3mg/ml and 1.5mg/ml dose where dinitrosalicylic acid (DNSA) method was adopted to estimate α-amylase inhibition. Stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis was taken as a measure of the anti-inflammatory activity and extract showed maximum 35.99% protection where standard drug diclofenac displayed 47.42% protection in 1000µg/ml dose. In antioxidant investigation, plant extract showed minor antioxidant property in DPPH radical scavenging activity where IC_{50} value was 211mg. In case of cytotoxicity study, In Brine Shrimp Lethality Bioassay, ethanolic extract of *Sterculia villosa* showed LC_{50} 0.639 µg/ml And LC_{90} 2.926 µg/ml. The mean percent clot lytic activity of plant extract was found 15.36%, 5.61% and 6.46% respectively for 1000µg/ml, 500µg/ml and negative control which revealed that the extract is pharmacologically active for thrombolytic action. The results of our exploration disclosed that ethanolic extract of *Sterculia villosa* possess significant antidiabetic, anti- inflammatory, cytotoxic and thrombolytic action although extract showed minor antioxidant specialty in our method. Further study is suggested to uncover mechanism of those actions as well as different method is suggested to ensure its antioxidant activity.

Keywords: *Sterculisa villosa*, anti-diabetic, anti-inflammatory, antioxidant, thrombolytic, cytotoxic, phytochemical screening

Introduction
Medicinal plants are gaining global owing to the fact that the herbal drugs are cost effective, easily available and most importantly, with negligible side effects. The international market for the medicinal plants is around US $60 billion with an annual growth rate of 7%. Indian exports in medicinal plants are valued at US $4.63 billion annually [1]. Currently, the raw material is supplied by contractors who employ landless local people to collect these plants from the wild. The method of collection is extremely exploitative and destructive, posing severe threat to the biodiversity besides, the real beneficiaries are the middlemen and the pharmaceutical companies while the local people remain poor and socio-economically backward. Therefore the need of the hour is to harness this natural resource sustainably for the socio-economic development of the local and indigenous communities while protecting the biodiversity at the same time. The twin strategies of cultivation of medicinal plants combined with sustainable collection practices from the wild would be effective in achieving this goal. Presently, the poor rural population relies on the local healer for their treatment who in turn collects the medicinal plants from the forests. Since the poor people in these communities cannot afford to pay high prices, this system needs to be preserved. Therefore, a sustainable collection strategy needs to be adopted that will conserve the valuable medicinal plants in situ while addressing to the needs of the people. The cultivation of medicinal plants is a promising option as there is availability of suitable dry lands and research and development support is available from governmental and non-governmental organizations. The requirement today is a cooperative society of medicinal plants that would ensure its members the availability of bank loans, development of value added products like cosmetics, nutraceuticals etc, and quality control and marketing strategies.

With the implementation of these strategies, the sustainable development of the local communities can be envisaged. Plants and other living organisms have great potential to treat human disease. There are two distinct types of biomedical research that seek to develop this potential. One type of research explores the value of medicinal plants as traditionally used, which constitute the only available medicines for most people in poor countries. Studies of these plants have the potential to determine which plants are most potent, optimize dosages and dose forms, and identify safety risks. Another type of research uses bioassays to identify single molecules from plants that have interesting bioactivities in isolation and might be useful lead compounds for the development of pharmaceutical drugs [2, 3].

Globally, as of 2013, an projected 382 million people have diabetes, where type 2 diabetes is more common making up about 90% [4, 5]. This is equal to 8.3% of the adults' people, [6] with almost equivalent rates in both women and men [7]. Globally, in 2012 and 2013 diabetes remains the 8th prime cause for death and nearly 1.5 to 5.1 million deaths per year due to diabetes [8, 9]. This is so alarming that the number of diabetes patient is expected to increase to 592 million by 2035 [10]. Not only human health, diabetics is also consuming a great part of economy. In 2013, worldwide cost for diabetics was $548 billion [9] and in the United States $245 billion (2012) [11]. Inflammation, a response triggered by damage to living tissues. The inflammatory response is a defense mechanism that evolved in higher organisms to protect them from infection and injury [12]. Acute inflammation is a short-term process, usually appearing within a few minutes or hours and ceasing upon the removal of the injurious stimulus [13]. Diclofenac is a nonsteroidal anti- inflammatory drug (NSAID) taken or applied to reduce inflammation and as an analgesic reducing pain in certain conditions. Reactive oxygen species (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions ($O_{2^{\cdot}}$) and reactive hydroxyl radicals ($OH^{\cdot}$); as well as non-free radical species such as hydrogen peroxide (H_2O_2) [15]. In living organisms free radicals are generated as part of the body's normal metabolic process and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neuro-degeneration. Thrombolysis is the breakdown (*lysis*) of blood clots by pharmacological means. It is colloquially referred to as *clot 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 [16]. Bioactive compounds are always toxic to the living body at some higher doses and it justifies the statement "Pharmacology is simply toxicology at higher doses and toxicology is simply pharmacology at lower doses". There are several established methods of assaying biological activity of a compound. Here in the study the Brine shrimp lethality bioassay is a rapid and comprehensive bioassay for the bioactive compound of the natural and synthetic origin. By this method, natural product extracts, fractions as well as the pure compounds can be tested for their bioactivity. In this method, *in vivo* lethality in a simple zoological organism (Brine shrimp nauplii) is used as a favorable monitor for screening and fractionation in the discovery of new bioactive natural products [17-20].

Sterculia villosa (Bengali: *Udal*) is one of the very well-known plants which can be available in Chittagong and forest of Chittagong Hill Tracts and some other part throughout Bangladesh. This plant can be 15– 18 m tall with grey bark and about 2.50–2.65 cm thick. [21] The plant had been reported for significant therapeutic activity including diuretic, cooling and aphrodisiac properties. Sherbet is recommended in urinary problems and rheumatism which is prepared by prepared from the petiole along with water and sugar. The bark and the petiole are used as a medication in seminal weakness and leaves is used in impotency [22, 23]. White exudates of the tree are applied for throat infection and whole plant extract is helpful for skin diseases [24].

Our research work was carried out to evaluate Anti diabetic activity, Anti-inflammatory activity, anti-Oxidant activity, cytotoxic activity and thrombolytic activity of *Sterculia villosa.*

Materials and Methods

Phytochemical screening

By phytochemical screening we can find out the chemical constituents of *Sterculia villosa*. *Sterculia villosa* contain flavanoids, glycosides in leaf part, alkaloids, saponins carbohydrates, and tannins only root part.

The present phytochemical study indicates the presence of some chemical constituents in the plant parts which are responsible for various pharmacological activity of the plant.

Anti-Diabetics activity test

α-amylase inhibitory assay is one of the methods to study the anti-diabetic effect of the drug in vitro. α-amylase stimulates the hydrolysis of starch, glycogen and other oligosaccharides into monosaccharide which can be freely accessible for intestinal absorption. Inhibition of this enzyme resulting in decreased absorption of glucose from starch is being considered to be the effective in management of post-prandial hyperglycemia. In our study we calculated the concentration of starch in the sample to estimate α-amylase inhibition by the sample. There were 8 different concentration were taken like 1.5 mg/ml, 1.3mg/ml, 1.1mg/ml, 0.9mg/ml, 0.7mg/ml, 0.5mg/ml, 0.3mg/ml, and 0.1mg/ml. Moreover, for more precise value we have taken two samples for each dose. Inhibition rate of plant extract were compared to standard drug acarbose as well as there were a control.

In brief, test extract was allowed to react with 20µL of α-amylase enzyme and 80µL of phosphate buff er (pH=6.9-7.2). After 10 minute incubation, 200µL of 1% starch solution was added. The same was performed for the controls where 200µL of the enzyme was replaced by buff er. After incubation for 15 minutes, than 40µL hydrochloric acid and 200 µL iodine reagent was added to both control and test. Blue to deep violet ring were observed and the absorbance was recorded at 620nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated.

Anti-inflammation test

Preparation of human red blood cells (HRBC) suspension:

Fresh whole human blood was collected and mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosaline

(0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline.

Heat induced hemolysis: The principle involved here is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36%], 0.5 ml HRBC suspension [10% v/v] with 0.5 ml of plant extracts and standard drug diclofenac sodium of various concentrations (125, 250, 500, 1000, µg/ml) and control (distilled water instead of hypo saline to produce 100% hemolysis) were incubated at 37ºC for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.

Preparation of test samples: Samples of ethanolic extract were taken and dissolved in 200 µl of distilled methanol in vials to get mother solution (Conc. 1000 µg/ml). Then 100 µl of solution was taken in test tube each containing 2ml of distilled methanol. Thus, final concentration of the prepared solution in the first test tube was 500 µg/ml. Then a series of solutions of varying concentrations were prepared from the mother solution by serial dilution method. In each case 100 µl sample was added to test tube and fresh 100µl methanol was added to vial. Thus the concentrations of the obtained solution in each test tube were 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml, 7.8125 µg/ml, 3.90625 µg/ml, 1.9531µg/ml and 0.9766 µg/ml for 10 dilutions.

Antioxidant

Preparation of Control Sample

Control groups are used in antioxidant study to validate the test method and ensure that the results obtained are only due to the activity of the test agent and the effects of the other possible factors are nullified. Usually two types of control groups are used i) Positive control ii) Negative control

Preparation of positive control sample: Positive control in an antioxidant study is a widely accepted antioxidant agent and the result of the test agent is compared with the result obtained for the positive control. In the present study *tert*-butyl-1-hydroxytoluene (BHT) is used as the positive control. Measured amount 2 mg of the BHT is dissolved in 200 µl methanol to get an initial concentration of 500 µg/ml from which serial dilutions are made using methanol to get concentrations of 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml, 7.8125 µg/ml,
3.90625 µg/ml, 1.9531µg/ml and 0.9766 µg/ml for 10 dilutions.

Preparation of negative control sample: 20 mg of DPPH was weighed and dissolved in methanol to get a DPPH solution having a concentration 20 µg/ml. The solution was prepared in the amber reagent bottle and kept in the light proof box.

Assay of free radical scavenging activity: 2.0 ml of a ethanol solution of the sample (extractives / control) at different concentration (500 µg/ml to 0·977 µg/ml) were mixed with 3.0 ml of a DPPH eethanol solution (20 µg/ml). After 30 minute reaction period at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer.

Inhibition of free radical DPPH in percent (I%) was calculated as follows: $(I\%) = (1 - A_{sample} / A_{blank}) \times 100$

Where, A_{sample} is the absorbance of the test material, A_{blank} is the absorbance of the control reaction (containing all reagents except the test material)

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

Thrombolytic

Plant sample preparation: 100mg of extract was suspended in 10ml of distilled water, kept over- night and finally the soluble supernatant was decanted and filtered.100µl of this aqueous preparation was added to each micro-centrifuge tubes.

Blood specimen preparation: 10 pieces micro centrifuge tubes were taken, sterilized and weighed (let n=1). 5ml of blood was drawn from volunteer and the blood was distributed in 10 different per weighed (W_1) micro-centrifuge tube, each tube.05ml. The blood specimen was centrifuged at 2500rpm for 5 minutes. incubated the blood for 45 minutes at 37 ^{0}C. After clot formation i.e. incubation, the serum was completely removed by decantation, capillary absorption and by removing the serum from the inner surface of the tube carefully by cotton bar or by use of cotton bound at top of a glass rod without disturbing the clot and ensure complete removal of serum or the result will be erroneous. Kept the tubes at lying position on a tray for 6 minutes after first removal of serum and then removed the liquids of the tube surface by the cotton rod. Each tube was weighed (W_2) again. Weight of clot was found as, weight of clot = weight of containing tube (W_2) – weight of tube alone (W_1). Finally weighed very carefully, because result varies for inappropriate weighing, checked the balance before weighing. To each micro-centrifuge tube containing pre-weighed clot, 100µl of each organic solvent extract of plant was added separately. As a positive control, 100µl of streptokinase was added to clot of tube no. 5 (standard). As a negative control, 100µl water is added to clot of tube no. 4 (blank).

All the tubes were incubated at 37 ^{0}C for 90 minutes and observed if clot lysis has occurred. After 90 minutes of incubation, the released fluid was completely removed by decanted clot containing liquid from the inner surface of the tube carefully by cotton bar or by use of cotton tightly bound at top of a glass rod without disrupting the clot. The tubes were then weighed again and ensured complete removal of released fluid or the result will be erroneous. Kept the tubes at lying position on a tray for 6 minutes after first removal of released clot and then removed the liquids of the tube surface by the cotton rod. Weighed the tubes (W3) very carefully, because result varies for inappropriate weighing.

The difference obtained in weight taken before and after clot lysis is expressed as percentage of clot lysis.

Percentage of clot lysis = *Weight of clot after lysis/ Weight of clot before lysis* X 10

Cytotoxic activity

Preparation of Sample: In a small beaker, measured amount of the sample was accurately weighed and dissolved in DMSO (Dimethylsulfoxide) to give a final concentration of 10mg/ml (10µg/µl).

Application of test sample to the test tube containing brine shrimp nauplii: 8 test tubes for the sample were taken where each contained 5ml of seawater and 10 nauplii. These test tubes were marked from 1 to 8 for the sample. To test tubes different concentration of the sample was added. In test tube 1 sample was taken which concentration is 400 µg/ml. Similarly 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml Then the samples were subjected to brine shrimp lethality bioassay.

Preparation of control solution: Two control groups were used in cytotoxicity study, to validate the test method and results obtained due to the activity of the test agent.

Negative control test: In this case, only 30µl DMSO was added in 5ml sea water containing 10 Nauplii. No extract was added to prepare control solution.

Positive control test: Measured amount of the Vincristine Sulphate (VINCRIRST ®, Techno Drugs Ltd., and Bangladesh) was dissolved in DMSO to get an initial concentration of 0.3125µg/µl. 9 test tubes for the standard sample were taken where each contained 5ml of seawater and 10 nauplii. These test tubes were marked from 1 to 9. In test tube no.1, 160µl of the Vincristine Sulphate solution was added to give a concentration of 10µg/ml. In a similar way, 80 µl, 40 µl, 20 µl,10 µl, 5µl, 2.5µl, 1.25µl and 0.625µl of the sample were added to test tubes 2, 3, 4, 5, 6, 7, 8 & 9 to give the concentration of 5µg/ml, 2.50 µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml, 0.78125 µg/ml & 0.039 µg/ml of the standard sample solution respectively
After 24 hours, test tubes were observed and the numbers of dead nauplii were counted and the LC_{50} value was calculated.

Result and Discussion
Phytochemical screening: Phyochemical Screening ensures the presence of Alkaloids, Glycosides, Tannins, Flavonoids and reducing sugar.
In case of anti-diabetic observation, plant extract showed dose depended significant ($p < 0.001$) hypoglycemic action at 0.9 mg/ml,1.1mg/ml1.3mg/ml and 1.5mg/ml dose where dinitrosalicylic acid (DNSA) method was adopted to estimate α-amylase inhibition. Maximum inhibition was 22.51% in 1.5 mg/ml concentration. All other concentration of sample also showed alpha amylase inhibitory activity. All value was compared with standard drug acarbose where maximum inhibition was 38.28% in 1.3mg/ml.

Table 1: Calculation of% of inhibition by ethanolic extract of *Sterculia villosa.*

Concentration (mg/ml)	Sample 1 (Absorbance)	Sample 2 (Absorbance)	Average	Control	Final Control	Final Sample	% of inhibition
0.1	0.147	0.196	0.17	0.127	0.87	0.83	5.10
0.3	0.166	0.2	0.18	0.127	0.87	0.82	6.41
0.5	0.206	0.199	0.20	0.127	0.87	0.80	8.65
0.7	0.192	0.156	0.17	0.127	0.87	0.83	5.38
0.9	0.235	0.239	0.24	0.127	0.87	0.76	12.60**
1.1	0.209	0.289	0.25	0.127	0.87	0.75	13.97***
1.3	0.272	0.294	0.28	0.127	0.87	0.72	17.87***
1.5	0.304	0.343	0.32	0.127	0.87	0.68	22.51***

Table 2: Calculation of % of inhibition by standard drug Acarbose

Concentration (mg/ml)	Acarbose (Absorbance)	Control	Final Sample	Final control	% inhibition
0.1	0.36	0.347	0.64	0.65	1.99
0.3	0.39	0.347	0.61	0.65	6.74
0.5	0.41	0.347	0.59	0.65	9.80
0.7	0.44	0.347	0.56	0.65	14.70
0.9	0.43	0.347	0.57	0.65	12.10
1.1	0.53	0.347	0.47	0.65	28.33
1.3	0.60	0.347	0.40	0.65	38.28
1.5	0.60	0.347	0.40	0.65	38.13

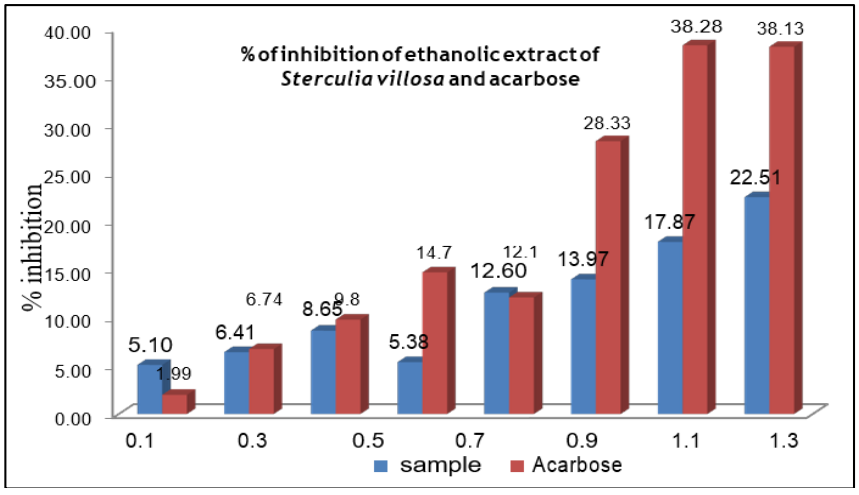

Fig 1:% of inhibition of ethanolic extract of *Sterculia villosa* and acarbose

Results ensured that ethanolic extract of Sterculia villosa possess dose dependent ant diabetic activity. Further study is suggested to discover responsible bioactive compound for alpha amylase inhibition as well as to know the mechanism of action.

In case of Anti-inflammatory activity test, the percentage of hemolysis of HRBC membrane can be calculated as follows:

% Hemolysis = (Optical density of Test sample / Optical density of Control) X 100 The percentage of HRBC membrane stabilization can be calculated as follows:

% Protection = 100 – [(Optical density of Test sample / Optical density of Control) X 100]

Table 3: Membrane Stabilization by ethanolic extract of *Sterculia villosa*.

sample	concentration (µg/ml)	Absorbance of Sample	% of hemolysis	% of protection	% of protection By Diclofenac Na
1	1000	0.354	64.01447	35.99	47.42
2	500	0.465	84.0868	15.91	45.83
3	250	0.477	86.25678	13.74	45.05
4	125	0.478	86.43761	13.56	44.44
control	1000	0.553			

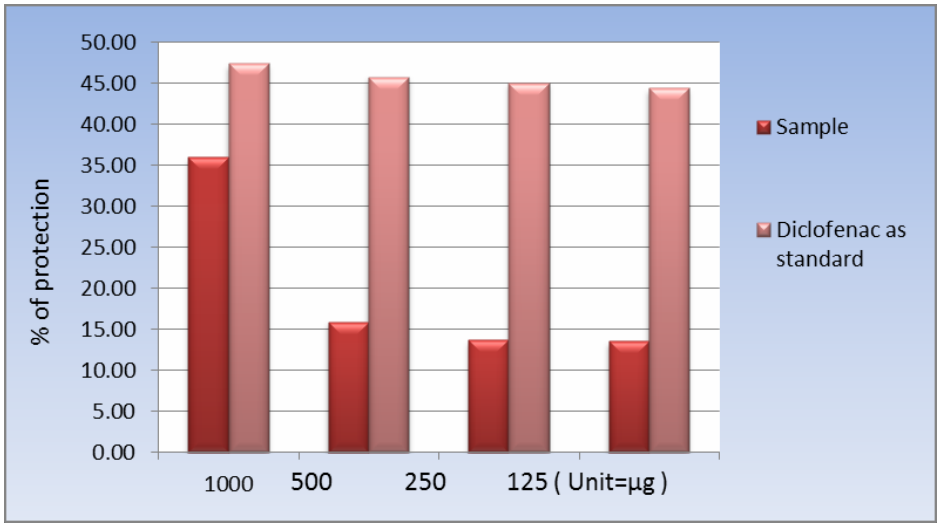

Fig 2: Comparison of membrane stabilization activity of sample and standard

Stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis was taken as a measure of the anti-inflammatory activity and extract showed maximum 35.99% protection where standard drug diclofenac displayed 47.42% protection in 1000µg/ml dose. Moreover other dose like 500 µg/ml, 250 µg/ml, 125 µg/ml also showed membrane stabilization activity and protection is 15.91%, 13.74% and 13.56% respectively. Thereto, all results were compare with standard drug diclofenac Na where protection rate was 47.42%,45.83%, 45.05% and 44.44% for 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml doses respectively.

In antioxidant investigation, extract showed minor antioxidant property in DPPH radical scavenging activity where IC_{50} value was 211mg. The result was compared with standard drug ascorbic acid where LC_{50} 49.56 µg/ml.

Table 4: DPPH radical scavenging activity in plant extract

SL	Concentration (µg/ml)	Absorbance	Absorbance of control	% of inhibition	IC_{50}	IC_{50} Of Ascorbic acid (µg/ml)
1	1000	0.191	0.321	40.50		
2	500	0.255	0.321	20.56		
3	250	0.287	0.321	10.59		
4	125	0.304	0.321	5.30		
5	62.50	0.315	0.321	1.87	211mg/ml	49.56 µg/ml
6	31.25	0.321	0.321	0.00		
7	15.63	0.313	0.321	2.49		
8	7.81	0.321	0.321	0.00		
9	3.91	0.320	0.321	0.31		
10	1.95	0.319	0.321	0062		

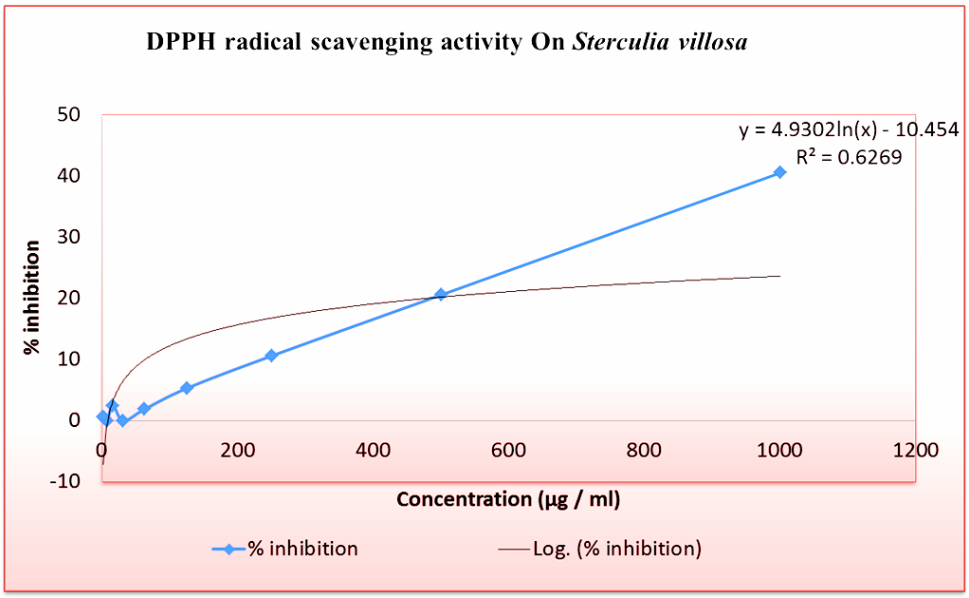

Fig 3: DPPH radical scavenging activity in plant extract

In our study, we have taken two different doses (10mg/ml; 5mg/ml) of ethanolic extract of *Serculia villosa* to investigate thrombolytic activity of our plant extract.For, precise results we have taken 4 alpine tube for each doses. Moreover, there were four alpine tubes as control and four other for standard. All data is given below,

Table 5: Results of thrombolytic activity of *Sterculia villosa*

No of alpine tube	Dose	Weight of blank alpine tube (W1 mg)	Weight of alpine tube with clot (W2 mg)	Weight of alpine tube after lysis (W3 mg)	Weight of clot (W2-W1) mg	Weight of lysis (W2-W3)mg	Avarge mg	% of lysis
Control 1	Normal saline	0.799	1.144	1.122	0.345	0.022	0.0303 ±.0085	6.46
Control 1	Normal saline	0.805	1.071	1.015	0.266	0.056		
Control 1	Normal saline	0.82	1.131	1.11	0.315	0.021		
Control 1	Normal saline	0.719	1.034	1.012	0.315	0.022		
Sample 1	10 mg/ml	0.806	1.093	1.042	0.287	0.051	0.0498 ±.0031	15.36
Sample 2	10 mg/ml	0.806	1.175	1.131	0.369	0.044		
Sample 3	10 mg/ml	0.816	1.1	1.054	0.284	0.046		
Sample 4	10 mg/ml	0.815	1.188	1.13	0.373	0.058		
Sample 5	5 mg/ml	0.809	1.169	1.128	0.360	0.041	0.0183± .0078	5.61
Sample 6	5 mg/ml	0.823	1.208	1.2	0.385	0.008		
Sample 7	5 mg/ml	0.834	1.121	1.113	0.287	0.008		
Sample 8	5 mg/ml	0.814	1.088	1.0711	0.274	0.016		
Standard	--	0.79	1.128	1.016	0.338	0.112	0.2202 ± 5.17	45.05
Standard	--	0.80	1.498	1.148	0.698	0.350		
Standard		0.799	1.272	1.019	0.473	0.253		
Standard		0.827	1.197	1.031	0.37	0.166		

From the table, we can conclude that ethanolic extract of Sterculia villosa possess thrombolytiv activity. For 10mg/ml dose,% of lysis is 15.3 and the result was found significant ($p< 0.05$). Moreover drug showed minor lysis activity at 5mg/ml. All results are compared with standard drug streptokaise where 45% of clot lysis were observed as maximum lysis for standard drug. In thrombolytic activity, Extract was found 15.36%, 5.61% and 6.46% respectively for 10mg/ml, 5mg/ml and negative control.

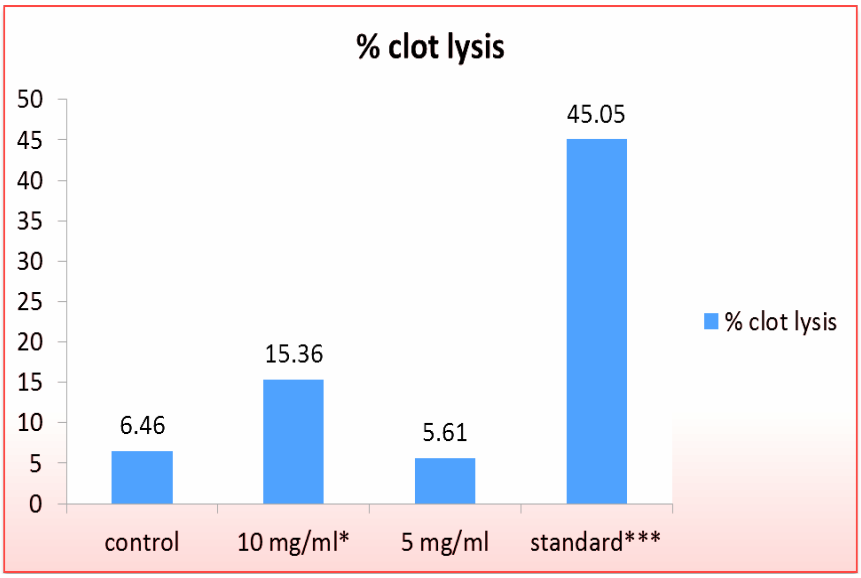

Fig 4: Comparative% clot lysis in vitro study of ethanolic extracts of Sterculia villosa with standard drug (streptokinase).

The ethanolic extracts of *Sterculia villosa* were tested for Brine shrimp lethality bioassay using brine shrimp nauplii and DMSO as a solvent. Extracts showed positive result on brine shrimp lethality bioassay with high concentration. In case of cytotoxicity study, extract showed LC_{50} 0.639 µg/ml And LC_{90} 2.926 µg/ml Control was used to see whether DMSO had any effect on brine shrimp lethality. The control group of brine shrimp nauplii with and without DMSO exhibited no mortality.

Table 6: Brine Shrimp Lethality Bioassay for the *Sterculia villosa*

SL No.	Conc. (µg/ml)	Log C	No. of Nauplii	Alive Nauplii	Dead	% of mortality	Corrected Mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
1	400	2.602	10	0	10	100	100		
2	200	2.301	10	2	8	80	77.77		
3	100	2.000	11	3	7	63.63	59.59		
4	50	1.699	11	4	6	54.54	49.49	0.639	2.926
5	25	1.398	10	2	8	80	77.77		
6	12.5	1.097	10	4	6	60	55.55		
7	6.25	0.796	11	3	7	63.63	59.60		
Control			10	9	1	10			

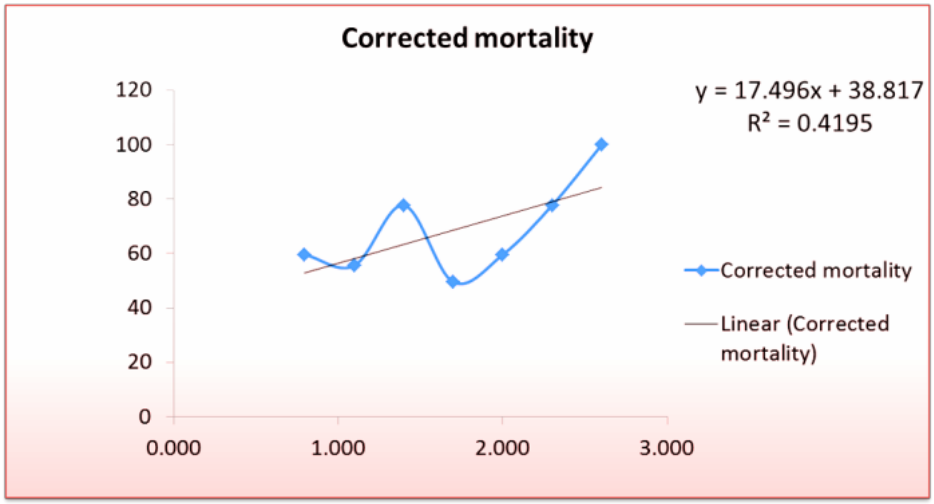

Fig 5: Determination of LC_{50} and LC_{90} of ethanolic extraxt of *Sterculia villosa.*

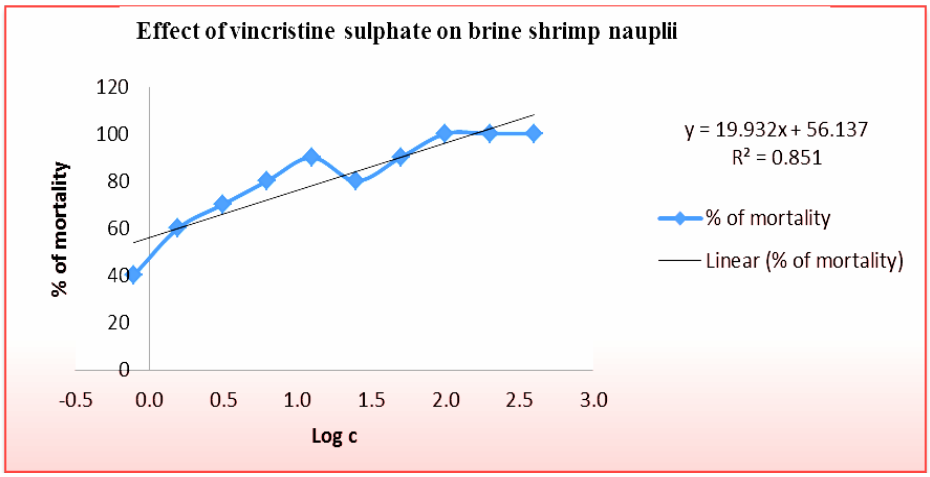

Fig 6: LC_{50} value of standard Vincritine sulphate was found 0.492 µg/ml

In Brine Shrimp Lethality Bioassay, extract showed LC_{50} 0.639 µg/ml And LC_{90} 2.926 µg/ml which revealed that the extract is pharmacologically active. The result was compared with standard drug Vincritine sulphate where LC_{50} value of standard Vincritine sulphate was found 0.492 µg/ml. The LC_{50} value of the extracts showed significant lethality against brine shrimp and it can be considered for compound isolation in order to detect future anti-tumor compounds. Moreover, this significant lethality of the ethanolic extract of Sterculia villosa to brine shrimp are indicative of the presence of potent cytotoxic and probably insecticidal compounds which warrants further investigation. This bioassay has good correlation with the human solid tumor cell lines.

Conclusion

This study conclude that ethanolic extract of *Sterculia villosa* showed dose depended significant ($p < 0.001$) hypoglycemic action at 0.9 mg/ml, 1.1mg/ml, 1.3mg/ml and 1.5mg/ml dose in dinitrosalicylic acid (DNSA) method. In anti-inflammatory activity, extract showed maximum 35.99% protection where standard drug diclofenac displayed 47.42% protection in 1000µg/ml dose.In antioxidant investigation, extract showed minor antioxidant property in DPPH radical scavenging activity where IC50 value was 211mg. In case of cytotoxicity study, extract showed LC50 0.639 µg/ml And LC90 2.926 µg/ml. In thrombolytic activity, Extract was found 15.36%, 5.61% and 6.46% respectively for 1000µg/ml, 500µg/ml and negative control which revealed that the extract is pharmacologically active for thrombolytic action.

The results of our exploration disclosed that ethanolic extract of *sterculia villosa* possess significant antidiabetic, anti-inflammatory, cytotoxic and thrombolytic action although extract showed minor antioxidant specialty in our method. Further study is suggested to uncover mechanism of those actions as well as different method is suggested to ensure its antioxidant activity. These findings show that the plant have potential therapeutic efficacy and may serve as potential source of chemically interesting and biologically important drug candidate and thus can be further screened against various diseases or using different in vitro as well as *in vivo* model in order to find out its unexplored efficacy as well as the mechanism of action of the explored bioactivities.

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