Phytochemical screening and study of analgesic potential of *Pandanus fascicularis* different root extract in experimental animal model

Project submitted
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This work is dedicated to my parents and siblings for their love and constant support……

Certification Statement

This is to certify that this project titled '**Phytochemical screening and study of analgesic potential of *Pandanus fascicularis* different root extract in experimental animal model**' 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 Farhana Alam Ripa, Senior Lecturer, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the foreign language, ideas or writings of another.

Signed,

_____

Countersigned by the supervisor

F.A.Ripa 20.09.16

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SaimaZaman Asha
September 2016

Abstract

The aim of this study was to investigate phytochemicalcompoundsand study of analgesic activity ofdifferent root extracts like [ethanol (EAPF), chloroform (CHPF) and ethyl acetate (EAPF)]of*Pandanusfascicularis*. For analgesic activity we have performed acetic acid induced writhing method and formalin induced pain method. Different extracts were tested for qualitative chemical analysis which reveals the presence of alkaloids, glycosides, carbohydrate and tannin.

In our present study we administered our experimented crude extracts at two doses 250 mg/kg and 500 mg/kg to evaluate the analgesic activity in rodents. Both doses of extracts significantly ($p<0.01$) inhibited writhing response induced by acetic acid and formalin. The experimental extracts have satisfactory analgesic effect compare to control group and represented by number of writhes in acetic acid induced pain method as well as number of licking time in formalin induced licking method. The EAPF extract, at 250 mg/kg dose showed 85.7 % and at 500 mg/kg showed 88.12 % inhibition of writing response compare to reference Indomethacine(58.50%) inhibition was observed. In conclusion we can say that the different root extracts of *Pandanusfascicularis* contain some active phytochemical constituents which are responsible for the observation of analgesic activity and this amply justify the traditional use of this plant as a pain reliever.

Chapter 1: Introduction

Chapter 2: Methodology

Chapter 3: Results

List of Tables

List of Figures

List of abbreviations

ETPF – Ethanol extract of *Pandanusfascicularis*

CHPF- Chloroform extract of *Pandanusfascicularis*

EAPF- Ethyl acetate extract of *Pandanusfascicularis*

ICDDRB- International Centre of Diarrheal Disease Research, Bangladesh

ml-Mililitre

mg-Miligram

kg- Kilogram

SEM- Standard Error of Mean

WHO- World Health Organization

1. Introduction

1.1 General introduction

From earlier times, humans depend on nature for his or her basic wants for the assembly of foodstuffs, shelter, clothing, suggests that of transportation, fertilizers, flavors and not least, medicines. Plants are victimization as medicines for treating diseases and ailments from the dates back to the start of human civilization. From starting of that age folks were compelled to use natural substances that they might notice to ease their stricken by acute and chronic health problem, physical discomfort, wounds and injuries and even terminal health problem. Since precedent days, plants with therapeutic properties have secured as vital place within the healing follow and treatment of diseases.

Plants are indispensable well springs of different shift of synthetic mixes. Some of these mixes having a decent fluctuate of pharmacologic exercises are either unrealistic or hard to incorporate inside the research facility. A phytochemist, WHO is revealing these assets are creating helpful materials for screening programs for medication disclosure. Development of most recent ailment additionally driving the researchers to venture out back to nature for more up to date successful atoms. As therapeutic use of plants continuing with the progress of civilization and development of human information, scientists endeavored to isolate completely different chemical constituents from plants, place them to biological and pharmacologic tests and establish therapeutically active natural compounds, that have eventually accustomed prepare fashionable medicines.

As therapeutic use of plants continued with the progress of civilization and development of human information, scientists endeavored to isolate totally different chemical constituents from plants, place them to biological and medical specialty tests and determine therapeutically active natural compounds, that have eventually wont to prepare trendy medicines. In course of time artificial analogues and derivatives of the natural compounds were additionallyready. In this way, ancient uses of asterid dicot genus plants have semiconductor diode to the isolation of hyoscinic, poisonous substance,atropine and tigloidine, tree barks to antimalarial and Quinidex,

Rauwolfia serpentina root to Sandril and rescinnamine, *Digitalis purpurea* to digitoxin and digoxin; controlled substance to morphine and analgesic, Ergot to alkaloid and ergametrine, Sena to sennosides, *Catharanthus roseus* to periwinkle plant derivative and *Velbanto saya* couple of. (Ghani, 1998)

1.1.1 Medicinal plants

Plants that possess therapeuticproperties or exert useful pharmacologic effects on the animal body are typically selected as medicinal plants. Plants that naturally synthesis and accumulate some secondary metabolites like lactones, quinones, saponines, glycosides, resine, tanin, cyanogenines, volatile oils, etc and minerals and vitamins, possess healthful properties. In step with World Health Organization (WHO) '**Amedicinal plants is any plant that is one or a lot ofof its organs contains substances that may be used for therapeutic purpose or thatareprecursors for synthesis of helpful drugs'**. (Sofowara, 1982). Restorative plants are the wealthiest bio-asset of solution of old frameworks of prescription, stylish drugs, nutraceuticals, nourishment supplements, people meds, pharmaceutical intermediates and synthetic elements for simulated medicine.Restorative plants speak to a fundamental abundance of a provincial. They assume a noteworthy part in giving essential human services and administrations to rustic people. They work fundamental restorative operators moreover as essential material for the production of old and in vogue drugs. Substantial amounts of interchange is saved and attained by industrial production of healthful plants in a very country and by mercantilism them to different country (Ghani, 1998).

1.1.2 History of the medicinal plant

Plants having medicative properties were such a material tha tindividual utilized to remain healthy and to fight against diseases right from the start of his rational living activities on this earth. In an attempt to accomplish these issues individual utilized and created use of any material that he found useful in his struggle for existence. Plants having medicative properties were such a cloth that individual utilized to remain healthy and to fight against diseases. so the history of use of medicative plants for assuaging diseases had its origin within the activities of the foremost primitive man of the remote past.

As civilizations progressed, a gaggle of individuals referred to aspriest-doctor, originated in every society United Nations agency specialized in featdata of medicative plants and their healing powers. Before the arrival of communication the valuable data of the medication men was transmitted from one generation to a different by verbal and experimental suggests that and afterward in written kind as papyri, baked clay tablets, parchments, manuscript, herbals and at last written herbals, material medica, pharmacopoeias and alternative written materials. The traditional scriptures on medicative plants of ancient city, Egypt, India, China, Greece and alternative countries of the globe bear testimony to the actual fact that medicative plants and flavoring medication were extremely revered in history.

1.1.2.1 Assyrians, Babylonians times:

As far of the records go, it seems that Babylonians were awake toan outsizedrange of medicative plants and their properties regarding 3000 before Christ. A number of the plants they used then square measure still in use in virtually constant manner and for constant functions. Historical records of Assyria and geographic area medicated that by 650 before Christ there have been regarding 250 plant medicines in use in this region (Obranwu, 1984)

1.1.2.2 Ancient Egyptian times:

The ancient Egyptians possessed a decent data of the medicative properties of many plants. Several of the current day vital plants like mandrake, physicand plenty of alternatives were in common use in Egypt regarding 4500 years past.

1.1.2.3 Ancient India:

The previous proof from the history of ancient Indian culture and medication is documented in several writingthe Vedas, specifically RgvedaSamaveda, Yajur veda and Atharva veda. the excellent Indian verba , the "Charaka Sanhita" contains 341 healthful plants. (Ali, 1994)

1.1.2.4 Ancient China:

The earliest legendary Chinese accumulation, the Pen Tsao, appeared around 1122 before Christ. Attributed to the legendry emperor Shen Nung, the authoritative work delineate the utilization of oil to treat Hansen's disease.

1.1.2.5 Greek civilization:

The apply drugs exploitation energizing plants thrived most all through the Greek development once chronicled identities like Hippocrates (born in 460 BC) and Theophrastus(born in 370 BC) Practiced flavoring medication.

Figure 1.1: Use if medicinal plant in Greek Civilization

1.1.2.6 Arabian Age:

The Arabian Muslim physicians, like Al–Raji and IbnSina (ninth to twelfth century AD), led to a revolution medicine by transfer new drugs of plants and minerals origin into general use.

Figure 1.2: Use of medicinal plants Arabian age

1.1.2.7 Ancient South American time:

The South American centuries have provided the planet with several helpful and medicative plants, full-grown naturally within their forests and planted in the healthful plant garden. Use of medicinal plants like coca and tobacco was common within these centuries in the fourteenth and fifteenth centuries.

The medicinal plants utilized by the Australian aborigines several centuries past hugely enriched the stock of medicinal plants of the planet. This list of medicinal plants growing round the world includes quite thousand item (Sofowora, 1982).

1.1.3 Importance of medicinal plants

The utilization of common item with helpful properties is as old as human development and for a broadened time, mineral, plant and creature material were the most well springs of medication (De Pasqual, 1984). Medicinal plants square measurewealthy sources of bioactive compounds and therefore function vital raw materials for drug production. They'll represent a valuable natural plus of a rustic and contribute an excellent deal to its health care systems. Plants will offer biologically active molecules and lead structure for the event of changed derivatives with increased activity and reduced toxicity. The little fraction of flowering plants that have to datebeen investigated have yielded regarding a hundred and twenty therapeutic agents of familiar structure from regardingenuity species of plants.

Anumber of medicinal plant drug include vinblastin, pilocarpine, gitoxigenin, digoxigeninxol, morphine, codein, aspirin, atropine, vincristine,allicine, taxol, ephedrine among others.The medicinal plants demonstrate their application in pharmaceuticals, sustenance industry and beauty care products industry. The utilization of the restorative herbs for curing infection has been archived in history of all developments. Around 25% of all recommended medication today is substances gotten from plants.

As of late research has bolstered natural exercises of some restorative herbs. Malignancy is such a section where specialists are expecting new atoms from herbs that can give us with apparatuses to battling this feared malady. All *Amanda cathratica* [allamandin], *Elephatop uselatus* [elephantpoin] , *Helenium autmnale* [helenalin] *Vernoniahym enlepis*, *Heliotropium indicum* [Indicine-N-oxide], *Daphne mezereum* (mezerien) and *Stereospermum suaveolans* [laphacol] are therapeutic plants that have indicated critical tumor restraining impact.

1.1.4 Medicinal plants of Bangladesh

Plants, plants parts and plant results of all portrayals, especially those with restorative properties are constantly utilized as foremost segments or elements of different conventional medicines. More than 500 wild and developed therapeutic plants of Bangladesh have so far been with specified with data on their restorative properties and employments(Yusuf et al, 1994).More than 250 of medicinal plants are now in common use in the preparation of tradition medicines in Bangladesh, which include plants like *Terminali aarjuna* for treating heart disease, *Andrographis paniculata* for the cure of fever and liver diseases, *Allium sativum* for reducing blood cholesterol, *Abruma angusta* for curing male and female disease, *Coccinea indica* for management of diabetes, *Cantella asiatica* for treating diarrhea and dysentery and many others. It is estimated that more than one thousand metric tons of medicinal plants are annually required by the industries involved in the manufacture of traditional medicine in Bangladesh.

Table 1.1: Medicinal plants of Bangladesh and their uses

Name of the plant	Scientific name and family	Parts used	Uses

Nayant 	*Catharanthusroseus*Family: Apocyanceae	Leaves	Cancer, insomnia, blood pressure, diabetes.
Shatamuli 	*Asparagus racemosus* Family: Liliseeae	Leaves	Cancer, bacterial and fungal diseases, tonic, appetizer, diabetes, jaundice.
Name of the plant	**Scientific name and family**	**Parts used**	**Uses**
Neem 	*Azadirachta indica* Family: Meliaceae	Leaves	Anathematic, dermatitis, fever, stomach disoerder, jaundice, nausea.
Tulsi 	*Ocimum sanctum* Family: Labiatae	Leaves , flower,seeds ,whole plant	Stomach disorder, stimulant, cough fever, malaria.
Ashwagandha 	*Withania somnifera* Family: Solanaceae	Roots and leaves	Piles, hemorrhages, headache, convulsion, insomnia, weakness
Ashok	*Saraca asoca* Family:Caesalpinanceac	Bark flower	Uterine, menstrual pain, disorder, diabetes.

			
Sarpagandha 	*Ranwolfia serpentin* Family: Apocynaccac	Roots	Hyper tension and insomnia

So plants have always fulfills many of essential needs of human including life-saving pharmaceutical agents.

1.2.1 Plant review

1.2.1 *Pandanus fascicularis*

Pandanus fascicularis is a plant of the family Pandanaceae. It grows in mangrove of Bangladesh. The name of the plant which is known by the local people is keora, keya. (Ghani, 2003)

Figure 1.3:*Pandanus fascicularis*

1.2.2 Scientific classification

Kingdom : Plantae

Division : Magnoliophyta

Class : Liliopsida

Order : Pandanales

Family : Pandanaceae

Genus : Pandanus

Species : *Pandanus tectorious, Pandanusod oratissimus*

1.2.3 Different name and synonym:

Pandanus fascicularis is familiar in different names at various places. They are given below-

Table 1.2: Synonyms and different names of *Pandanus fascicularis*

Botanical	*Pandanus odoratissimu s*Roxb**.**

Bengali	Keora, Keya, Ketaki
English	Umbrfella tree, Screwpine, Screw tree
French	Pandanus
Germen	Schraubenbaum, Schraubenpalme
Hindi	Kewra, Kewda, Pushpa-chamar. Keora, Panushka
Marathi	Ketaki, Kewda, Kegad
Oriya	Kia, Kiya
Urdu	Kiura, Kevera, Jambala, Jambul, Panshuka Ketaki

1.2.4. Description

Tremendously expanded bushes or little trees with various thick prop roots. Surrenders ensiform, over to 250×8 cm, caudateacuminate, thorny on the edges and midrib, lustrous green. Male inflorescence spicate, pedenculate, fragnant. Bracts straight lanceolate or lanceolate, yellowish, lower ones flagelliferous. Spikes 5-11, up to 10 cm long. Stamens numerous, racemose on stamenophores; anthers cuspidate. Female inflorescence lone, terminal, pedunculate, globose or ellipsoid. Bracts whitish yellow, lower ones leaf-like. Carpels blended in gatherings (phalanges) of 5-15; disgrace U-or V-formed. Organic product a syncarp, up to 25 cm long, orange or ruddy, phalanges turbinate, up to 8 x 4.5 cm.

Figure1.4: Roots of *Pandanus fascicularis*

1.2.5 Medicinal uses

- Roots are bitter, sweet, acrid, thermogenic, emollient, depurative, antiseptic, aphordistic, carminative, stomachic, supparative, anodyne, deodorant, urinary astringent, vulnerary, sudorific, febrifuge and tonic (Ghani, 1998).

- Useful in skin diseases, leprosy, headache, fever, colic, ulcers, wounds, diabetes, flatulence, coxalgia, sterility, threatened abortion, meno-metrorrhagia and general debility (Ghani, 1998).

- The leaves are acrid, bitter, alexerteric, aphrodistiac, depurative and somniferous. They are useful in tumors, leprosy, dysuria, syphilis, scabies, small pox, cardiac disorder and cereberal disorder due insomnia (Ghani, 1998).

- Flowers are acrid, bitter, anodyne and demulcent and are useful in pruritus, otalgia, headache, leycoderma and skin eruotions (Ghani, 1998).

1.2.6 Chemical constitution

Bracts of flowers yield an essential oil containing methyl ether of beta-phenylethylalcohol, blossoms yield an essential oil containing benzyl benzoate, benzyl salicylate, benzyl acetate, bromostyrene, guaicol, phenyl ethyl alcohol and aldehydes (CA, 1939b). the oil also contains terpinelol. (Ghani, 1998)

1.2.7 Plant part used in the study

For my study, *Pandanus fascicularis* roots extract was used for pharmacological investigation.

1.3 Related publication on *Pandanus fascicularies*

A small number of studies on this plants root, leaves, bark have done previously. The reported studies are shown below:

- Raj GG, Varghese HS, Pathan RK. Anticancer studies of Aaqueous extract of roots and leaves of *Pandanus odoratissimus* f ferreus (Y. Kimura) hatus: an invitro approach. J Tradit complement Med.2014;4(4):279-284.
- Rajeshwari J, Kesavan K, Jayakar B. Phytochemical and pharmacological evaluation of prop roots of *Pandanus fascicularis* lam. Asian pacific journal of tropical medicine 2011;649-653
- Gupta S, Ojeh N, Rajput R, Benrgal D, Rao S, S N, Rajput A. analgesic activity of *Pandanus fascicularis* lam. Pharmacologyonline. 2011; 2:837-840.

1.4 Rationale of the project

Resistance to invented and established a drug against wide number of diseases are quiet common in the evolution history of drug development. So it is very common that scientist and researchers are relentlessly working on finding new therapeutically effective compounds from plant kingdom. Though the evolution of synthetic and biologically active drugs are emerging on a great pace, significant risk of toxicity and virulence also associated with these drug products in comparison with therapeutically active compound obtained from plant kingdom. At present, an enormous number of plant metabolites are being with accomplishment used for the treatment of sort of disorders. Thus, union of pejoratively dynamic common item from plant was the prime purpose of enthusiasm for this study.This study will focus on phytochemical screening and to

evaluate analgesic activities from different extract of the roots of „*Pandanas facicularis'* due to its use in various in traditional medical treatment.

1.5 Aim of the project

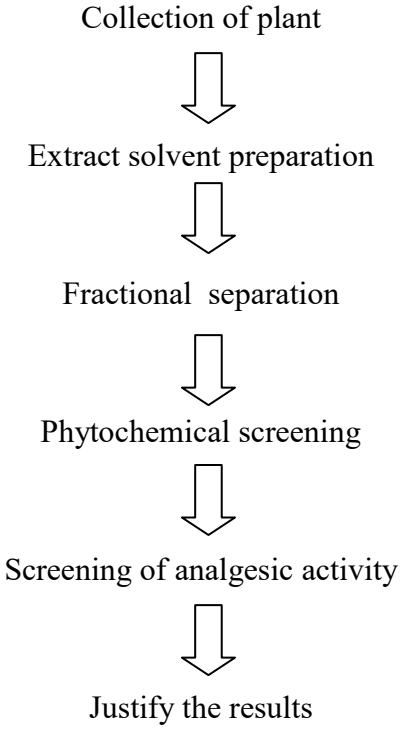
The aim of the study is the phytochemical screening and investigation of in vivo assessment of antinoceptive exercises of various concentrates of *Pandanus fascicularis* roots.

1.6 Objectives

The medicinal effect of different extract of roots of *Pandanus fascicularis* plant can be observed that is why we make our present study in such a way. The study convention comprises the accompanying strides:

- Phytochemical screening of that plant root.
- Screening of analgesic activity of crude extracts on Swiss albino mice.

1.7 Present study protocol:



2. Methods and Materials

2.1 Preparation of Plant Extract (*Pandanus fascicularis*)

2.2.1 Collection and identification

The fresh roots of *Pandanus fascicularis* was selected for pharmacological investigation. For this present investigation the roots of *Pandanus fascicularis* was collected from Shatkhira, Bangladesh on April, 2016 and then the plants were taxonomically identified at Bangladesh National Herbarium at mirpur, Dhaka, where the accession number 43172 was deposited.

2.1.2 Preparation of Plant Sample

From undesirable plant parts the roots were seperated and dried in the sun shed for seven days. Once completion of drying, dried roots were fine-grained into coarse powder by appropriate grinder machine. Powders were unbroken in clean airtight glass instrumentation for additional use. Here the grinder was properly cleanso contamination with previous ground material on alternative foreign matter will be avoided. The root powder was measured 678.56 gm. Finally the experiment until it begins placed in dry and cool space.

2.1.3 Fraction and extraction of plant

A glass container was washed legitimately thus flushed with aging alcohol in addition it was dried. In this manner dried roots powders were spot into the jug and maturation liquor was filled it up to one inch higher than the example surface. The holder was fixed with its substance and unbroken for seven days with intermittent shaking and blending. This shaking was done for satisfactory extraction.

Tweaked Kupchan parceling technique (Van Wagenen et al., 1993) was taken after for part. For that *Pandanus fascicularis* root concentrate were sifted by utilizing cleaned cotton plug took after by channel paper. Here the cotton were washed with ethanol and after that balanced in the pipe. After the extraction procedure, 700 ml concentrate was found; the cintents were vanished by turning vacuumed evaporator (RE 200 Sterling, UK) upto 1OO ml. Two measuring utensils are taken and square with measure of filtrate is taken in it. One is considered as ethanol concentrate, (ENLS) and the other one is utilized for getting different extractions.

The following a portion of extraction was taken in an isolating channel and 20 ml of N hexane was filled it. Then it was shaken for 15 min. meanwhile the plug of the carafe was opened after

few time interim so that the gas which was shaped inside the cup discharged effectively. At that point it was kept in a stand for 15 min open.Taken part was the unadulterated upper layer in a pre-measured container. The second divide in the measuring utencil was of course filled the isolating channel and 10ml of the pet ether is filled it, and shaken it for 15 minute. At that point it is permitted to remain for couple of minutes, and the same procedure of acquiring the second divide and the primary part is rehashed. This is petroleum ether extricate, (NHS). The second parcel got from the primary extraction is then permitted to be poured into the isolating pipe and 25 ml choloroform was added d to it and shaken for 15 min or more said system is taken after once more. However, here we have gathered the unadulterated lower layer as chloroform concentrate, (CHPF).

In conclusion the rest of the concentrate was blended with two fold amount of ethyl acetic acid derivation in isolating cup. At that point it was shaken for fifteen min, signifies fifteen min and got the lower unadulterated lower layer by utilizing isolating cup. This was ethyl acetic acid derivation. (EAPF)

After culmination of definite extraction every one of the concentrates were concentrated by dissipating under roof fan and water shower at 30° c to 40° c in water shower until dried. It rendered a sticky concentrate of cocoa shaded concentrate. Extract which were decided for examination was ethanol, choloroform and ethyl acetic acid derivation.

2.1.5 Drugs

Indomethacin was utilized for current study which was supplied from Square Pharmaceuticals Ltd, Bangladesh

2.2 Phytochemical analysis

Phyto" is that the Greek word for plant. There are several groups of phytochemicals and that theyfacilitate the anatomyin a verysort ofways in which. Phytochemicals mightshield human from infections. Phytochemicals are non-nutritive plant chemicals that have defensive or illnesses preventive properties.Phytochemical screening alludes to the extraction, screening and distinguishing proof of the therapeutically dynamic substances found in plants.

Table 2.1: Examples of some phytochemcals and their activities

Phytochemicals	Structural features	Example(s)	Activities
Phenols and Polyphenols	C3 side chain, - OH groups, phenol ring	Catechol, Epicatechin, Cinnamic acid	Antimicrobial, Anthelmintic, Antidiarrhoeal
Quinones	Aromatic rings, two ketone substitutions	Hypericin	Antimicrobial
Flavonoids	Hydroxylated phenols, C6-C3 unit linked to an aromatic ring	Chrysin, Quercetin, Rutin	Antidiarrhoeal
Tannins	Polymeric phenols (Mol. Wt. 500-3000)	Ellagitannin	Antimicrobial, Anthelmintic, Antidiarrhoeal
Terpenoids and essential oils	Acetate units + fatty acids, extensive branching and cyclized	Capsaicin	Antimicrobial Antidiarrhoeal
Glycosides	Sugar + non carbohydrate moiety	Amygdalin	Antidiarrhoeal
Alkaloids	Heterocyclic nitrogen compound	Berberine, Piperine, Palmetine, Tetrahydropalmetine	Antimicrobial, Anthelmintic, Antidiarrhoeal

2.2.1 Chemical Group Tests

Testing of various compound gatherings present in root concentrate of *Pandanus fascicularis* represent the preparatory phytochemical contemplates. Reagents utilized for various gathering tests are recorded in the accompanying table.

Table 2.2: Reagents used for different group test

Chemical group	Reagent	Tests
Carbohydrate	Molisch Reagent Fehling‟s Reagent	Molisch test Fehling‟s test
Tannins	10% Potassium dichromate 5% Ferric chloride 1% Lead acetate	Potassium dichromate test Ferric chloride test Lead acetate test
Saponine	Water	Forthing test
Alkaloids	Mayer‟s reagent Dragendroffs reagent Hager‟s reagent	Mayer‟s test Dragendroffs test Hager‟s test
Resin	Acetic anhydrate and sulfuric acid	General test of resin
Glycosides	Aqueous sodium hydroxide Fehling‟s reagent and Sulfuric acid	General test for glycosides Test for glycosides

2.2.2 Preparation of reagents

Preparation of reagents used for different chemical group tests reagents were prepared following standard procedure as described by Ghani A, 2005.

- **Mayer's Reagent:** 1.36 gm. mercuric iodide in 60 ml of water mixed with a solution contains 5 gm. of potassium iodide in 20 ml of water. Then 100 ml volume was adjusted .
- **Dragendroff's Reagent:** 7 gm. essential bismuth nitrate and 20 gm. tartaric corrosive were broken down in 80 ml water. This arrangement was blended with an answer contains 16 gm. Before use potassium iodide and 40 ml water and weakened 10 times with 10% picric corrosive.
- **Hager's Reagent:** A 1% solution of picric acid in water.
- **Fehling's solution A:** In a mixture of 0.50 ml of sulfuric acid and sufficient water 34.64 gm copper sulphate was dissolved to produce 500 ml.
- **Fehling's solution B:** 176 gm. of sodium potassium tartarate and 77 gm. of sodium hydroxide in adequate water to deliver 500 ml. Level with volume of above arrangement was at the season of utilization.

2.2.3 Phytochemical tests

1. Detection of alkaloids: Individually extracts were dissolved in dilute Hydrochloric acid and filtered.

(a) Mayer's Test: Filtrateswere treated with Mayer's reagent (Potassium Mercuric Iodide). Development of a yellow hued hasten shows the nearness of alkaloids.

(b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Arrangement of chestnut/rosy hasten shows the nearness of alkaloids**.**

(c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (arrangement of Potassium Bismuth Iodide). Red accelerate that shaped shows the nearness of alkaloids**.**

(d) Hager's Test: Filtrates were treated with Hager's reagent (immersed picric corrosive arrangement). The development of yellow shaded hasten affirmed the nearness of alkaloids.

2. Detection of carbohydrates: Concentrates were broken up exclusively in 5 ml refined water and separated. The filtrates were utilized to test for the nearness of carbohydrates.

(a) Molisch's Test (General test for Carbohydrates): 2ml solution of the concentrate of the plant material was taken in a test tube. Two drops of crisply arranged 10% alcoholic arrangement of α-Naphthol was given in test tube and altogether blended. 2ml of conc. Sulphuric corrosive was given to stream down the side of the slanted test tube so that the corrosive structures a layer underneath the fluid arrangement. On the off chance that a sugar is available a rosy violet ring will shaped at the intersection of the two layers. A dull purple arrangement was shaped on standing or shaking. The test tube was shaken and permitted to remain for 2 minutes, and afterward it was weakened with 5ml of water.A dull violet quicken was framed immediately which asserted the proximity of Carbohydrates.

(b) Fehling's Test: Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling‟s A & B solutions. Red precipitate that formed indicates the existence of reducing sugars.

3. Detection of tannin: 10 ml of refined water was added to 0.5 gm of powdered example in a test tube and bubbled for 3 min in a water shower. The blend was cool down and afterward separated with channel paper. This filtrate was utilized for the nearness of tannin.

(a)Ferric chloride test: 1 ml of the filtrate was weakened with 4ml of refined water and few drops of ferric chloride were included. Moment arrangement of blue-dark or green shading development demonstrate the nearness of tannins.

(b) Potassium dichromate test: 5ml arrangement of the concentrate was taken in a test tube. At that point 1ml of 10% Potassium dichromate arrangement was included. A yellow accelerate was framed demonstrate the nearness of tannins.

(c) Lead acetate test: 5ml of a watery concentrate of the plant material was taken in a test tube and included a couple drops of a 1% arrangement of lead acetic acid derivation. A yellow encourage was framed which affirmed the nearness of tannins.

4. Detectionof Resin: Utilizing sensitive warmth, a little measure of ethanolic concentrate of the plant was separated in 5 to 10 ml of acidic destructive anhydride. By then it was stand to cool and after that 0.05 ml of sulphuric destructive was incorporated. A splendid purplish shading, rapidly changing to violet shading was molded that demonstrate the closeness of pitch

5. Detection of Saponins:

(a) Froth Test: Extracts were weakened with refined water to 20ml and this was shaken in a graduated barrel for 15 minutes. Arrangement of 1 cm layer of froth shows the nearness of saponins.

(b) Foam Test: 0.5 gm of concentrate was shaken with 2 ml of water. On the off chance that froth delivered perseveres for ten minutes it shows the nearness of saponins.

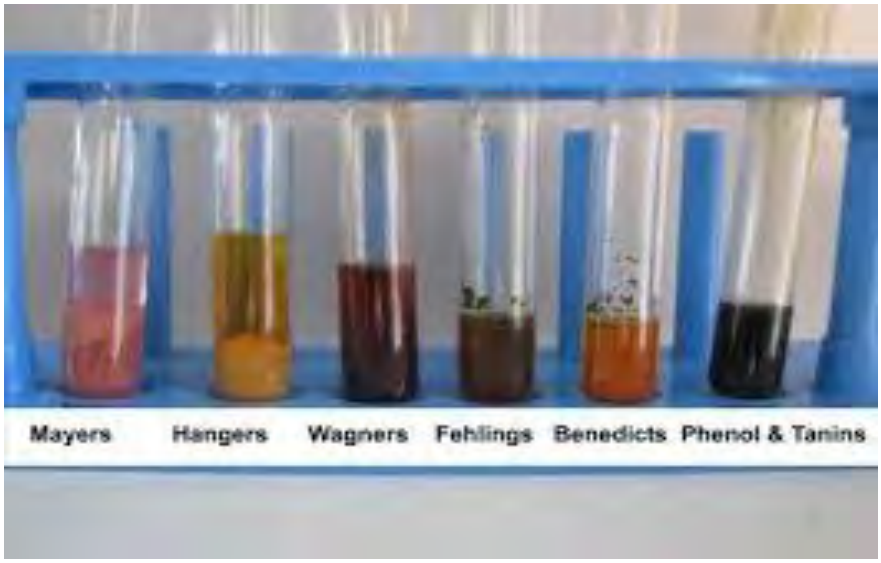


Figure 2.1: Different test results

6. Test for Glycosides (General test for glycosides): A little measure of alcoholic concentrate was broken down in1 ml of water and including a couple drops of fluid sodium hydroxide arrangement. A yellow shading creates within the sight of glycosides. Tests for Glucosides Dissolve a little measure of a drunkard concentrate of the plant material in water and liquor and overflow with Fehling's answers An and B.A yellow shading makes inside seeing glucosides. Separate another section of the gather in water and alcohol, flood with a few drops of debilitate

sulphuric destructive, murder with sodium hydroxide course of action and flood with Fehling's answer An and B. A square red rush was confined which demonstrated the proximity of glucosides.

Figure 2.2: Test result of glycosides

2.1.7 Experimental animal

For the pharmacological investigation we tend to used Swiss albino mice that were collected from animal laboratory of Jahangirnagar University and ICDDRB. Bangladesh normal weights of the mice were twenty eight to thirty three gm. The ecological situation was kept up to continue through to the end. The condition was 55-65% proportion, twelve hours light/dim cycle and twenty four. 0±0ºC temperature. Additionally adequate quantity of nourishment and water was provided all in time.

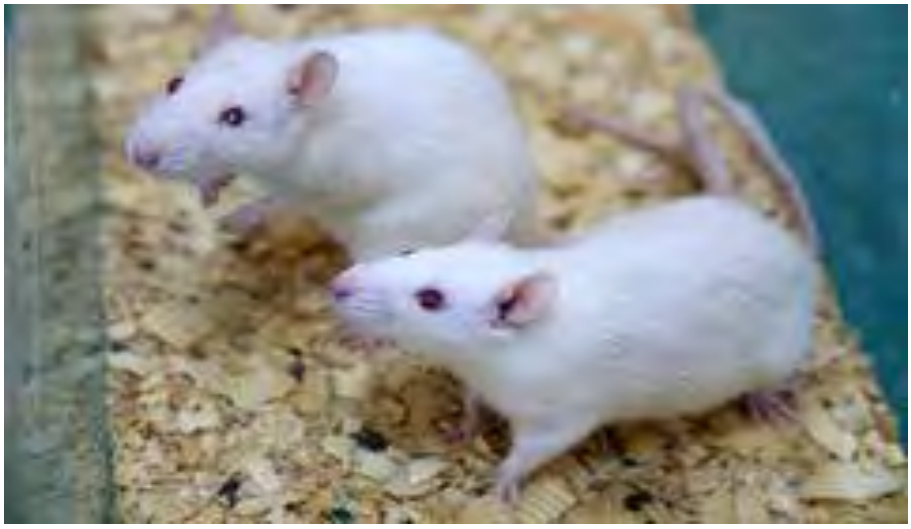


Figure 2.3: Swiss albino mice

2.1.8 Ethical approval

Institutional creature moral board of trustees acknowledged the rules which were taken after for creature test (Zimmermann, 1983).

2.2 Pharmacological investigation of plant extract

The following pharmacological investigation wasdone to determine the medicinal effect of the experimental extracts:

- Analgesic activity

2.2.1 Analgesic activity of *Pandanus fascicularis* plant extract

Absence of pain these medications are otherwise called torment killers.The pain relieving test was done two by taking after two techniques. These two techniques are:-

1. Acetic acid induced writhing technique.
2. Formalin induced pain technique.

2.2.1.1 Design of the analgesic experiments

Forty eight mice were picked and separated into 8 bunches where the gatherings were G1 to G8 in which 6 mice were in every gathering. Every gathering got a specific treatment. Prior to the treatment, every mouse were weighted appropriately and stamped. At that point the dose of the test and control materials was likewise settled by weight.

Group- G1- 1% Tween 80 in water

Group- G2- Indomethacin (standard)

Group- G3- ETPF 250 mg/kg

Group- G4- ETPF 500 mg/kg

Group- G5- CHPF 250 mg/kg

Group- G6- CHPF 500 mg/kg

Group- G7- EAPF 250 mg/kg

Group- G8- EAPF 500 mg/kg

2.2.1.2 Acetic acid-induced writhing technique:

Acidic corrosive prompted writhing check is a substance technique used to actuate torment of fringe source by infusion of punishment standards like phenylquinone, ethanoic corrosive in mice. Amid this methodology (Ahmed et al., 2001) intra-peritoneallyethanoic corrosive was managed to the mice all together that agony sensation produces. Here, indomethacin was considered as standard. Initially traditional saline, extricates at a measurement of 250 mg/kg and 500 mg/kg comparatively as standard medication were managed orally. After organization of determination of ethanoic corrosive, no writhing (turning) was meant five minutes. Along these lines five minutes, moving was meant quarter-hour.

2.2.1.3 Reagents, Chemicals and Equipments:

Table 2.3: Reagents, chemicals and types of gear use for acidic corrosive initiated pain relieving test

Reagents, Chemicals and Equipments	Source
Acetic acid	Merck, Germany
Indomethacin	Square Pharmaceuticals Ltd.
Tween 80 (as suspending agent)	BDH Chemicals Ltd.
Normal saline solution (0.9% NaCl)	Beximco Infusion Ltd.
Sterile disposable syringe (1ml, 100 divisions)	CHPL, India
Tuberculin syringe with ball shaped end	Merck, Germany
Electronic and digital balance	Denever Instrument M-220/USA

2.2.1.4 Preparation of drug and chemical solution

For the principal presentation of standard that is indomethacin (standard) at a dose of ten mg/kg, specific measure of this was suited. By then it totally was separated in zero. Saline water was 9%. By then at all measure of zero.5 ml, every mouse was managed standard orally.

Harsh assemble was set up at an estimations of 250 mg/kg and 500 mg/kg unsurprising with weight method for mice. For that, doses were measured so suspending administrator tween 80 was added to every arranging consequently mixed. After definite blend of those two, saline water was more additional and last volume was conjointly made. Furthermore, a short time later 0.5 ml was directed to every mouse.

Table 2.4: Test samples used in the evaluation of analgesic activity by acetic acid induced writhing method of *Pandanus fascicularis*.

Group	Medication	Dose	Route of administration
Group-G1 (Control)	1% tween 80 in water	0.1 ml/10gm body weight	Orally
Group-G2 (Standard)	Indomethacin	10 mg/kg	Orally
Group- G3 (Extract)	ETPF	250 mg/kg	Orally
Group-G4 (Extract)	ETPF	500 mg/kg	Orally
Group-G5 (Extract)	CHPF	250 mg/kg	Orally
Group-G6 (Extract)	CHPF	500 mg/kg	Orally
Group-G7 (Extract)	EAPF	250 mg/kg	Orally
Group-G8 (Extract)	EAPF	500 mg/kg	Orally

2.2.1.5. Procedure of analgesic activity of *Pandanus fascicularis* extract by acetic acid induced writhing (twisting) technique.

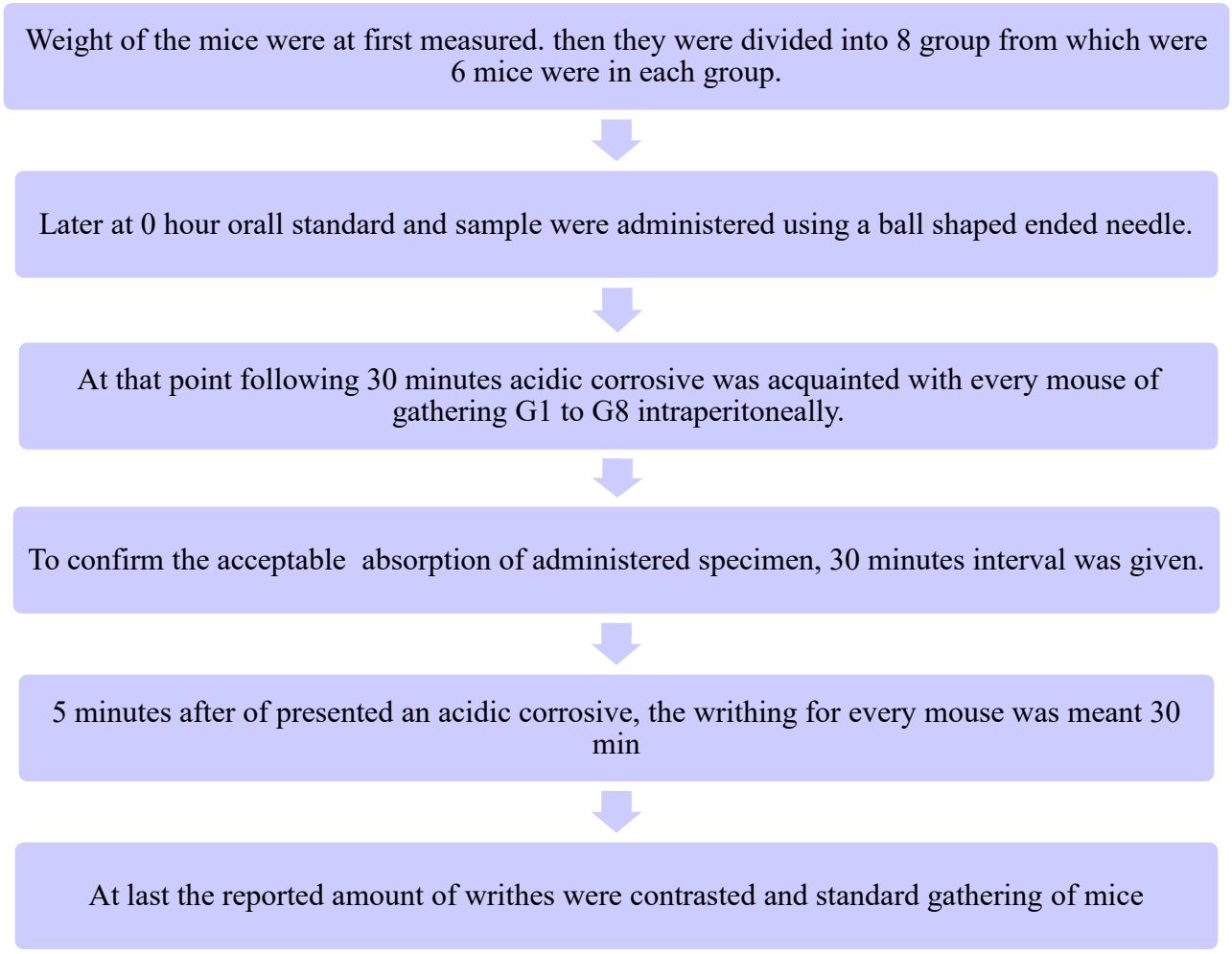


Figure 2.4: Flow chart of process for analgesic activity on mice by acetic acid induced technique (Ahmed et al., 2001)

2.2.2.1 Formalin induced pain method

Formalin test is another system (Sharma et al., 2010) by strategy pain relieving movement served. Amid this case, formalin infusion is prompted to mice's correct paw. Thus biophasic torment is made. For standard, indomethacin (NSAID) was picked that has torment sensation hindrance administration. Then the standard was contrasted and test tests and control.

2.2.2.2 Reagents, Chemicals and Equipments:

Table 2.5: Reagents, Chemicals and Equipments used for formalin induced analgesic test

Reagents Chemicals and Equipments	Source
Indomethacin	Square Pharmaceuticals Ltd.
Tween 80 (as suspending agent)	BDH Chemicals Ltd.
Normal saline solution (0.9% NaCl)	Beximco Infusion Ltd.
Formalin	Merck, Germany
Sterile disposable syringe (1ml, 100 division)	CHPL, India
Tuberculin syringe with ball shaped end	Merck, Germany
Electronic and digital balance	Denver Instrument M-220/USA

2.2.2.3 Preparation of drug and chemical solution

At first needed quantity of indomethacin (NSAID, standard) was weighed with ten mg/kg measurements and soready by dissolving with saline water. At that time zero. 5 ml in amount was managed to each mouse orally.

To make 250 mg/kg and 500 mg/kg dosage of rough concentrate, they were measured and extra with 2 drops of tween 80. Once good and correct mixing, ordinary saline was included gradually. At last 0.5 milliliter of planning was regulated orally to each mouse.

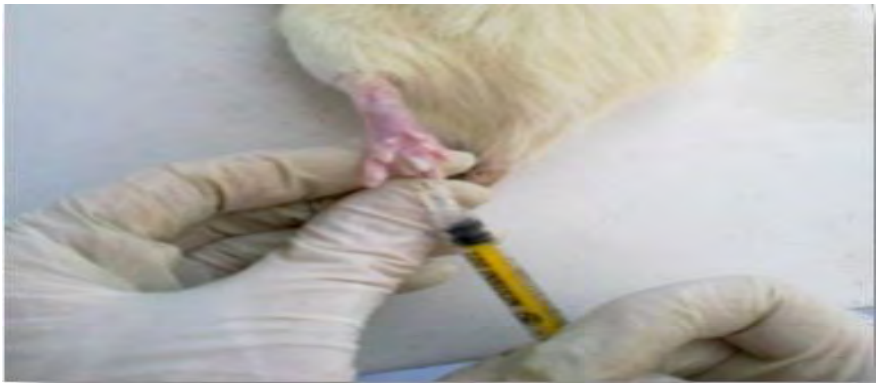


Figure 2.5: Injected in the mice's paw.

Table 2.6: Test samples used in the evaluation of analgesic activity of the extracts by formalin induced method

Group	Treatment	Dose	Route of administration
Group-G1 (Control)	1% Tween 80 in water	0.1 ml/10gm body weight	Orally
Group-G2 (Standard)	Indomethacin	10 mg/kg body Weight	Orally
Group- G3 (Extract)	ETPF	250 mg/kg	Orally
Group-G4 (Extract)	ETPF	500 mg/kg	Orally
Group-G5 (Extract)	CHPF	250 mg/kg	Orally
Group-G6 (Extract)	CHPF	500 mg/kg	Orally
Group-G7 (Extract)	EAPF	250 mg/kg	Orally
Group-G8 (Extract)	EAPF	500 mg/kg	Orally

2.2.2.4. Process of analgesic activity of *Pandanus fascicularis* by formalin test:

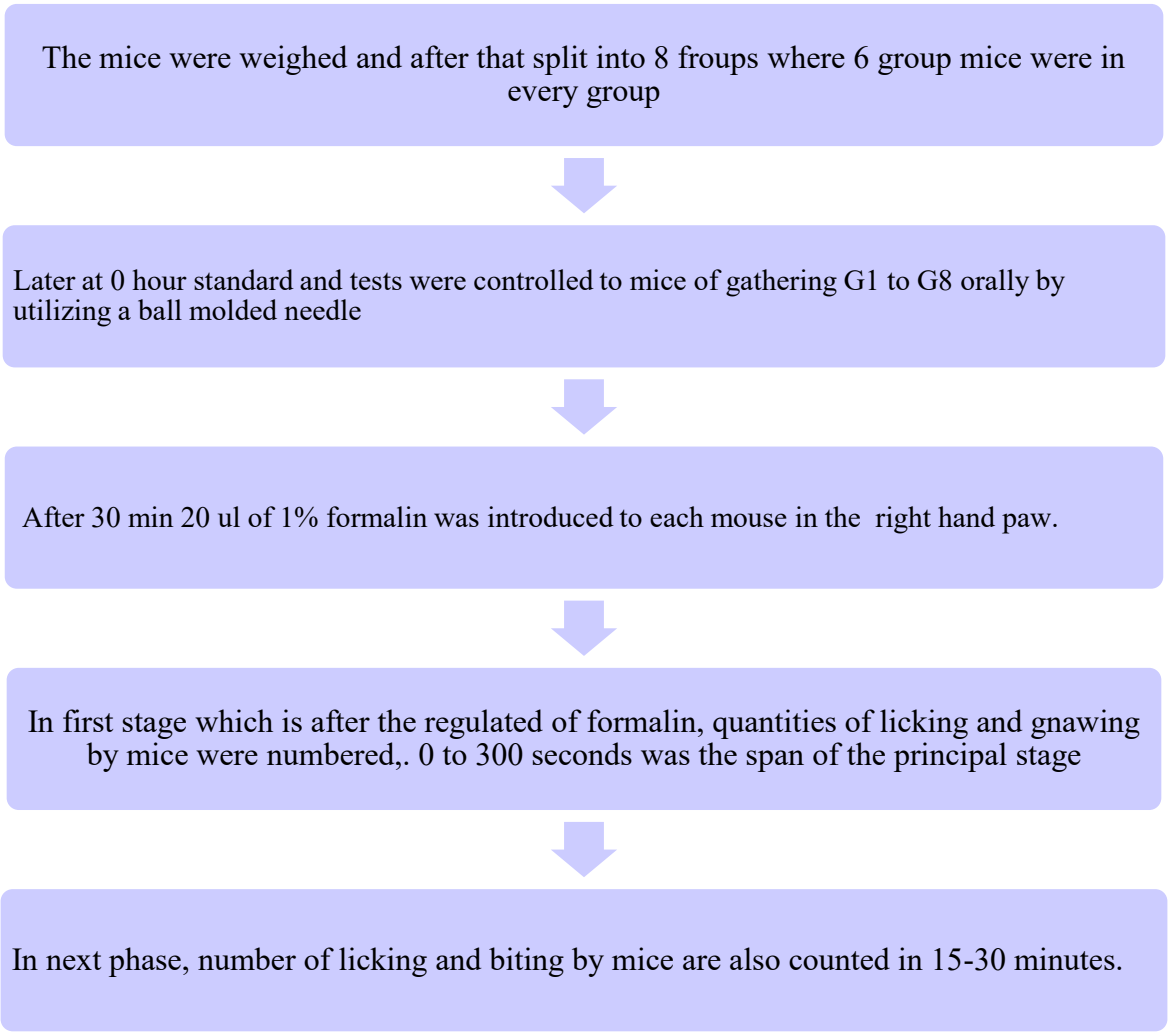


Figure 2.6: Flow chart of process for analgesic activity on mice by formalin test (Sharma et al., 2010)

2.2.2.5 Counting of licking and biting of paws

Inducing of formalin mice bite and lick the wounded space of paw and this made up our minds by facilitate of stopwatch.

Figure 2.7: Mice licking its paw

2.3 Statistical analysis

All out qualities that were controlled from the analyses are described as mean ± standard mistake of the mean (SEM). Measurably acquired information was measurable by using ANOVA (Analysis of difference) and post-hoc Dunnett's check that was connected with SPSS program (SPSS 16.0, USA).

3. Results

3.1Results of phytochemical screening of root extract

Table 3.1: Showing results of phytochemical screening of the different root extract of *Pandanus fascicularis*

Chemical group	Tests	Reagents	Results
Alkaloids	Dragendroff‟s test	ETPF	++
		CHPF	+
		EAPF	+
Alkaloids	Mayer‟s test	ETPF	++
		CHPF	+
		EAPF	+
Alkaloids	Wagners‟s test	ETPF	++
		CHPF	+
		EAPF	+
Alkaloids	Hager‟s test	ETPF	+
		CHPF	+
		EAPF	+
Glycosides	General glycoside test	ETPF	++
		CHPF	+
		EAPF	+
Carbohydrate	Molish test	ETPF	++
		CHPF	+
		EAPF	+

Table 3.1: Showing results of phytochemical screening of the different root extract of *Pandanus fascicularis* continued

Chemical group	Tests	Extract	Results
Carbohydrate		CHPF	+
		EAPF	+
Resin	General resin test	ETPF	−
		CHPF	−
		EAPF	−
Tannin	Ferric test	ETPF	++
		CHPF	+
		EAPF	+
Tannin	Potassium test	ETPF	++
		CHPF	+
		EAPF	+
Saponine	Foam test	ETPF	−
		CHPF	−
		EAPF	−−
Saponine	Forth test	ETPF	−
		CHPF	−−
		EAPF	−

From the above table we can see that there is presence of alkaloids, glycosides and tannins in the plant root extract. But the saponine and resin were not present.

3.2 Results of Analgesic activity of plant extract on mice

For the trial of pain relieving action, two strategies were taken after. They were acidic corrosive instigated and writhing strategy and formalin initiated torment technique.

3.2.1 Acetic acid induced writhing test (peripheral pain)

In this examination, the analgesic effects of plant *Pandanus fascicularis* was researched by controlling 250 mg/kg and 500 mg/kg estimations to occur. By applying this test it totally was seen that there was enormous delayed consequence of plant concentrate stand out from controlled pharmaceutical (Indomethacin). Among the examples of harsh concentrate, group G8 showed best results appear differently in relation to the standard that was ethanol (ETPF) 500 mg/kg in estimation depending way**.**

Table 3.2.1: Data of Analgesic activity test of *Pandanus fascicularis* plant root extract by acetic acid induced writhing method.

Goups	Treatment	Dose (unit)	No. of writhing	Percent inhibition %
Group-G1(Control)	1% Tween 80 in water	0.1 ml/10gm body weight	24.5 ± 1.20416*	---
Group-G2 (Standard)	Indomethacin	10mg/kg body Weight	10.167± .79232*	58.50
Group-G3 (Extract)	ETPF	250mg/kg	4.66 ± .24721*	80.97
Group-G4 (Extract)	ETPF	500mg/kg	3.16 ± .21082*	87.10
Group-G5 (Extract)	CHPF	250mg/kg	5.33 ± .42164*	78.24
Group-G6 (Extract)	CHPF	500mg/kg	4.5 ± .15366*	81.63
Group-G7 (Extract)	EAPF	250mg/kg	3.5 ± .51640*	85.71
Group- G8 (Extract)	EAPF	500mg/kg	2.91±.37454*	88.12

*All values are expressed as mean ± STD (n=6); One-way Analysis of Variance (ANOVA) followed by Dunnet"s test. P<0.01 significant compared to control

3.1.2 Formalin induced pain method

The pain relieving movement by agony strategy by formalin evoked torment technique determined by numeration paws licking and gnawing occasions. The root concentrates of the trial plant at a dosage of 250 mg/kg and 500 mg/kg stop the licking and gnawing action of mice amid measurements depending on the way. These occasions are given inside the accompanying table no 3.2.2.

Table 3.2.2: Analgesic activity of extracts ETPF, CHPF and EAPF in the mice by formalin induced pain method

Groups	Treatment	Dose, route	Early phase (Sec)	Late phase (Sec)
Group-I (Control)	Distilled water	10 ml/kg	25.17 ± .70317*	42.5 ± .76376*
Group-II (Standard)	Indomethacin	10 mg/kg	12.83 ± .30732*	24 ± .51640*
Group-III A (Extract)	ETPF	250 mg/kg	4.33 ± .42164*	10.66 ± .33333*
Group-IV B (Extract)	ETPF	500mg/kg	4.83 ± .30732*	7.16 ± .60093*
Group-VA (Extract)	CHPF	250 mg/kg	9.16 ± .47726*	6.33 ± .49441*
Group-VIB (Extract)	CHPF	500mg/kg	8.33 ± .33333*	7.16 ± .47726*
Group-VII A (Extract)	EAPF	250 mg/kg	5.5 ± .42817*	10.83 ± .70317*
Group-VIII A (Extract)	EAPF	500mg/kg	4.66 ± .33333*	5.16 ± 1.13774*

*All values are expressed as mean ± STD (n=6); One-way Analysis of Variance (ANOVA) followed by Dunnet"s test. P<0.01 significant compared to control

3. Discussion

The advanced relationship between pain and injury turns the perception of pain in an important analysis issue. It is progressively evident that his becomes a transmission of pain to the brain is below numerous psychological control. This becomes a tough challenge within the discovery of forms and compounds capable of inhibiting the pain feeling while not side effects. Most of the drugs that are used for depression have an effect on the standard lifetime of diseased person. Oppositely herbal medicines have less toxicity, sensible absorption and have a lower side effect profile. That is why it has been used since terribly previous time (Li et al., 2003). And currently to keep pace with the growing world have to be compelled to discover a lot of new compounds from the plants to find new drugs with less side effects.

Introductory, phytochemical screening of plant root extract was done where tannin, glycosides, alkaloids, resin were noticed. From other journals it has been seen that plants with these types of constituents shows medicinal effect. Each phytochemical compounds have their own and different medicinal activities. Therefore there is a possibility that *Pandanus fascicularis* may contain medicinal effect which may help to cure some diseases. That is why two types of experiments have done to determine the analgesic activity of this plant.

The analgesic activity of the root extracts of ETPF, CHPF & EAPF of the roots of *Pandanas fascicularis,* was investigated for its peripheral pharmacological action using both formalin induced pain method and acetic acid induced writhing method in mice. Here the percentages of inhibition for acetic acid induced pain method in respect to standard Indomethacin showed significant effect. The percent inhibitions for lower dose were 80.97%, 78.24% and 85.71% for ETPF, CHPF and EAPF respectively. Again the percent inhibitions for the higher dose were 87.10%, 81.63% and 88.12% for ETPF, CHPF and EAPF respectively. Whereas in case of formalin induced pain method after the administration of same doses significantly ($p<0.01$) suppression of licking and biting activity in early phase in 250 mg/kg 10.66%, 6.33% and 10.83% for ETPF, CHPF and EAPF respectively and in 500 mg/kg dose were 7.16%, 7.16 and 5.16% in pharmaceutical field for controlling pain ETPF, CHPF and EAPF respectively were

observed and showed significant ($p<0.01$) analgesic activity compared to standard Indomethacin (24 percent).

The phytochemical ingredients that we found in different root extracts of this plant like, tannin and alkaloids could contribute to the analgesic action of the plant. The results therefore suggest that *Pandanus fascicularis* different root extracts could become promising natural analgesic agents with potential applications.

5. Conclusion

Medicative plants assume a huge part in turning away various illnesses. The antidiuretic, mitigating, pain relieving, anticancer, hostile to viral, against malarial, hostile to bacterial and hostile to contagious exercises of the medicative plants are owing to the nearness of the above mentioned secondary metabolites. Therapeutic plants are utilized for finding and screening of the phytochemical constituent that square measure terribly useful for the producing of latest medicine. The phytochemical investigation of restorative plants additionally are necessary and have modern interest in each analysis institutes and pharmaceuticals partnerships for the manufacturing of the new medications for treatment of different ailments. Through examination we have a tendency to come to comprehend that the *Pandanus fascicularis* extracts contains verities of phytochemical constituents. Once more different concentrates of *Pandanus fascicularis* was set up a characteristic safe solution for the treatment of algesia (agony or offensive sensation). After observing the aftereffects of late study, it is a fore mentioned that ETPF, CHPF and EAPF concentrates of our exploratory plant roots at measurements 250 and 500 mg/kg indicated noteworthy ($P<0.01$) pain relieving movement contrast with control compound.

From this experiment it can be declared that, *Pandanus fascicularis* different root extracts have organic activity and constituents. But, as we have conducted our studies with crude extracts we still do not know which particular extracts shows potency specifically. So, in future our concern is to conduct further comprehensive investigation as well as illustration of active components and necessitates assumption studies for development of the potential dosage form.

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