



***In vitro* Study of Antioxidant and Anticoagulant Activities of Ethanol Extract of *Pandanus tectorius* Leaves**

O. D. Omodamiro^{1*} and C. O. Ikekemma¹

¹Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author ODO designed the study, wrote the protocol, wrote the first draft of the manuscript, managed the literature searches and analyses of the study performed the spectroscopy analysis. Author COI managed the experimental process and identified the species of plant. Both authors read and approved the final manuscript.

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ABSTRACT

Pandanus tectorius of the family *Pandanaceae* is a medicinal plant that grows in Nigeria. The *Pandanus tectorius* extract was tested for; its acute toxicity using mice and, its antioxidant activities (using DPPH spectrometric assay, anti-lipid peroxidation activity and Nitric oxide scavengin assay) and anticoagulant activities using fresh human plasma. The acute toxicity test gaved LD50 for 176.78 mg/ml.DPPH (1, 1-Diphenyl-2-picrylhydrazyl) spectrophotometric assay showed that at different concentrations of the extract (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml), there is different percentage inhibition (95.01, 81.39, 59.26, 25.81) respectively compared to standard drug using vitamin C at 300 mg/ml which gives 98.14% inhibition. Anti-lipid peroxidation activity of the extract showed that at different concentration (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml), there is different percentage inhibition (99.27, 94.99, 77.86, 29.92) respectively compared to standard drug using vitamin C at 300 mg/ml which gives 99.71% inhibition. Nitric Oxide scavenging

*Corresponding author: E-mail: majekdamiro@yahoo.com;

activity of the extract showed that at different (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml), will give different percentage inhibition (98.77, 88.15, 78.07, 45.52) respectively compared to standard drug using vitamin C at 300 mg/ml which gives 99.61% inhibition. The antioxidant activity test shows that at increasing concentration, there is increasing inhibition and revealed that the crude leaf extract possesses appreciable high antioxidant potentials. The crude ethanol extract of *Pandanus tectorius* leaves induced significantly ($p < 0.05$) increase in the clotting times (26.23 ± 1.34 mins, 15.95 ± 0.53 mins, 9.37 ± 0.14 mins, 6.75 ± 0.75 mins, 5.71 ± 0.58 mins) of different concentrations (500 mg, 250 mg, 62.25 mg, 125 mg, 31.125 mg) of the extract respectively compared to the baseline clotting time of 83.5 ± 2.12 secs for human plasma. However, the invitro anticoagulant activities using fresh human plasma showed that the extract demonstrated a reduced potency compared to warfarin. *Pandanus tectorius* leaves could be a source of novel anticoagulant and antioxidant compounds for the management of various hematological and biochemical disorders.

Keywords: Antioxidant; anticoagulant; *Pandanus tectorius*; human plasma; thrombin time.

1. INTRODUCTION

About 80% world's population depends on traditional medicine for their health benefits. Traditional medicine contains a wide range of materials which is used to treat several disorder [1]. There is a critical need to explore for effective and alternative anticoagulants and antioxidants from natural products with minimal effect. Plant offer prospect as sources of various medicaments and effective chemotherapeutic agents.

Pandanus tectorius of the family *Pandanaceae*, is a wide spread diocious, palm-like tree that exist throughout rock and sandy shores Nigeria. This plant belongs to its own monocotyledonous family. Although it is called "screw pine" it is not related to the pine family. It has conspicuous prop root at the base of the trunk which support these in water -logged soils and strong winds. Pandanus leaves are used in treatments for cold/flu, hepatitis, dysuria, asthma, boils, and cancer, while the roots are used in a decoction to treat hemorrhoids and the main parts used in making traditional medicines are the fruits, male flowers, and aerial roots [2]. These are used individually or in combination with other ingredients to treat a wide range of illnesses, including digestive and respiratory disorders. The root is used in Palau to make a drink that alleviates stomach cramps, and the leaves are used to alleviate vomiting. The root is also known for its use in traditional medicine [3].

Reactive oxygen species (ROS) are produced from molecular oxygen as a result of normal cellular metabolism [4]. ROS can be divided into 2 groups: free radicals and non radicals.

Molecules containing one or more unpaired electrons and thus giving reactivity to the molecule are called free radicals. When 2 free radicals share their unpaired electrons, non radical forms are created. The 3 major ROS that are of physiological significance are superoxide anion, hydroxyl radical (OH), and hydrogen peroxide (H_2O_2) [4].

ROS can induce lipid peroxidation and disrupt the membrane lipid bilayer arrangement that may inactivate membrane-bound receptors and enzymes and increase tissue permeability [5]. Lipid peroxidation products, such as isoprostanes and thiobarbituric acid reactive substances, have been used as indirect biomarkers of oxidative stress, and increased levels were shown in the exhaled breath condensate or bronchoalveolar lavage fluid or lung of chronic obstructive pulmonary disease patients or smokers [6,7].

Antioxidative materials have recently attracted considerable attention because of their potential as reactive oxygen species scavengers [8]. Thus, many antioxidants have been identified for use as food additives or medical supplements as reactive oxygen species scavengers that will nullify the harmful effects from ROS. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate have been used as food antioxidants. However, the use of these synthetic antioxidants is negatively perceived by the users due to their potential toxicity and connotation as chemicals in food [9]. Natural antioxidants have awakened a great deal of interest because of their health effects and positive image as substances acting against degenerative diseases and certain cancers.

Therefore, owing to consumer concerns about the safety of synthetic antioxidants, the promotion of natural antioxidants has been greater than ever.

Plants are the main source of natural antioxidants, including several edible species. Among antioxidant molecules, tocopherols, carotenoids, ascorbic acids, and phenolic compounds stand out. Additionally, the antioxidants present in fruits and vegetables have been found to exhibit anticarcinogenic and antimutagenic [10].

Antioxidants that are rich in fruits and vegetables generally fight against free radicals and delay or prevent oxidation which help the body to protect against reactive oxygen species (ROS). These reactive oxygen species (ROS) cause major diseases such as cancer, atherosclerosis, diabetes, liver disorder, pesticide toxicity and inflammatory disease. They also cause damage to cell membrane and denature proteins which results in cell death and aging [11].

The concept of blood coagulation dates back to the 1960s, when Davie, Ratnoff, and Macfarlane published articles in *Nature* and *Science* outlining the fundamental principle of a cascade of proenzymes activated through proteolytic cleavage that in turn activate "downstream" enzymes [12,13]. Schematically, the coagulation system is divided into the extrinsic and intrinsic pathways. The extrinsic pathway is triggered in response to tissue trauma and is initiated with exposure of tissue factor. The role of the intrinsic pathway is less clear in vivo but becomes important when the blood is activated via contact with artificial surfaces, such as a cardiopulmonary bypass circuit or a mechanical circulatory assist device (MCAD) [14]. These pathways are interconnected on many levels and converge at the prothrombinase complex, which consists of factors Xa and Va bound together by calcium ions on a phospholipid membrane. The prothrombinase complex converts prothrombin (factor II) to thrombin (factor IIa). Thrombin activates factor XIII to XIIIa, which stabilizes the fibrin clot by covalently cross-linking fibrin. Injured endothelial cells quickly become prothrombotic [15]. Anticoagulants are indicated for strokes, transient ischaemic attacks, deep vein thrombosis and pulmonary embolism [16]. Oral anticoagulants have been used in the management of arthrothrombotic stroke treatment [17] which accounts for all strokes and have

been relied upon for prevention and treatment for several decades. These drugs however, produce a highly variable anticoagulant effect in patients requiring their effect to be measured by special blood test and their dose adjusted according to the results [18] frequently development of Immune thrombocytopenia, haemorrhages and idiosyncratic adverse reactions during treatment of patients with drugs [19] are some of the limitations encountered in anticoagulant therapy. But each of these drugs has distinct pharmacological properties that could influence optimal use in clinical practice [20].

Today, substances containing antithrombotic agents namely anticoagulants, antiplatelets and thrombolytics that decrease clot in the blood by dissolving already formed clot or prevent clot formation [21] and substances containing antioxidants are a part of drug preparations to treat diseases.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Matured inbred albino mice of both sexes weighing between 23-27 were obtained from the Laboratory Animal Unit of the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria. The animals were kept in different cages in the same room with a temperature varying between 28 and 30°C. They were supplied clean drinking water and fed standard feed (Grower mash pellets, Vital feeds®, Nigeria). The animals were allowed two weeks to acclimatize prior to commencement of the experiments. The laboratory animals were used in accordance with laboratory practice regulation and the principle of laboratory animal care.

2.2 Plant Specimen

2.2.1 Plant collection and Identification

Fresh leaves of *Pandanus tectorius* were collected from Umuda Isingwu village, in Umuahia North Local Government Area of Abia State, Nigeria in May, 2015. The plant leaves were authenticated by Dr. Garuba, Department of Plant Science and Biotechnology, Michael Okpara University Umudike, Ikwuano Local Government Area, Abia State, Nigeria.

2.2.2 Preparation and extraction of plant materials

The plant leaves were dried under mild sunlight, and then reduced to coarse particles with mortar and pestle before been pulverized into fine particles using a laboratory hammer mill. The plant material was exhaustively extracted by ethanol with intermittent shaking at 2 h intervals for 48 h. The extract was filtered and concentrated *in vacuo* using a vacuum rotary evaporator. The concentration and percentage yield of the extract were determined. The concentrated *Pandanus tectorius* extract, subsequently denoted as PTE was stored in a refrigerator at 40C until further use.

2.3 Acute Toxicity Test

The acute toxicity of the extract was done using twenty swiss mice weighing 23-27 g of both sex, and the were divided into five groups each having four mice. The groups were labelled A, B, C, D and E.

Group A recieved 1000 mg/ml of the extract, while group B, C, D and E recieved 500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml of the extract repectively. The route of administration of the extract was through intra-peritoneal route of administration. The number of death in each group was being monitored and recorded within a period of 72 hours. The lethal dose 50 (LD₅₀) was calculated using the following formular [22]

$$LD_{50} = \frac{\text{Lowest conc. with high motality} \times \text{Highest conc. Without motallity}}$$

2.4 Antioxidant Capacity of *Pandanus tectorius* Leaf Extract

2.4.1 Determiration of DPPH radical scavenging activities

This is carried out according to the 2,2 diphenyl - 2- picrylhydrazyl (DPPH) assay system [23]. Extract at different concentrations was mixed with 2 ml of absolute ethanol (100%). 1 ml of DPPH was added to the solution and shaken immediately. The mixture was allowed to stand at room temperature in the dark for 30 minutes. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA%) using the formular;

$$\% \text{ Inhibition} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Absorbance of control}} \times \frac{100}{1}$$

All determinations were performed in triplicates.

2.5 Anti-lipid Peroxidation Activity

This was determined by the method described by Dinakarn, 2010. Ethanolic extracts of *Pandanus tectorius* was used in different concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.25 mg/ml) respectively. 3 ml of liver homogenate obtained from the Veterinary Medicine Laboratory MOUAU, was added with 100 µl of 15 mM ferric chloride and was shaken foe 30 minutes. From the collected mixture, 100 µl was added with 1ml of different concentration of the plant extract respectively in different test tubes. The same procedure was followed for standard and blank. Ascorbic acid was used as standard and TBARS as control. All the test tubes were incubated for 4 hours at 37°C. After incubation, 20% trichloroacetic acid (TCA) was added to all the test tubes containing the mixture in 1:1 ratio and was centrifuged for 30 minutes. The supernated liquid was collected and 0.6% thiobarbituric acid (TBA) was added in 1:1 ratio and heated for 1hour in a water bath. The mixture was cooled and absorbance measured at 530 nm. The percentage of anti-liquid peroxidation activity was calculated using the formular:

$$\% \text{ Inhibition} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Absorbance of control}} \times \frac{100}{1}$$

All determinations were performed in triplicates.

2.6 Nitric Oxide Radical Scavenging Assay

Nitric oxide scavenging activity, 2.0 ml of 10 µm sodium nitroprusside and 5.0 ml of phosphosphate buffer were mixed with 0.5ml of different concentration of plant extracts and incubated at 25°C for 150mins. After incubation, 2 ml of incubated solution was added to 2 ml of Greiss reagent (1% Sulphanilamid, 0.1% α-naphthylethylenediamine Dihydrochloride and 3% phosphoric acid) and incubated at room temperature for a period of 30 minutes.

The absorbance of the pink chromophore formed by diazotization of nitrite with α-naphthyl ethylene diamine dihydrochloride was measured at 540 nm. Ascorbic acid was used as standard, sodium nitroprusside as control and results were

expressed as percentage inhibition of nitric oxide.

$$\% \text{ Inhibition} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Absorbance of control}} \times \frac{100}{1}$$

All determinations were performed in triplicates.

2.7 Anticoagulant Activities Test

2.7.1 Study population

Blood samples obtained from my dear groupmates, were used to assess the anticoagulant effects of *Pandanus tectorius*. Participants were 20-25 years old. They had been chosen for the study according to the following criteria: having normal prothrombin time, not suffering from any cardiovascular disease (hypertension, congestive heart failure, coagulation disorders such as, Hemophilia A or B) or diabetes, not recently using nonsteroidal anti-inflammatory drugs, not obese or smokers and free from dyslipidemic disorders.

2.7.2 Collection of blood samples

The blood samples were obtained from normal individuals by using sterile syringes, withdrawn from vein of right arm of each individual and placed separately in containers containing tri-sodium citrate to prevent the scavenging process. Centrifugation (15 minutes at rate 3000 rpm) was carried out to separate the blood cells from plasma in order to obtain Pure Platelet Plasma (PPP) for prothrombin time test. The obtained plasma sample of each individual were poured separately in plane containers using automatic pipette and stored at room temperature [24,25].

2.7.3 Prothrombin time test

0.2 ml plasma, 0.1 ml plant extract of different concentration and 0.3 ml $\text{CaCl}_2(25\text{mM})$ were added together in a clean fussion test tube. 0.1 ml of 0.9% saline, 0.2 ml plasma and 0.3 ml $\text{CaCl}_2(25\text{mM})$ was added to the second test tube for negative control. 0.1 ml warfarin, 0.2 ml plasma and 0.3 ml $\text{CaCl}_2(25\text{mM})$ was added to the third test tube for positive control. All test tubes were incubated at 37°C in water bath. The clotting time was recorded with stop watch by tilting the test tubes every 5 seconds. The time of clotting is called the prothrombin time. Each of

the test was carried out twice and the average scavenging time were noted [26,27].

3. RESULTS

3.1 Description of Plant Extract

The ethanol extract of *Pandanus tectorius* leaves was dark green in color. The extraction process gave a yield of 4.50 g.

3.2 Acute Toxicity of Plant Extract

Pandanus tectorius caused death in all the experimental mice which were administered 2000 mg/ml and 1000 mg/ml. The extract caused death in two out of four animals administered with 500 mg/ml and at 250 mg/ml one in four mice died. No death was recorded on animals administered extract at 125 mg/ml.

Table 1. Acute toxicity test of *Pandanus tectorius* using mice

Group	Conc. mg/ml	No. of mice	No. of mortality
A	2000 mg/ml	4	4
B	1000 mg/ml	4	4
C	500 mg/ml	4	2
D	250 mg/ml	4	1
E	125 mg/ml	4	0

$$LD_{50} = 176.78\text{mg/ml}$$

3.3 The 1,1- Diphenyl-2-Picrylhydrazyl Radical (DPPH) Spectrophotometric Assay

The crude ethanol extract of *Pandanus tectorius* leaves demonstrated appreciable but reduced antioxidant activity relative to the reference values of ascorbic with DPPH radical scavenging method (Table 2).

3.4 Anti- lipid Peroxidation Activity

The crude ethanol extract of *Pandanus tectorius* leaves demonstrated appreciable but reduced antioxidant activity relative to the reference values of ascorbic with Anti-lipidperoxidation method (Table 3).

3.5 Nitric Oxide Radical Scavenging

The crude ethanol extract of *Pandanus tectorius* leaves demonstrated appreciable but reduced antioxidant activity relative to the reference values of ascorbic with nitric oxide scavenging method (Table 4).

Table 2. The antioxidant activity of *padanus tectorius* leaf extract determined by DPPH spectrophotometric assay

Sample	Conc. mg/ml	% Scavenging	IC ₅₀
+ctrl Vit.C	300 mg/ml	98.14±0.420	110.87 mg/ml
P. extract	500 mg/ml	95.01±2.34	
P. extract	250 mg/ml	81.39±4.16	
P. extract	125 mg/ml	59.26±3.21	
P. extract	62.5 mg/ml	25.81±5.49	
-ctrl		0.0	

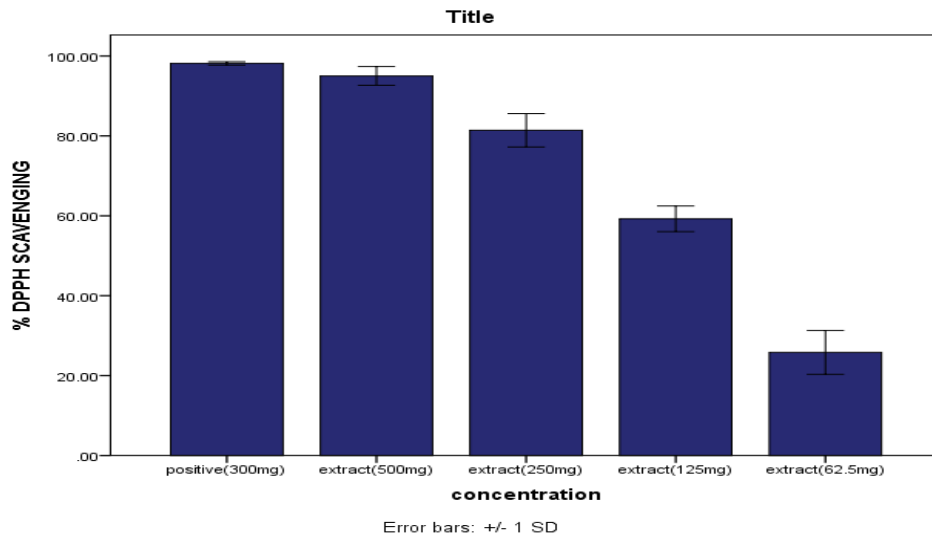


Fig. 1. The antioxidant activity of *padanus tectorius* leaf extract determined by DPPH spectrophotometric assay

Table 3. The antioxidant activity of *padanus tectorius* leaf extract determined by Anti-lipid peroxidation

Sample	Conc. mg/ml	% Scavenging	IC ₅₀
+ctrl Vit. C	300 mg/ml	99.70±0.129	56.28 mg/ml
P. extract	500 mg/ml	99.27±0.087	
P. extract	250 mg/ml	94.99±0.325	
P. extract	125 mg/ml	77.86±5.066	
P. extract	62.5 mg/ml	26.92±8.97	
-ctrl		0.0	

Table 4. The antioxidant activity of *padanus tectorius* leaf extract determined by Nitric oxide radical scavenging

Sample	Conc. mg/ml	% Scavenging	IC ₅₀
+ctrl Vit. C	300 mg/ml	99.61±0.096	59.82 mg/ml
P. extract	500 mg/ml	98.77±0.308	
P.t extract	250 mg/ml	88.15±11.39	
P.t extract	125 mg/ml	78.07±0.33	
P. extract	62.5 mg/ml	45.52±4.05	
-ctrl		0.0	

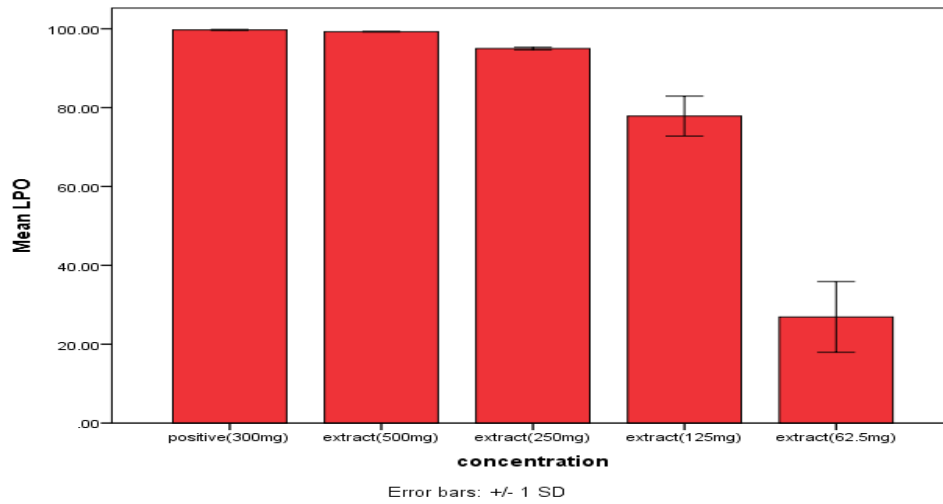


Fig. 2. The antioxidant activity of *padanus tectorius* leaf extract determined by anti-lipid peroxidation

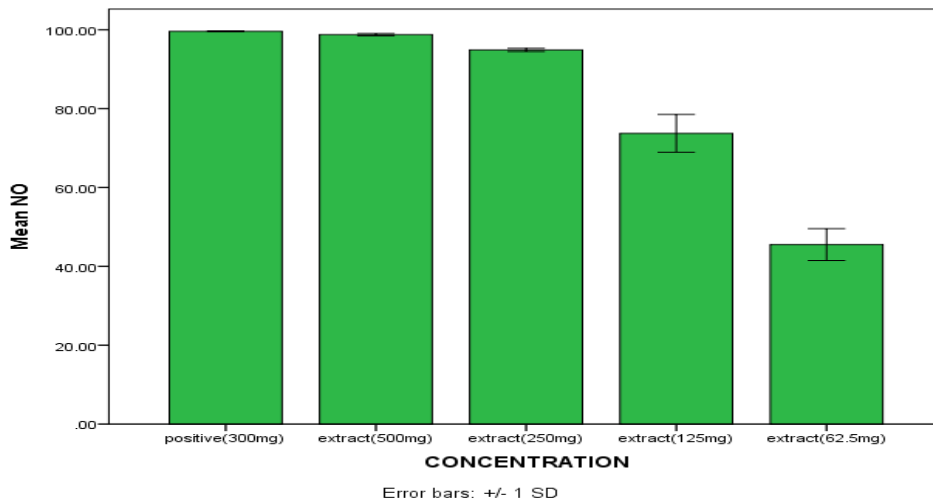


Fig. 3. The antioxidant activity of *padanus tectorius* leaf extract determined by nitric oxide radical scavenging

Table 5. The *in vitro* effect of PTE at different concentration on clotting time of plasma in a clotting time test

Sample	Conc. of sample	Mean±std. D time of coagulation
+ctrl Warfarin	5 mg/ml	57.88±0.06 mins
Plant extract	500 mg/ml	26.23±1.34 mins
Plant extract	250 mg/ml	15.95±0.53 mins
Plant extract	125 mg/ml	9.38±0.14 min
Plant extract	62.25 mg/ml	6.75±0.75 mins
Plant extract	31.125 mg/ml	5.71±0.58 mins
-ctrl 0.9% Saline		83.5±2.12 secs

The coagulation time was recorded as mean time of coagulation±standard deviation. Significant ($p>0.05$) difference between the samples and the control (blood alone)

3.6 Coagulation Time Using Ethanol Plant Extract and Warfarin

PTE at different concentrations produced increased clotting times compared to the baseline clotting time of 83.5 ± 2.12 s for the blood sample. However, the blood added to warfarin has more increased clotting time (Table 5).

4. DISCUSSION

The percentage yield of ethanol extract of the *Pandanus tectorius* was 10.65%w/w. The plant extract which was administered to five groups of four mice each represented as groups A, B, C, D and E at 2000 mg/ml, 1000 mg/ml, 500 mg/ml, 250 mg/ml and 125 mg/ml respectively. All the animals in groups A and B died. Two animals in group C and one animal in group D died within 48 hours. After 72 hours, no other animal was killed by the extract. All the animals in group E survived. The LD₅₀ was calculated to give 176.78 mg/ml, which is the lethal dose that can kill 50% of the animals used for experiment.

The *Pandanus tectorius* leaf extract from the present study of antioxidant activities revealed a remarkable antioxidant activities. The DPPH scavenging activity shows that the extract at different concentration (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml), will give different percentage inhibition (95.01, 81.39, 59.26, 25.81) respectively compared to standard drug using vitamin C at 300 mg/ml which gives 98.14% inhibition. This shows that at increasing concentration, there is increasing inhibition but the standard Vitamin C have more scavenging effect using DPPH method. The result was represented as %inhibition \pm standard deviation. The IC₅₀ was calculated to give 110.87 mg/ml. The IC₅₀ represent the concentration of the extracts that caused 50% inhibition of the radical. DPPH assay reveals the ability of the extract to scavenge free radicals. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [28]. The reduction capability of DPPH is determined by the decrease in the absorbance at 517 nm induced by antioxidants. This system is valid for the primary characterization of the scavenging potential compound [29]. PTE's ability to concentration dependently reduce DPPH forming yellowish colored diphenylpicrylhydrazine suggests that, it is a free radical scavenger and probably acts so by donating electron or hydrogen radical.

The Anti-Lipid peroxidation activity shows that the extract at different concentration (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml), will give different percentage inhibition (99.27, 94.99, 77.86, 29.92) respectively compared to standard drug using vitamin C at 300 mg/ml which gives 99.71% inhibition. This shows that at increasing concentration, there is increasing inhibition but the standard Vitamin C have more scavenging effect using Anti-Lipid peroxidation activity method. The result was represented as %inhibition \pm standard deviation. The IC₅₀ was calculated to give 56.28 mg/ml. The IC₅₀ represent the concentration of the extracts that caused 50% inhibition of the radical. The peroxidation of lipids is basically damaging because the formation of lipid peroxidation products leads to spread of free radicals reactions. The important role of lipids in cellular components emphasizes the significance of understanding the mechanisms and consequences of lipid peroxidation in biological systems. Polyunsaturated fatty acids (PUFAs) serve as excellent substrates for lipid peroxidation because of the presence of active bis-allylic methylene groups. The carbon-hydrogen bonds on these activated methylene units have lower bond dissociation energies, making these hydrogen atoms more easily abstracted in radical reactions. The susceptibility of a particular PUFA toward peroxidation increases with an increase in the number of unsaturated sites in the lipid chain [30]. PTE's ability to concentration dependently reduce lipid peroxyl radicals suggests that, it is a free radical scavenger and probably acts so by donating electron or hydrogen radical.

The nitric oxide scavenging activity shows that the extract at different concentration (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml), will give different percentage inhibition (98.77, 88.15, 78.07, 45.52) respectively compared to standard drug using vitamin C at 300 mg/ml which gives 99.61% inhibition. This shows that at increasing concentration, there is increasing inhibition but the standard Vitamin C have more scavenging effect using nitric oxide scavenging activity. The result was represented as %inhibition \pm standard deviation. The IC₅₀ was calculated to give 59.82 mg/ml. The IC₅₀ represent the concentration of the extracts that caused 50% inhibition of the radical. Nitric oxide interacts with other biomolecules and can combine with superoxide anion (another free radical) to form an unstable intermediate peroxynitrite which may initiate tissue injury. Peroxynitrite may also

decompose to form a strong oxidant hydroxyl radical. Nitric oxide, peroxy nitrite and hydroxyl radical are capable of oxidizing lipids, proteins, and nucleic acids. Nitric oxide is also a major signaling molecule in neurons and immune system, either acting on the cell in which it is produced or by penetrating cell membranes to affect adjacent cells [31]. PTE's ability to concentration dependently reduce nitric oxide suggests that, it is a free radical scavenger and probably acts so by donating electron or hydrogen radical.

The anticoagulant study of ethanol extract of *Pandanus tectorius* was carried out and the present study revealed that the extract have remarkable anticoagulant activity than the negative control solution (0.9% saline water). The extract was tested in different concentrations (500 mg/ml, 250 mg/ml, 31.125 mg/ml, 62.25 mg/ml, 125 mg/ml) giving a mean clotting time of (26.23, 15.95, 9.38, 6.75, 5.71) minutes respectively which proved that the time of clotting increases with increase in amount (concentration) of extract. Warfarin which was used as the positive control also revealed to have more than 50% anticoagulant activity than the ethanol plant leave extract of *Pandanus tectorius* and still remain the standard as already determined. This is logical since warfarin is a pure compound but the extract is a crude and mixture of many compounds. The mechanism by which the extract exhibited anticoagulant activity is not understood but chelating agents, warfarin and vitamin K antagonists are known to interfere with blood clotting processes. Warfarin can inhibit clotting factor IXa, XIa and thrombin but its action on factor Xa accounts for its potency as an anticoagulant [32]. Heparin can inhibit both the generation of thrombin and also the formed thrombin. The synthesis of clotting factors II, VII, IX and X in the liver depends on adequate amounts of vitamin K. Coumarin and inadenedione group of oral anticoagulant drugs antagonizes the synthesis of non functional forms of coagulant proteins and thereby prevents blood clot formation [33].

Blood coagulation is an important part of homeostasis in which platelets; a non nucleated cell derived from megakaryocyte plays a prominent role to prevent excessive bleeding [34]. Platelets change shape and adhere to damaged endothelium or aggregate to themselves resulting in plug formation which is strengthened by fibrin containing clot to stop

bleeding and for repair of damaged vessel to begin. Disorders of coagulation are known to cause haemorrhage or thrombosis [35]. Fibrin is the more important component of clots that form in veins but platelets are the major component of clots that form in arteries where they can cause heart attacks and strokes by blocking the flow of blood in the heart and brain, respectively [18].

5. CONCLUSION

The ethanol extract of *Pandanus tectorius* have been revealed to have a higher acute toxicity effect with higher concentration. From the experiment carried out, it has been found that *Pandanus tectorius* leave extract may be useful as antioxidants and anticoagulants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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