Investigation of *in vitro* antioxidant and cytotoxic activity of different fractions of root extracts of *Pandanus fascicularis*

A project submitted
by
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ID: 12146001
Session: Spring 2012
to
The Department of Pharmacy
in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy

Dhaka, Bangladesh
September 2016

Dedicated to my parents to whom I owe my achievements.
May Almighty keep you as well as you have kept me.

Certification Statement

This is to certify that this project titled '**Investigation of *in vitro* antioxidant and cytotoxic activity of different fractions of root extracts of *Pandanus fascicularis***' submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Ms. Farhana Alam Ripa, Senior Lecturer, Department of Pharmacy, BRAC University and this project is the result of the author's original research and has not previously been submitted for a degree or diploma in any university.

Signed,

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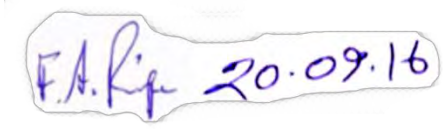
Countersigned by the supervisor



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Acknowledgement

I would like to express my gratitude to Almighty Allah for the help in completion of this research and preparation of this dissertation.

I would like to express my heartiest pleasure and honor to work with the very dedicated teacher of the department, my supervisor Ms. Farhana Alam Ripa, Senior Lecturer, Department of Pharmacy, BRAC University for the continuous support, kind guidance and patience. Her skilled advice and constructive criticism greatly helped me to finish my project work.

I am really grateful to our Chairperson, Dr. Eva Rahman Kabir, Department of Pharmacy, BRAC University, for her support, encouragement and kind cooperation all through the project.

Finally, I would like to thank the laboratory assistants and staffs for their prompt assistance and cooperative attitude.

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Abstract

The plant *P. fascicularis* has significant therapeutic activities in the different parts including bark, stem, leaves, trunk, seed, fruit and flower. Every part shows unique medicinal activities. They exert medicinal efficiency that can be incorporated to treat diverse ailments and can develop new formulas essential to treat newer afflictions. But the root did not show any significance yet so we are trying to emphasize on the antioxidant and cytotoxic activity of the roots. Ethanol (EPF), ethyl acetate (EAPF) and chloroform extract (CLPF) of the grinded root were prepared for running the experiments. The anti-oxidant study was observed *in vitro* by DPPH scavenging radical in comparison to ascorbic acid as standard at 517 nm. The IC_{50} value of standard Ascorbic acid EAPF, EPF and CLPF extracts were 56.27, 46.35, 35.5, 48.25 and 50.39, respectively. From the obtained results of the extracts are considered to have antioxidant effect and CHPF was found to be higher than the other extracts. And in the case of brine shrimp lethality bioassay the EPF showed higher value of LC_{50} compared with the other two extracts. Here vincristine sulphate which was used as positive control. It was observed that the EAPF gave higher value of LC_{50}. It means that using small amount of EPF will give more toxic effect. The LC_{50} values of the vincristine sulphate, EAPF, EPF and CLPF extracts were 6.73, 8.09, 9.015, and 7.046, respectively. So it can be claimed that the results obtained from the experimental data were satisfactory enough to run further studies considering different parameters such as solvent type- methanol, acetone, pet ether etc. We can conclude that the root extract of *P. fascicularis* contains active antioxidant and cytotoxic functions with great potential for producing a revolutionary drug.

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Abbreviation:

WHO – World health organization

ROS – Reactive oxygen species

DMSO- Dimethyl sulphoxide

DNA- Deoxy ribonucleic acid

EAPF- Ethyl acetate extract of *P. fascicularis*

CLPF- Chloroform extract of *P. fascicularis*

EPF- Ethanolic extract of *P. fascicularis*

DPPH- 1, 1-diphenyl-2-picrylhydrazyl

ASA- Ascorbic acid

Chapter 1: Introduction

1.1 Medicinal Plants:

Medicinal plants have been used all over the world from the beginning of time to heal illness and treat various forms of diseases. The main purpose of using medicinal plant is to get natural medicines because it has less side effects and better than synthetic one. For the betterment of human culture around the whole world medicinal plants play a very significant role. According to WHO they defines medicinal plant as "A medicinal plant is any plant in which one or more of its parts, comprises ingredients that are used as healing purposes, or which are pioneers for chemo therapeutic semi-synthesis". They are considered as a great source of ingredients which are used for drug development and synthesis. Medicinal plant includes such type of plants which are used in herbalism and which have medicinal activities. The term herbalism refers to plants used for medicinal purpose (Hassan,2012).

1.1.1 History of medicinal plant:

It is assumed that from the search of some remedy probably medicinal plant was used but nobody exactly knows when and where medicinal plants were used for the first time. India has been always a source for drugs for thousands of years as evidence of using medical plants was found on a Sumerian clay slab at Nagpur. Poppy, henbane and mandrake were popular alkaloid among over 250 various plants which lead to 12 recipes of creating drugs thousand years back (Petrovska, 2012).

The principal of medicinal plants was used by the Egyptians in a controlled and systematic way. The most interesting printed document is the Papyrus of Ebers from 1700 A.C. more than 700 formulas are over there with known plants. The Chinese book "Pen T'Sao," which is based on roots and grasses is a good example of medicinal plants. This book includes the study of more than 300plants (Botanical-online, 1999-2016). The other books such as the Indian holy book Vedas and the holy Jewish book the Talmud consist of treatment with plants. A military physician and pharmacognosist of nero's army dioscorides who was titled as "the

father of pharmacognosy". He wrote the work "De Material Medica" in which 944 drugs described among which 657 are of plant origin. In present days there are national and international pharmacopoeias which include the drugs and preparations, their description, formulation, analytic composition, chemical properties for identifications, standards for purity, dosage etc. Countries such as the United Kingdom, Russia, Germany they have their separate herbal pharmacopoeias. Unofficial drugs always find their way to pop up their head in the field of medicine though they got banished mostly because of the popular and conventional medicines. There are hundreds of medical plants which can be used as a independent medicine or a supportive one that can work with some other synthetic drugs. It is true that after finding authentic information about particular diseases and knowing the perfect way of therapy it can be said that these medicinal plants are essential for their pharmacological purpose. By the phrase therapeutic means we understand that plant drugs and phyto preparations got the ability to function properly when they get connected with defined active objects with verified action and have therapeutic efficiency. Germany is one of the major producer of these herbal medications, and the efficiency depends and corroborated by applied dose, identified active objects and clinical tests respectively and it is obvious that those drugs were powered by the standard drug extracts gathered from large number of authentic herbal plants and they successfully fill out all the pharmaceutical requirements needed for a quality drug (Petrovska, 2012).

1.2 Traditional medicine: Practice in Bangladesh:

In Bangladesh folk medicinal practitioners also known as 'kavirajes' are treats a huge number of people especially in the rural areas. Nowadays the age old traditional medicines are being adopted by modern medical science. The main difference between the folk medicinal practitioners and modern doctors are that their use of simple formula using herbs as the main and the only ingredient. Different kavirajes uses different kinds of formulations that they have learnt from their masters in this field. And this chain is going for thousands of years (Rahmatullah, 2016). Bangladesh is a subtropical country and has a good repository of medicinal plants. About 5000 angiosperms are distributed among 200 families. Among those approximately 500 are being used for the treatment of different types of diseases as traditional medicines. In the 'Materia Medica' 2000 medicinal plants are included of the subcontinent. Various regions of the country like- Dhaka, Rajshahi, Sylhet and Chittagong, more than 500

are growing (IUCN, 2003). Leaves are the leading part which is used in a majority of medicinal plants and other used parts also include seeds, rhizome, fruits, barks, whole plant and inflorescence. Bangladesh is rich flora of herbal medicines. Almost 5000species are found in this region and about 1000 are used in medicinal purpose. Generation after generation people has been using these plants as their source of medication and still now it is being used. Even in this era of allopathic medication 70-80% people are still using traditional medicines. This use of traditional medicines is very involved in the life of Bangladeshi people. The method of the application of traditional medicine in Bangladeshi people varies widely among the different ethnic groups. The medicaments which are prepared from plant materials and other natural products, sometimes they also include some other substances of animal origin. While introducing the medicines like infusions, pastes, powders, dried pills, decoctions, creams and poultices etc. various dosage forms are used. From tribe to tribe the way of treatment and dosage form varies. There are few examples of method of application from tribe to tribe. Among the methods one is the old method and the original form is based on old knowledge, experience and belief of the older generations.

This includes: i) **Folk medicine:** They mainly use the plant and animal parts and their products are medicines for treating different types of diseases and also include treatments like bone-setting, hot and cold baths, therapeutic fasting and cauterization etc.
ii) **Religious medicine:** This type of medicine includes the use of verses from religious books. They are written on papers and are given as amulets, religious verses recited and water to drink or on food to eat etc. iii) **Spiritual medicine**: This method utilizes communication with the supernatural beings, spirits or ancestors through human media, torturous treatment of the patient along with incantations to minimize the imaginary evil spirits.

The other is the modified form which is based on the two following main systems:

a) Unani-Tibb or Graeco-Arab system: This system is developed by the Arab and also by the Muslim scholars from the ancient Greek system.

b) Ayurvedic system: This is the old Indian system and it is based on the *Vedas*.

The Ayurvedic and Unani system of medication both are well known and practiced in rural areas mostly. Though they are use in some parts of the urban area by those who still goes with traditional medication even after having hospitals and allopathic medicines. These both med-

ication uses plant extracts mainly and also some synthetic organic substances. Both indigenous and modern techniques are being used now days to make these medicines. These medicines are nicely packed in appropriate packaging system, aluminum foil, plastic or metallic container and mostly bottles. When they are packed they are also labeled with indication, contraindication, expiry date, ingredients used just like available allopathy medicines present in the market (Ghani, 1990).

1.3 Antioxidants:

Antioxidants are naturally occurring plant substances. They are found in lots of plants also in foods we eat everyday such as vitamins, minerals, and other compounds in foods. Fruits, vegetables, grains and nuts are rich source of antioxidants. Antioxidant helps to protect the body from harmful molecules and the harmful molecules are called free radicals. Antioxidant also helps to prevent oxidation (Antioxidants, 2010). Oxidation is a typical reaction that happens in our body every day and they cause damage to our cell. Oxidation is the loss of electrons from an oxidizing agent. Oxidation reaction is responsible for the formation of free radicals which further starts spontaneous chain reaction to damage the cells. By eradicating the free radicals intermediates antioxidant stops this chain reaction and they also stops other oxidation reaction by oxidizing themselves. There are primary or natural antioxidants, secondary or synthetic antioxidants and tertiary antioxidants. Chain breaking antioxidants are called natural antioxidants. Minerals such as selenium, copper, iron, zinc, manganese. Vitamins such as vitamin B, vitamin C, vitamin E. Phytochemicals such as phenolic compound flavonoids. Synthetic antioxidants have the function of capturing free radicals and they are the reason to demolish the chain reaction. Butylated hydroxyl anisole, butylated hydroxyrotoluene, propyl gallate, metal chelating agent, tertiary butyl hydroquinone, nordihydroguaretic acid are examples of synthetic antioxidants (Hamid, 2010).

The mechanism of three main types of antioxidants is described below.

(1) **Primary antioxidants:** The basic mechanism of this antioxidants is that they cause the prevention and the formation of new radicals. It converts them into less harmful molecules and prevent the formation of free radicals into other molecules. For example: Enzyme superoxide dismutase (SOD) which converts $O_2 \bullet -$ to hydrogen peroxide (H_2O_2) and lipid peroxides

to harmless molecules before they form free radicals, Catalases, Glutathione reductase, Glutathione S transferase, Proteins that bind to metals (ferritin, transferrin and ceruloplasmin) limit the availability of iron necessary to form the radical OH.

(2) **Secondary antioxidants:** It prevents the chain reaction, captures the free radicals. For example: vitamin E or alphatocopherol, vitamin C or ascorbic acid, uric acid beta-carotene, bilirubin, albumin, ubiq-uinol-10, methionine.

(3) **Tertiary antioxidants:** Damages biomolecules are repaired by free radicals. For example: DNA repair enzymes and methionine sulfoxidereductase (Pérez, 2013).

1.3.1 Free Radicals:

Molecules with unpaired electron in the outer shell are free radicals and they are able to exist independently. Because of the presence of free electron free radicals are remarkably reactive and they are also very unstable. The fact that they are highly reactive means that they can react with most molecules in its vicinity. This includes protein, lipid, carbohydrates and DNA. There are stable radicals, persistent radicals and diradicals. The production route of free radicals are the immune system in which cells create oxy-radicals and reactive oxygen species, during energy production cell generates oxy-radicals and ROS, stress responses to increase the number of free radicals. Other factors such as aging, metabolism, stress. Dietary factors, toxins, drugs are also responsible for the production route of free radicals. There are three steps involving free radical generation. Initiation reaction, propagation reaction and termination reaction. Free radical mainly targets lipids, proteins, and DNA. They can cause other diseases like cancer cardiac reperfusion abnormalities, kidney disease, Alzheimer's disease, fibrosis and many more (Sarma, 2010). As free radicals cause damage to our system we need to counter them. Synthesized antioxidants have little or no effect on countering free radicals. In fact when they are used they turn into harmful agent in our bodies. Hence, we turn our interests on natural means. Everything that we need to be healthy can be found in nature. This is the reason why plants and foods have been used from the beginning of time for medical purposes.

3.2 Antioxidant metabolites present in plants:

There are different secondary metabolites. They are produced from plants. Many of them are potent antioxidants.

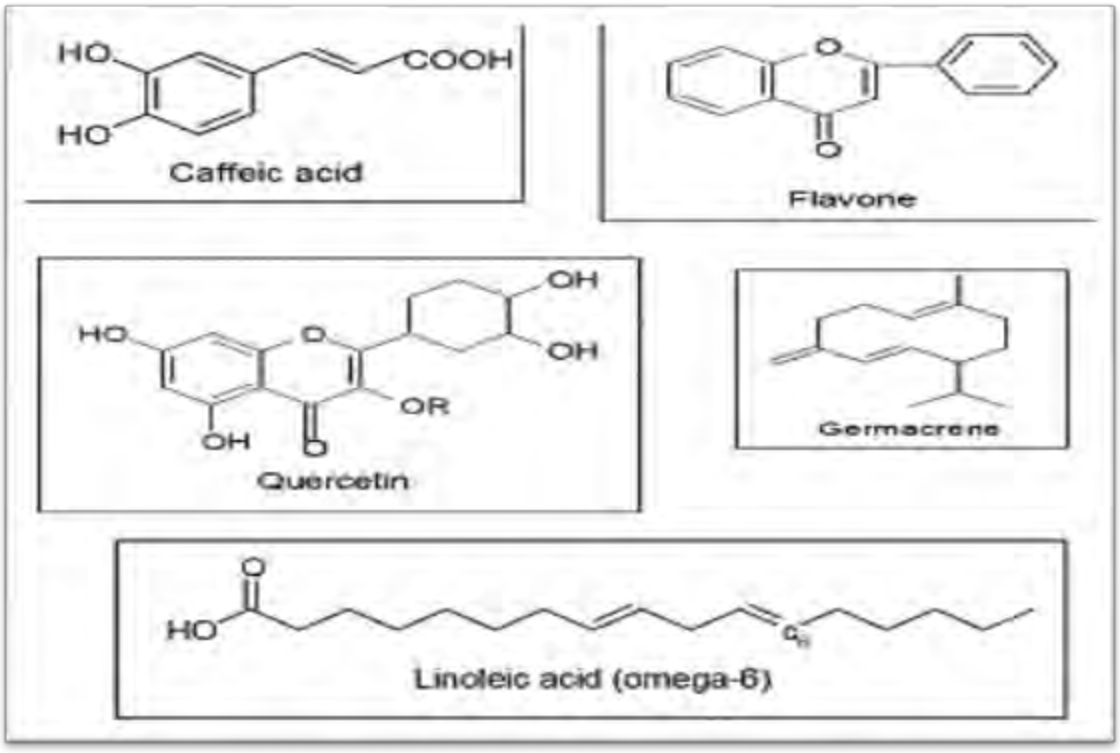


Figure 1.1: Different types of plant antioxidant metabolites (Pérez, 2013).

1.4 Cytotoxicity:

The term cytotoxicity is the quality of being toxic to cell. Cytotoxicity describes the detrimental effects of substances and also environmental changes on cell health. If a cell gets in contact to a cytotoxic stimulus it may compromise to metabolic activity which can inhibit cell growth or division or which causes cell death (Essen Bioscience, 2016). One of the most widely used modes of testing cytotoxicity is the brine shrimp lethality test (BST). This is a simple method for screening and fractionation of active plant extract. This test is carried out with zoological organism. In this test there is only one criterion: either alive or dead (Montanher, 2002).

1.5 Rationale of the current study:

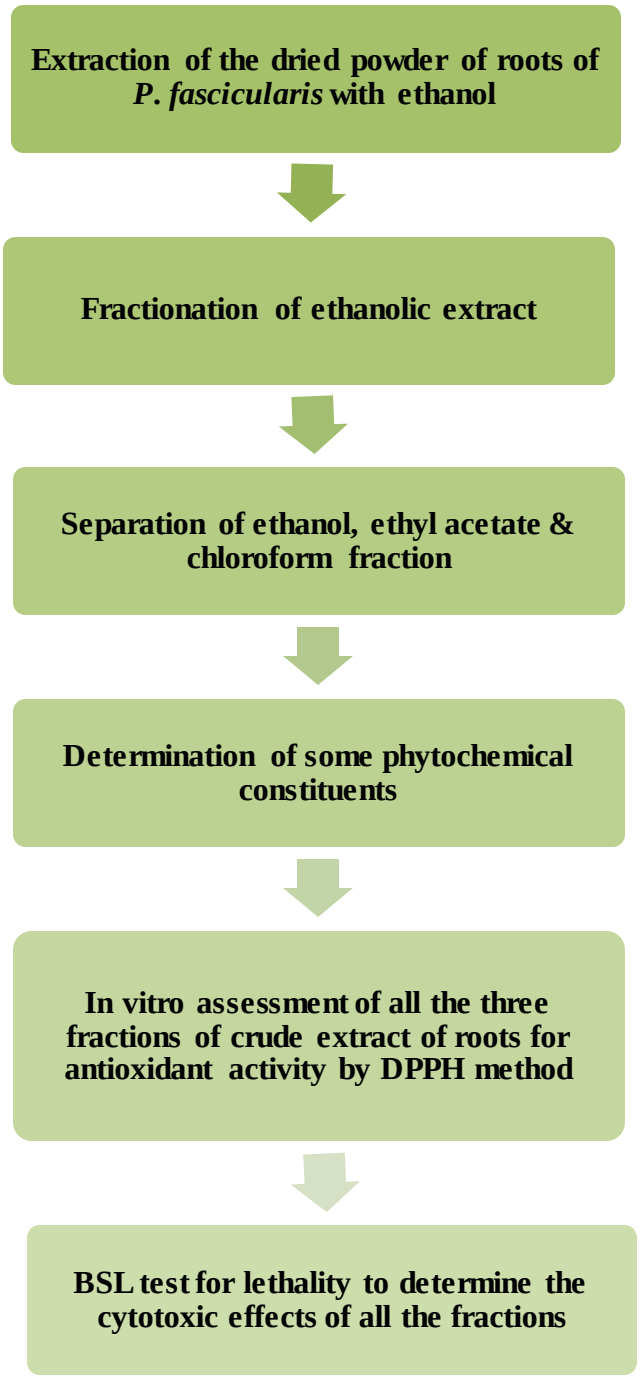
The present study focuses into the antioxidant and cytotoxic investigation of root extract of *P. fascicularis*. This tree is commonly referred to as screw pine. Other parts of this plant are also used. They are the leaves, fruits, spadices, flowers, roots, bracts. They are being used in the treatment of smallpox, syphilis, scabies, heat of body, leprosy, diseases of the heart, pain, brain and in leucoderma (Udupa, 2011). Previous study shows that the various parts of this plant has given various therapeutic activities such as anti-inflammatory activity, analgesic activity, hypoglycemic activity. The rationale behind choosing such experimental work is to investigate whether the root of this plant has both the antioxidant and cytotoxic activity. As the other parts already has given other therapeutic activities.

1.6 Aim of this study:

Aim of this study is to explore free radical scavenging product (antioxidants) and to see the cytotoxic effect in the root extract of *P. fascicularis* .

Consumption of medicinal herbs is considered as an alternative approaches to maintain good health. It has been used tremendously to improve the quality of life over a past decade. From ancient time's plant of medicinal values have been used for treating human diseases. Depend ing on the result of the phytochemical screening two activity tests are performed with the root extract of *P. fascicularis*. The antioxidant activity is performed by using DPPH (1, 1-diphenyl-2-picrylhydrazyl) free scavenging assay. This assay is widely performed and it is easy and simple. To see the cytotoxic activity brine shrimp lethality bioassay is performed. The other plant parts of Screw pine (*P. fascicularis*) is used previously for its therapeutic activity but work regarding on the root of this plant is not done precisely.

1.7 Study protocol:



1.8 Plant description and literature review:

1.8.1 *Pandanaceae* family:

Pandanaceae is known as screw pine family. This is a paleotropical group of arborescent or lianoiddioecious monocotyledonous plant. The family contains four genera, *Freycinetia* (200 species), *Pandanus* (500 species), *Sararanga* (two species) and *Martellidendron (*six species). Genus *Pandanus* has the largest geographical spreading among the four genera (Callmander, 2012).

1.8.2 *P. fascicularis*:

P. fascicularis commonly known as screw pine. This is a branched palm like plant with ever-green shrub and its steam is supported by the aerial roots (Figure 1.2). *Pandanus* genus comprises of about 500-600 species. The individual plants may have height of 20 m. These species are distributed mainly in subtropical and tropical regions (Rajeswari, 2011). This tree can have a height of about 15-18 m. Angling of this tree can be dichotomous, trichotomous or irregular. Leaves are glaucous-green, 0.9-1.5 m, 4 to 7 cm wide and they are sword like and uniform, acuminate with three different rows of prickles each on the margins and on midrib beneath. Approximately 80 to 110 cm in length and 6 to 8 cm in width are the adult leaves. Both female and male flowers grow in separate trees. Male inflorescence is a raceme of spikes, and male flowers are tiny, white, and it last only for about a day, with the inflorescence decaying within three to four days. Female inflorescence looks like pineapple and composed of free or joined carpels. When unripe fruits are green and orange or red or vermilion when ripe (Trees and shrubs of the Maldives, 2016).

1.8.3 Chemical constituents:

The principle constituent is the kewda oil which is isolated from the inflorescences of *P. fascicularis.* The chemical composition of this essential oil has been shown to contain many (>60) components, among which is 98.7% of the total oils. The major components of the kewda oil are 2-phenyl ethyl methyl ether (37.7%), gesmacrene B (8.3%), terpene-4-ol

(18.6%), benzyl benzoate (11%), viridine (8.8%), α –terpeniol (8.3%) and 2- phenyl ethylalcohol (7.5%), and with a little amount of benzyl acetate, benzyl salicylate, benzyl alcohol etc. (Udupa, 2012).

Figure 1.2: Screw pine tree

1.8.4 Distribution:

This plant is mostly allocated in India over coastal districts of Orissa, Tamil Nadu, Andhra Pradesh, and in some parts of Uttar Pradesh. Screw pine plants are found expanding along seashores, in the banks of ponds, canals, rivers and so forth. Screw pine grows mainly in tropical climate, where it can withstand drought salty spray and strong wind. This plant is naturally occurs in Southeast Asia, extending eastward through Papua New Guinea and northern Australia, including the Philippines and Indonesia and throughout the pacific ocean beaches, including Melanesia (Solomon Islands, Vanuatu, New Caledonia, and Fiji), and Polynesia (Wallis and Futuna, Tokelau, Samoa, American Samoa, Tonga, Niue, Cook Islands, French Polynesia, and Hawaii). *P. fascicularis* is indigenous to South Asia and it has significant presence in the mangrove swamps (Adkar, 2014).

Figure 1.3: Root of Screw pine

1.8.5 Taxonomical classification:

Kingdom - Plantae – Plants

Subkingdom - Tracheobionta – Vascular plants

Superdivision - Spermatophyta – Seed plants

Division - Magnoliophyta – Flowering plants

Class - Liliopsida – Monocotyledons

Subclass - Arecidae

Order - Pandanales

Family- Pandanaceae – Screw-pine family

Genus - *Pandanus* Parkinson

Species – *Pandanus fascicularis*

1.8.6 Synonyms:

Some substitutional synonyms of *P. fascicularis* are:

Pandanus odorifer

Pandanus odoratissima,

Pandanus tectorius

1.8.7 Different names:

Common English names: Screw pine, Umbrella tree, fragrant screw pine, Screw tree

Local names: Kewara, Kewada, Ketki, Kiya etc.

1.8.8 Use of this plant:

1) Medicinal uses include:

Diuretic: Taking extraction of fresh and also dried root as tea. It is also used for Headache, stomach ache, arthritis. Particularly for headache essence of fresh leaves mixed with oil can be used (Healing wonders of Philippine medicinal plants, 2015).

Healing purpose: The extract of its leaf can be applied to heal wounds. Cream of cabbage plant can be mixed with juice and shrubs of microcarpa is used for cavities. Arthritis is also treated with its root for the prevention of abortion. The root can be chewed to make the gum stronger as well. It has uses for urinary problems also. Extraction of its root mixed with banana sap can be used for the problems related with urine (Healing wonders of Philippine medicinal plants, 2015).

2) Other uses: The fruit of *Pandanus* produce food and medicine (Figure 1.4). It can be used as a ingredient for the scent of rice dishes for its aroma. Perfume is made from the spadices of male screw pine flowers (Figure 1.5). The leaves are scented and good quality papers are made from the leaves (Figure 1.6).

Figure 1.4: Fruit of *P. fascicularis*

Figure 1.5: Flower of *P. fascicularis*

Figure 1.6: Leaves of *P. fascicularis*

1.8.9 Literature review on *P. fascicularis*:

To determine the medicinal importance of the different parts of *P. fascicularis* various experiments were carried out.

1) The anti-inflammatory and analgesic activities of the ethanol and aqueous extracts of prop roots of *P. fascicularis* (*Pandanaceae*) by Rajeswari, 2011. According to this investigation the root of *P. fascicularis* produce significant analgesic and anti-inflammatory activities, supporting the traditional application of this herb in treating various diseases associated with inflammation and pain.

2) The analgesic activity of aqueous extract of *P. fasicularis* was evaluated in rodents by Udupa et al, 2011. This study confirms that the aqueous extracts of *P. fascicularis* possess analgesic activity which is comparable to that of codeine and aspirin and this favors the use of *P. fascicularis* in rheumatism and rheumatoid arthritis in traditional medicine

3) Anticancer activity of *P. fascicularis* was studied on Ehrlich Ascites Carcinoma (EAC) in Mice by Kumari et al, 2008. The result shows significant anticancer activity on dose dependent manner.

4) Hypoglycemic activity of aqueous extracts of roots of *P. fascicularis* was investigated in alloxan induced diabetic rats by Madhavan et al, 2015. The results of this study substantiate the traditional use of this drug in the treatment of diabetes and thus the roots of *P. fascicularis* may be a potential source of drug for management of diabetes mellitus

5) Antioxidant activity of methanol extract of *P. fascicularis* was examined by Sanjeeva et al, 2016. It was concluded that the methanolic extract of leaves of *P. fascicularis* has significant antioxidant activity.

6) Antitumor activity *of P. fascicularis* on Dalton's Ascites Lymphoma (DAL) in Mice was studied by Mani, 2016. The result of this study shows that it possesses significant antitumor activity in dose dependent manner.

Chapter 2: Methodology

2.1 Preparation of plant extracts:

2.1.1 Collection of plant parts and identification:

The screw pine tree *P. fascicularis* has been selected for phytochemical and pharmacological investigation. The roots along with leaves have been collected from Shatkhira, Khulna district of Bangladesh on March. The roots chosen for this investigation were taxonomically recognized by the Bangladesh National Herbarium, Dhaka and the accession number is 43172 for our specimen.

2.1.2 Preparation of extract:

The roots of *P. fascicularis* has been collected. It was cleaned in dry condition for grinding. After that the roots were separated from undesired parts and dried. When totally dried the roots were crushed into powder. Around 800 gm of powdered root was kept for further investigation.

To soak the powder about 1 liter of ethanol was added to the glass container. The container was handled carefully and the caps were opened to allow the gaseous substance pass out after shaking the contents. More ethanol was joined by continuous shaking and stirring. This technique was allowed to be followed for 7 days and on 8^{th} day the mixture of both containers were filtered. The whole mixture was filtered at first in a cloth. Then it was filtered by a piece of cotton and then it was filtered through filter paper. The ethanolic extract was obtained by evaporating 800 mL of one of the container's filtrate to 100 mL in rotary vacuum evaporator (Model Hei-vapAdv Rotatory Valve Tech, Gwalior, India) along with fan dry to obtain the desired extract. The filtrate of the other container was passed into fractional separation to obtain three identical type of extract. The first solvent was ethanol then chloroform and lastly ethyl acetate. They were separated by their value of specific gravity. Specific gravity is the ratio of the density of a substance to the density of a reference substance. Under reduced pressure by using a rotary vacuum evaporator the solvents were removed from the extracts. After that the filtrate was allowed to evaporate off under room temperature and then in a temperature of 40-50ºC under water-bath.

At last all the experimented extracts were shifted to enclosed vials for further use and named the ethanolic extract of *P. fascicularis* roots as ETPF, chloroform extract leaves as CLPF and the ethyl acetate extract of leaves as EAPF.

2.2 Screening of antioxidant and cytotoxic activities of *P. fascicularis* root extracts:

The investigations were carried on the *P. fascicularis* roots for the determination of the possible medicinal effects. Among various medicinal activities following two activities were tested:

(1) Antioxidant activity,

(2) Cytotoxic activity.

2.2.1 DPPH free radical scavenging Assay:

There are various methods for screening antioxidant activity.

(i) Hydrogen atom transfer methods (HAT), (ii) Electron transfer methods (ET) and (iii) Other assays. DPPH free radical scavenging assay is one method from electron transfer methods. Among all the methods electron transfer method, DPPH free radical scavenging assay is widely used because it is a simple and rapid method to measure antioxidant capacity. It runs with the use of the free radical (DPPH) which is used to test the ability of compounds to act as free radical scavengers to evaluate antioxidant activity (Shekhar et al, 2014). This is effective and easy way to investigate the profile of plant extract.

2.2.1.1 Principal:

This method of investigation is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H. A freshly prepared DPPH solution exhibits a deep purple color. The transformation results in the decolourization and this are measured spectrophotometric ally. Thus the antioxidant molecules will neutralize DPPH free radicals through converting them into colorless

products (2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine) resulting in a decrease absorbance. Therefore, the potency of antioxidant activity is inversely proportional to the rate of decrease in wavelength. In this experiment, ASA (ascorbic acid) is used as standard.

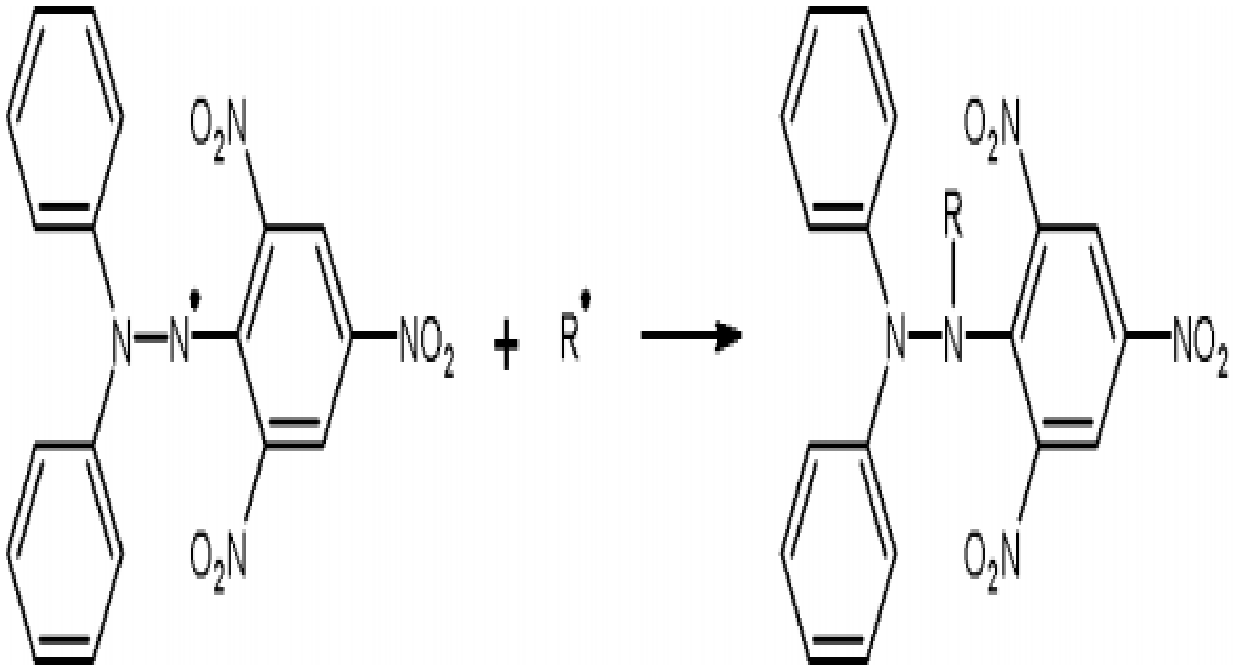


Figure 2.1: The mode of action of DPPH

The capacity to scavenge the DPPH compound was calculated by the following equation:

DPPH scavenged (%) = A (control) - A (test)/ A (control) x 100

Here,

A (control) = absorbance of the control reaction

A (test) = absorbance in the presence of the sample of the extracts

Inhibition percentages obtained were plotted in a graph against the used concentrations. The IC_{50} was calculated by using a standard which is potential antioxidant ascorbic acid.

2.2.1.2 Instrument and reagent:

(1) UV/VIS Spectrophotometer 200V, Hitachi technologies, Model no. U-2910 Part no. 2J1-0012

(2) DPPH

(3) Ascorbic acid (ASA)

Figure 2.2: UV Spectrophotometer

2.2.1.3 Experimental procedure of screening antioxidant activity by DPPH:

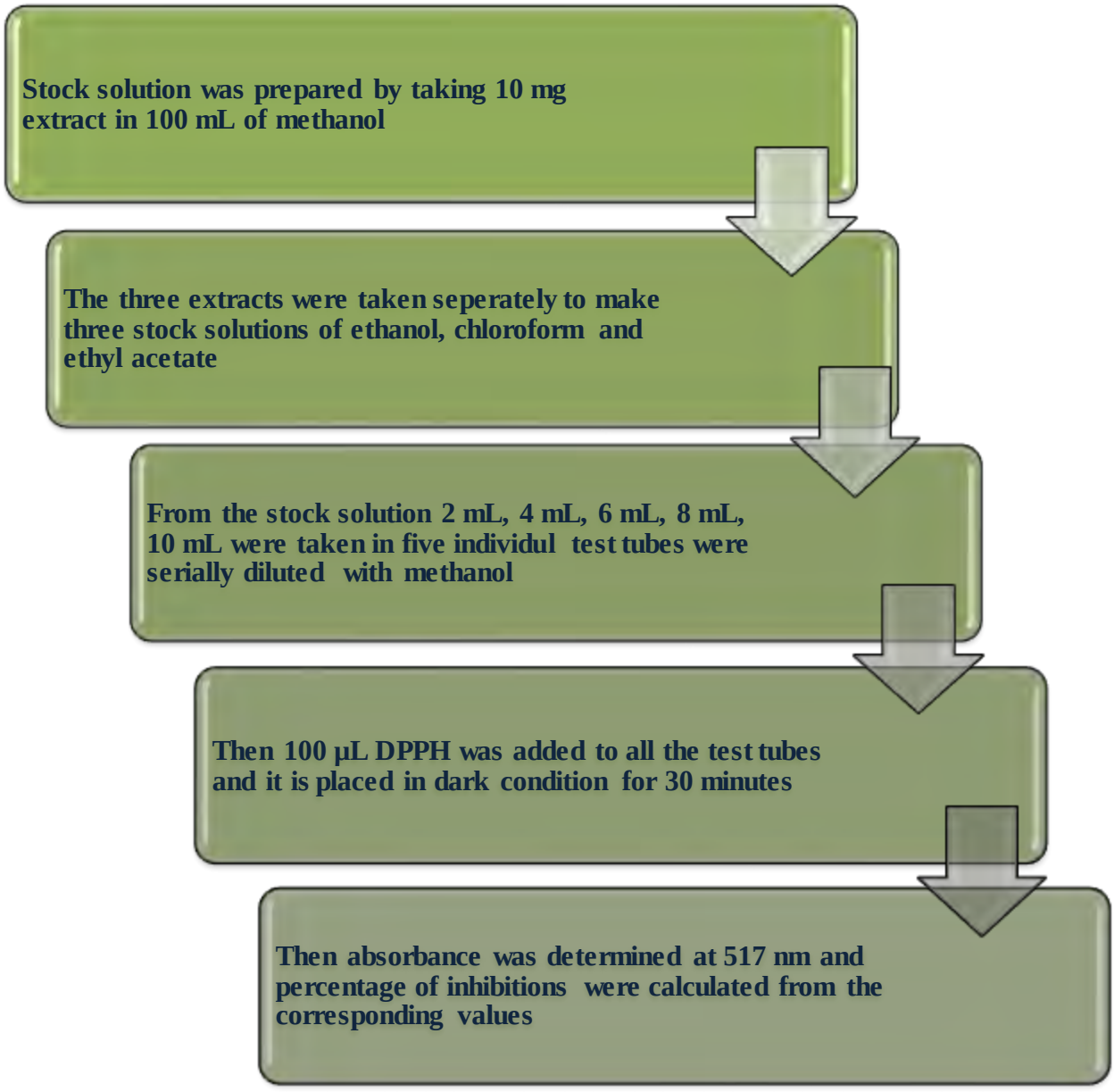


Figure 2.3: Diagram for the procedure for antioxidant screening by DPPH method

2.2.2 Screening of cytotoxic activity by BSL:

2.2.2.1 Brine shrimp lethality bioassay:

Brines shrimp bioassay is the most recognized and accepted method for determining cytotoxic effect of extract of any plant constitute. At first, in order to get nauplii, the brine shrimp eggs are hatched in simulated sea water. Then required amount of dimethyl sulphoxide (DMSO) were added so that the desired concentration of the test samples can be prepared. When the hatching was done, the nauplii were counted by inspecting visually. After that the vials were kept about for a day and survivors are counted after 24 hours. The advantages of this analysis are-Rapid, Inexpensive, In-house and less time consuming (Meyer, 1982).

2.2.2.2 Instruments and materials:

Brine shrimp eggs (*Artemiasalina* leach)

Test samples of experimented plants

Sea salt (NaCl)

Lamp to attract shrimps

Micropipette pipettes

Test tubes

Table 2.1: Test samples of experimental plant:

Plant Part	Sample Code	Test Sample	Amount (mg)
Root of *P. fascicularis*	EPF	Ethanolic extract	10
	EAPF	Ethyl acetate extract	10
	CLPF	Chloroform extract	10

2.2.2.3 Experimental Procedure for cytotoxic activity:

Preparation of seawater:

At first 3.8% NaCl was weighed and then dissolved in one liter of distilled water.

Hatching of Brine Shrimps:

From nearby pet shops, the brine shrimp eggs of the test organism named *Artemiasalina* leach were collected and added to the small tank containing sea water. Then the eggs were allowed to get hatched and matured into nauplii by 24 to 48 hours. Throughout the hatching time, constant oxygen supply was provided to the tank. The tank was under the lam about ten living shrimps were added to all of the test tubes containing different sample concentrations with the help of Pasteur pipette.

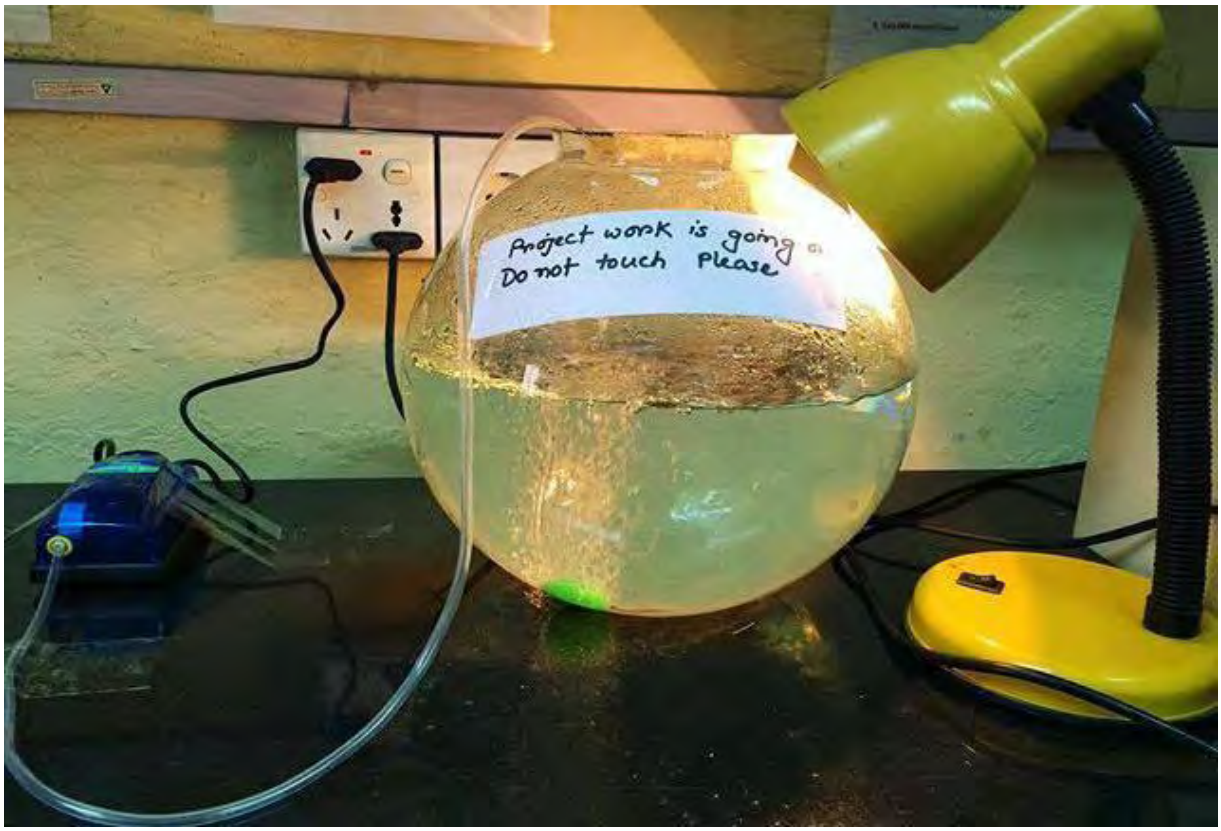


Figure 2.4: Hatching of brine shrimp

Counting of nauplii:

After a day (24 hours) DMSO was added into each test tubes and let it stand for 30 minutes and the number of survivors were counted through inspection. For each diluted concentrations, the percentages of mortality were counted. The effectiveness of the concentration-mortality relationship of plant product is usually expresses as median lethal concentration (LC_{50}) value. This indicates the concentration causing death in half of the test subjects after a fixed exposure period.

Figure 2.5 Brine shrimp

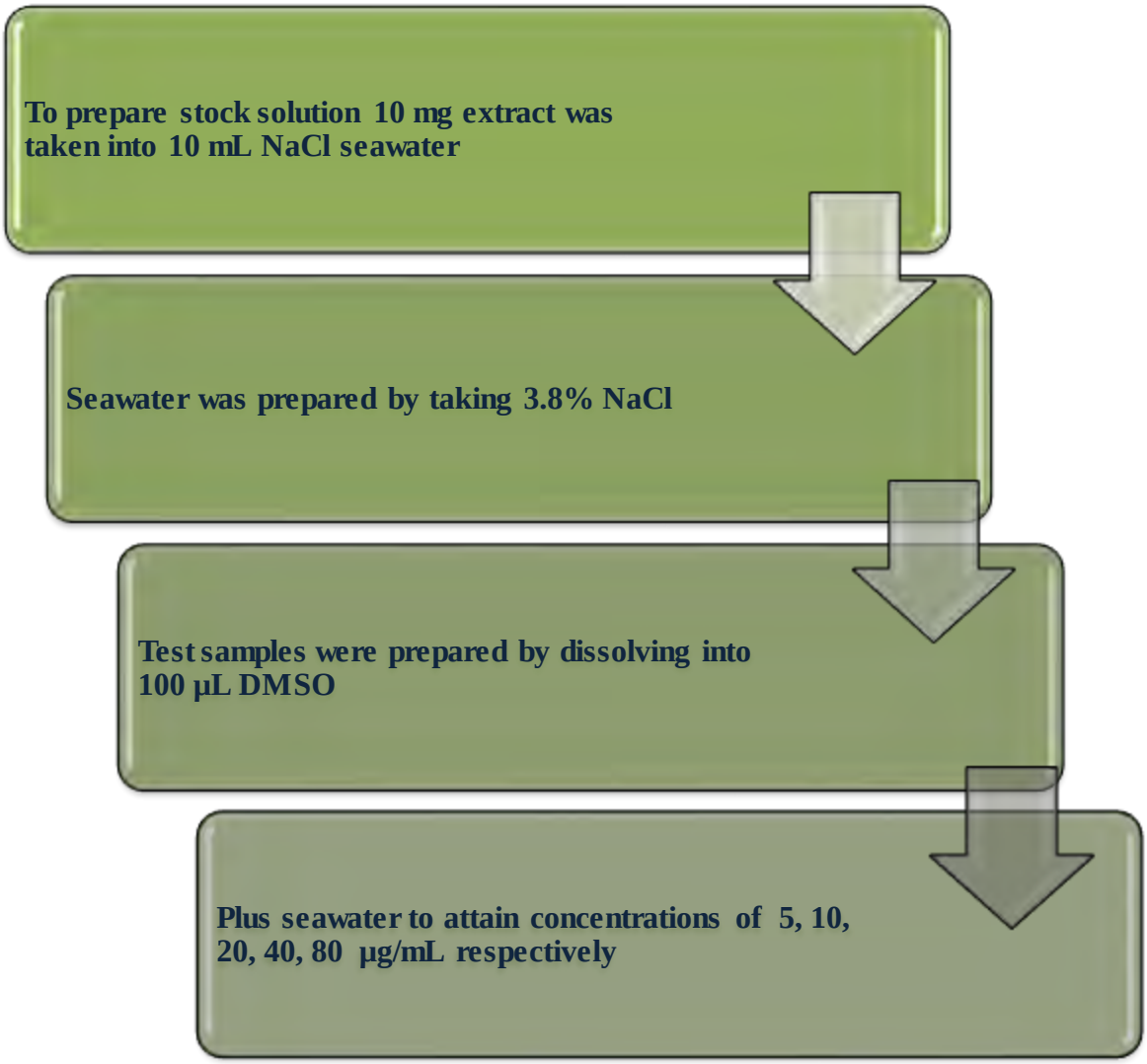


Figure 2.6: Diagram of the procedure for cytotoxic activity by brine shrimp lethality bioassay.

Chapter 3: Results and Discussion

3.1 Results of the *in-vitro* screening of the antioxidant activity:

The percentage of inhibitions of the extracts were found by the application of Brand William's modified DPPH technique method,

The capacity to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenged (%) = A (control) - A (test) / A (control) x 100

Where, A is the absorbance of the control reaction. The percentage inhibitions obtained were plotted in a graph given below. The graph is drawn against the used concentrations

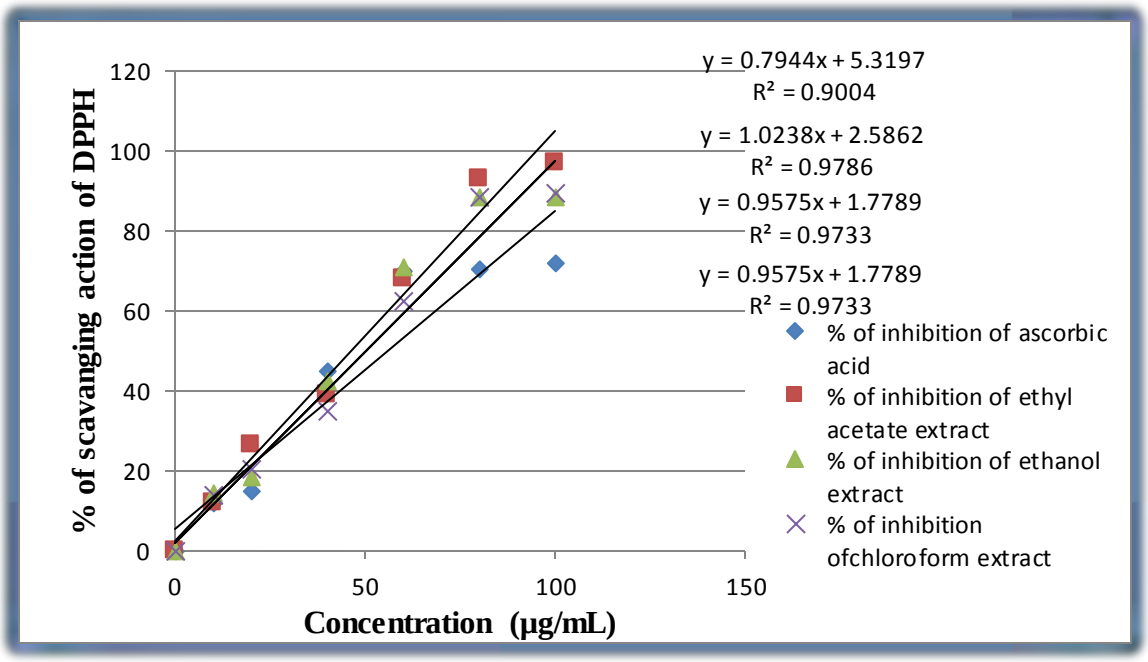


Figure 3.1: Graphical representation of % inhibition Vs concentration

Table 3.1: IC_{50} Value of ASA, EAPF, CLPF, EPF

Samples	IC_{50} values
ASA	56.27
EAPF	46.35
EPF	48.25
CLPF	50.39

DPPH antioxidant assay was written on the capacity of 1,1diphenyl-2-picryl-hydrazyl, a steady free radical, to change the color in the presence of antioxidants. DPPH contains a lose electron which gives it an absorbance at 517 nm and was responsible for its dark purple appearance. Upon accepting an electron, DPPH changes color and this is measured from the change in absorbance and subsequently the percentage scavenging ability can be measured. This was catalyzed by increasing the concentration of the sample. The antioxidant activity of the crude EPF, crude CLPF and crude EAPF of *P. fascicularis* were evaluated by DPPH radical scavenging assay. In this investigation, the crude EPF, crude CLPF and crude EAPF were subjected for estimating the free radical scavenging activity. The IC_{50} value was found lowest in EAPF that was 46.35 and the highest value was found in CLPF which was 50.39 (Table 3.1). The ASA was used as standard against the partitionate and the IC_{50} value was found 56.27.

3.2 Results of brine shrimp lethality bioassay:

From brine shrimp lethality bioassay the values were obtained and the percentage of mortality was plotted against the logarithm of concentration. By linear regression equation using the software "Microsoft Excel-2007" the concentration that would kill 50% of the nauplii (LC_{50}) was determined and % death were calculated by using the following formula:

% of death= (Total nauplii-alive nauplii/ Total nauplii)*100

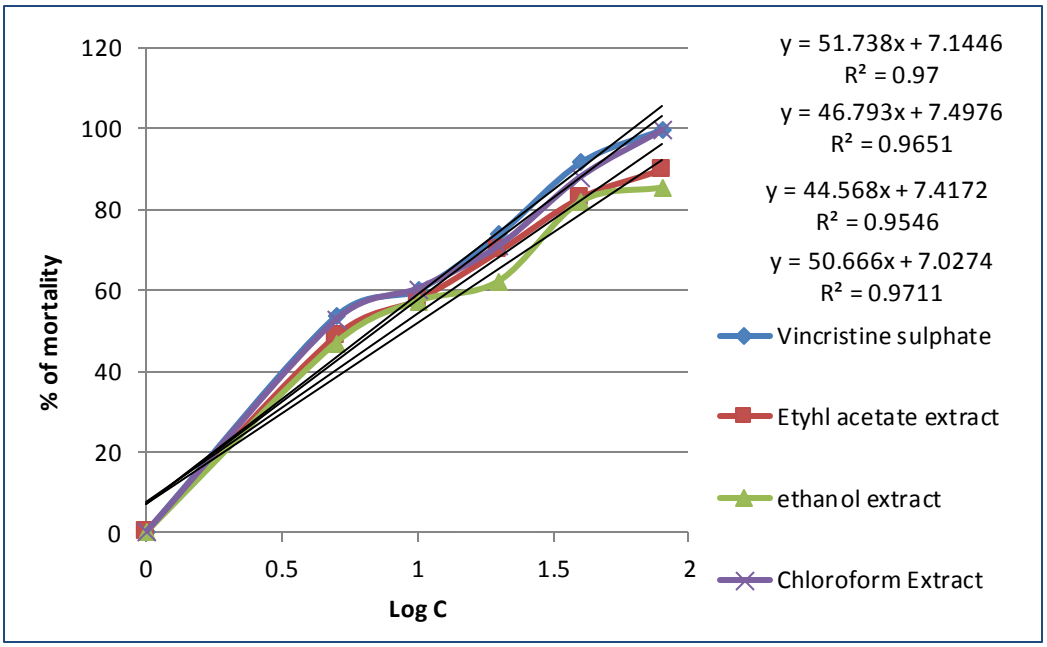


Figure 3.2: Graphical relation between log concentration and % mortality

Table 3.2: LC_{50} values of vincristine sulphate, EAPF, CLPF, EPF

Samples	LC_{50} values
Vincristine sulphate	6.73
EAPF	8.09
EPF	9.015
CLPF	7.046

The investigation was done using ethanol root extract of *P. fascicularis* to justify the cytotoxic activity. While considering cytotoxic analysis using brine shrimp, the extracts impart significant cytotoxic activity. It was observed from the data analysis, that all the fractions gave LC_{50} values. Here vincristine sulphate was used as positive control. It was observed that EPF of *P. fasciuclaris* gave higher value of LC_{50} (Table 3.2). It means that using small amount of EPF will give more toxic effect. Here it is noticed that the CLPF gives less toxicity than the others.

Chapter 4: Conclusion

Conclusion:

The study was focused to investigate antioxidant and cytotoxic activity of different fractions of root extracts of *P. fascicularis*. The antioxidant study was performed with the DPPH method to understand the capacity of hydrogen scavenging of the extracts in comparison with a standard. In this method CLPF showed the highest potential to inhibit than the other extracts. The value of CLPF at a high dose had stronger effects than the EPF and EAPF. Furthermore, the cytotoxic study was done by brine shrimp lethality bioassay to observe the toxic action. The EPF gave highest value of LC_{50}. In the investigation of antioxidant and cytotoxic property of the roots of the tree *P. fascicularis* promising results have been interpreted. Further broad level analysis and study is required to observe the mechanism and characterizing the active compounds to draw effective conclusions.

References

Adkar, B. (2014). *Pandanus odoratissimus* (Kewda): A review on ethnopharmacology, phytochemistry, and nutritional aspects. *Pharmacoline* , 1-19.

Botanical-online. (1999-2016). Retrieved from www.botanical-online.com:

http://www.botanical-online.com/english/historyofmedicinalplants.htm

Callmander, L. I. (2012). Benstonea Callm. & Buerki (*Pandanaceae*): characterization, circumscription, and distribution of a new genus of screw-pines, with a synopsis of accepted species . *Journal international de botanique systématique*, 323-345.

Essen Bioscience . (2016). Retrieved from www.essenbioscience.com:

http://www.essenbioscience.com/applications/cytotoxicity.

Food and agriculture organization of United Nations. (2016). Retrieved from:

www.fao.org: http://www.fao.org/docrep/010/ai387e/AI387E08.htm

Ghani, A. (1990). *Present state-of- The art of traditional medicine practice in Bangladesh.*

Hamid, A. U. (2010, June 25). Antioxidants: Its medicinal and pharmacological applications. *African journal of pure and applied chemistry*, 4(8), 142-151.

Hassan, B. A. (2012). Medicinal plants (Importance and uses). *Pharmaceutica Analytica Acta, 3*(10).

Healing wonders of Philippine medicinal plants. (2015). Retrieved from:

www.filipinoherbshealingwonders.filipinovegetarianrecipe.com/pandan.htm

Jothimani Rajeswari, K. K. (2011). Phytochemical and pharmacological evaluation of prop roots of *Pandanus fascicularis* Lam. *Asian pacific journal of tropical medicine*, 649-653.

Kumari, M. N. (2008). Anticancer activity of *Pandanus fascicularis* Lam.on Ehrlich ascites carcinoma (EAC) in mice. *Biosciences, Biotechnology Research Asia*, 389-394.

L.Udupa., D. A. (2011). Analgesic activity of *Pandanus fascicularis* Lam. *Pharmacologyonline*, 837-840.

Madhavan, N. M. (2015). Antihyperglycemic activity of alcohol and aqueous extracts of *Pandanus fascicularis* lam. roots in alloxan induced diabetic rats. *Pharmacologyonline* , 529-536.

Mani, K. S. (2016). Antitumor activity of *Pandanus fascicularis* Lam. on Dalton's ascites lymphoma (DAL) in Mice. *Hamdard medicus*, 27-31.

Meyer, B. F. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *Journal of medicinal plant research*, 31-34.

Pérez, A. (2013). Chemistry of natural antioxidants and studies performed with different plants collected in Mexico. *In oxidative stress and chronic degenerative diseases - A role for antioxidants,*830-833.

Petrovska, B. B. (2012). Historical review of medicinal plant's usage . *Pharmacognosy Review*, 1-5.

Pimentel, P. C. (2002). An application of the brine shrimp bioassay for general screening of Brazilian medicinal plants. *Acta farm. Bonaerense,* 21(3), 175-8.

Rahmatullah, M. A. (2016). A survey of medicinal plants used by folk medicinal practitioners in two villages of Tangail district, Bangladesh. *American-Eurasian journal of sustainable agriculture* ,415-422.

Rajeswari, K. J. (2011). Phytochemical and pharmacological evaluation of prop roots of *Pandanus fascicularis* Lam. *Asian Pacific journal of tropical medicine*, 649-653.

Reference. (2016). Retrieved from www.reference.com: https://www.reference.com/science/phytochemical-screening-6c0234abce82bf22#

Sanjeeva, K. A. (2016). Antioxidant activity of methanol extract of *Pandanus fascicularis* Lam. *Pharmacologyonline*, 833-841.

Sarma, M. G. (2010). Free radicals and their role in different clinical conditions: An overview. *International journal of pharma sciences and research,* 1(3), 185-192.

Saxena, S. N. (2013). Phytochemistry of medicinal plants. *Journal of pharmacognosy and phytochemistry,* 1(6), 168-182.

Shalaby, S. (2013). Antioxidant compounds, assays of determination and mode of action. *African journal of pharmacy and pharmacology*, 528-539.

Shekhar, A. (2014). Antioxidant activity by DPPH radical scavenging method of ageratum conyzoides Linn. Leaves . *American journal of ethnomedicine*, 244-249.

Tiwari, P. K. (2011). Phytochemical screening and extraction: A review. *Internationale pharmaceutica sciencea,* 1(1), 98-106.

Udupa. (2012). Analgesic activity of *Pandanus fascicularis* Lam. *Pharmacologyonline*, 837-840.

Udupa AL, N. O. (2011). Analgesic activity of *Pandanus fascicularis* Lam. *Pharmacologyonline 2*, 837-840.