Study of Pharmacological activities of methanolic extract of *Jatropha gossypifolia* fruits

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A thesis report, submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements of the degree of Pharmacy

RESEARCH REPORT ON

Study of Pharmacological activities of methanolic extract of *Jatropha gossypifolia* fruits

DECLARATION BY THE RESEARCH CANDIDATE

I, Md. Faruq Hossain, hereby declare that the dissertation entitled 'Study of Pharmacological activities of methanolic extract of *Jatropha gossypifolia* fruits*'*, submitted by me to the Department of Pharmacy, East West University , in the partial fulfillment of the requirement for the award of the degree of Bachelor of Pharmacy (Honors) is a legitimate record of original research work carried out by me under the supervision and guidance of **Mr. Apurba Sarker Apu**, Senior Lecturer, Dept. of Pharmacy, East West University. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any Degree or Diploma of Fellowship.

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CERTIFICATE BY THE INVIGILATOR

This is to certify that the dissertation, entitled 'Study of Pharmacological activities of methanolic extract of *Jatropha gossypifolia* fruits*'*, is a legitimate research work done by **Md. Faruq Hossain**, ID: 2008-3-70-078 in partial fulfillment of the requirement for the Degree of Bachelor of Pharmacy.

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ENDORSEMENT BY THE CHIRPERSON

This is to certify that the dissertation, entitled 'Study of Pharmacological activities of methanolic extract of *Jatropha gossypifolia* fruits*'*, is a legitimate research work done by **Md. Faruq Hossain**, ID: 2008-3-70-078 under the guidance of **Mr. Apurba Sarker Apu**, Senior Lecturer, Department of Pharmacy, East West University, Dhaka.

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ABSTRACT

Purpose: The research work was carried out to determine the pharmacological activities of methanolic extract of *Jatropha gossypifolia* fruits.

Method: Methanolic fruit extract was administered orally to the animal model (*Swiss albino*) and the effects were determined by comparing with respect to control group which were treated with 1% Tween 80 in saline. For every experiment positive control was used. Different experiments were used to determine the pharmacological profile which was collected from internationally published publications and journals.

Result: Oral administration of fruit extract of *J. gossypifolia* was found to decrease the writhing response which was very highly significant ($p<0.001$). In brine shrimp lethality bioassay the lowest LC_{50} values was found 2.84 μg/ml. A very highly significant ($p<0.001$) antidiarrheal activity was found in the sample. The percentage of protection of the diarrheal stool by the extract at the doses 200 mg/kg body weight was 61.11%. In hole cross test, two data were very highly significant ($p<0.001$) and three were highly significant ($p<0.01$). Three noticeable data were observed in hole board test which were very highly significant ($p<0.001$). A significant ($p<0.5$) difference was found in grooming and time spent in open arm in elevated plus maze test compared to control.

Conclusion: After summarize all the results it can say that fruit of *Jatropha gossypifolia* may have several pharmacological activities but to prove the hypothesis it need further higher studies.

Keywords: *Jatropha gossypifolia*, analgesic effect, antidiarrheal effect, cytotoxic effect, neuropharmacological effect.

Chapter 1

Introduction

INTRODUCTION

1.1 Medicinal Plants

Medicinal plants are various plants thought by some to have medicinal properties, but few plants or their phytochemical constituents have been proven by rigorous science or approved by regulatory agencies such as the United States Food and Drug administration or European Food Safety Authority to have medicinal effects (Newman DJ *et al.,* 2003).

World Health Organization (WHO) has provided a definition of medicinal plants, that is "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for synthesis of useful drug" (Sofowora, 1982). World Health Organization (WHO) reported that 80% of the world's population depends on medicinal plants for their primary health care. In the Plant Kingdom, Medicinal plants form the largest single grouping of plants. It is estimated that 30,000 species worldwide fall in this group, of which around 33% are trees.

Plants are known to be the source of many chemical compounds. Medicinal plants were used by people of ancient cultures without knowledge of their active ingredients. The common practice of taking crude extract orally is laden with hazards as the extracts may contain some toxic constituents. There is an ever increasing need to limit toxic clinical drugs. In modern times, the active ingredients and curative actions of medicinal plants were first investigated through the use of European Scientific methods (Herborn, 1998). The most important ingredients present in plant communities turn out to be alkaloids, terpenoids, steriods, phenols glycosides and tannins (Abayomi, 1993). The information obtained from extracts of medicinal plants makes pharmacological studies possible. The mode of action of plants producing therapeutic effects can also be better investigated if the active ingredients are characterized.

Infectious diseases are the leading cause of death worldwide. The clinical efficiency of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens (Bandow *et al*., 2003). Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents and resistance to old and newly produced drug is on the rise. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio, 1996; Scazzocchio *et al.*, 2001). There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants (El-seedi *et al.*, 2002).

Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health care since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play a vital role in modern drug development in the pharmaceutical industry.

1.1.1 History of plants in medicine

The earliest known medical document is a 4000-year-old Sumerian clay tablet that recorded plant remedies for various illnesses. The ancient Egyptian Ebers papyrus from 3500 year ago lists hundreds of remedies. The Pun-tsao contains thousands of herbal cures attributed to Shen-nung, China's legendary emperor who lived 4500 years ago. In India, herbal medicine dates back several thousand years to the Rig-Veda, the collection of Hindu sacred verses. The Badianus Manuscript is an illustrated document that reports the traditional medical knowledge of the Aztecs.

Western medicine can be traced back to the Greek physician Hippocrates, who believed that disease had natural causes and used various herbal remedies in his treatments. Early Roman

writings also influenced the development of western medicine, especially the works of Dioscorides, who compiled information on more than 600 species of plants with medicinal value in De Materia Medica. Many of the herbal remedies used by the Greeks and Romans were effective treatments that have become incorporated into modern medicine (e.g., willow bark tea, the precursor to aspirin). Dioscorides' work remained the standard medical reference in most of Europe for the next 1500 years.

The beginning of the Renaissance saw a revival of herbalism in the identification of medicinally useful plants. This coupled with the invention of the printing press in 1450 ushered in the Age of Herbals. Many of the herbals were richly illustrated; all of them focused on the medicinal uses of plants, but also included much misinformation and superstition. The Doctrine of Signatures, for example, held that the medicinal use of plants could be ascertained by recognizing features of the plant that corresponded to human anatomy. For example, the red juice of bloodwort suggests that it should be used for blood disorders; the lobed appearance of liverworts suggests that it should be used to treat liver complaints; the "humanoid" form of mandrake root suggests that is should be used to promote male virility and ensure conception.

Many of the remedies employed by the herbalists provided effective treatments. Studies of foxglove for the treatment of dropsy (congestive heart failure) set the standard for pharmaceutical chemistry. In the 19th century, scientists began purifying the active extracts from medicinal plants (e.g. the isolation of morphine from the opium poppy). Advances in the field of pharmacology led to the formulation of the first purely synthetic drugs based on natural products in the middle of the 19th century. In 1839, for example, salicylic acid was identified as the active ingredient in a number of plants known for their pain-relieving qualities; salicylic acid was synthesized in 1853, eventually leading to the development of aspirin. It is estimated that 25% of prescriptions written in the U.S. contain plant-derived

ingredients (close to 50% if fungal products are included); an even greater percentage are based on semi synthetic or wholly synthetic ingredients originally isolated from plants. While Western medicine strayed away from herbalism, 75% to 90% of the rural population of the rest world still relies on herbal medicine as their only health care. In many village marketplaces, medicinal herbs are sold alongside vegetables and other wares. The People's Republic of China is the leading country for incorporating traditional herbal medicine into a modern health care system; the result is a blend of herbal medicine, acupuncture, and Western medicine. Plantations exist in China for the cultivation of medicinal plants, and thousands of species are thus available for the Chinese herbalist; prescriptions are filled with measured amounts of specific herbs rather than with pills or ointments. In India, traditional systems have remained quite separate from Western medicine. In addition to Ayurvedic medicine, which has a Hindu origin, Unani medicine, with its Muslim and Greek roots, is another widely practiced herbal tradition in India. The renewed interest in medicinal plants has focused on herbal cures among indigenous populations around the world, especially those in the tropical rain forests. It is hoped that these investigations will add new medicinal plants to the world's pharmacopoeia before they are lost forever. In addition to the destruction of the forests, the erosion of tribal cultures is also a threat to herbal practices (Levetin and Mahon M, 2003).

1.1.2 Traditional medicine

Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine. Traditional preparation comprises medicinal plants, minerals and organic matters etc. Herbal drug constitutes only those traditional medicines that primarily use medicinal plant preparations for therapy. The ancient record is evidencing their use by Indian, Chinese, Egyptian, Greek, Roman and Syrian dates back to about 5000 years.

About 500 plants with medicinal use are mentioned in ancient texts and around 800 plants have been used in indigenous systems of medicine. Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments (Chopra *et al.*, 1956), which also forms a rich source of knowledge. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments (Rabe and Staden, 1997). In India around 20,000 medicinal plant species have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases (Kamboj, 2000).Currently 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects. Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources.

The use of traditional medicine has increased in developed countries also, mainly due to the failure of modern medicine to provide effective treatment for chronic diseases and emergence of multi-drug resistant bacteria and parasites. The adverse effects of chemical drugs, questioning of the approaches and assumptions of allopathic medicine, their increasing costs and greater public access to information on traditional medicine has also led to an increase in interest in alternative treatments (WHO, 2002). Plant extracts have become a source of hope as a wide group of medicinal plant preparations are available that have been used over the centuries almost exclusively on the basis of empirical evidence. Hence, it has become necessary to revisit the importance of these herbal medicines. Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses. Traditional medicine that has been

adopted by other populations (outside its indigenous culture) is often termed alternative or complementary medicine. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants or other plant materials as active ingredients.

1.1.3 Population using traditional medicine

In some Asian and African countries, 80% of the population depends on traditional medicine for primary health care. In many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine (e.g. acupuncture). Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international marketplace. Annual revenues in Western Europe reached US$ 5 billion in 2003-2004. In China, sales of products totaled US$ 14 billion in 2005. Herbal medicine revenue in Brazil was US$ 160 million in 2007 (Chaudhary SA *et al*., 2010).

1.1.4 Modern medicine from medicinal plants

Medicinal plants play a vital role for the development of new drugs. During 1950-1970 approximately 100 plants based new drugs were introduced in the USA drug market including deserpidine, reseinnamine, reserpine, vinblastine and vincristine which are derived from higher plants. From 1971 to 1990 new drugs such as ectoposide, E-guggulsterone, teniposide, nabilone, plaunotol, Z-guggulsterone, lectinan, artemisinin and ginkgolides appeared all over the world. 2% of drugs were introduced from 1991 to 1995 including paciltaxel, toptecan, gomishin, irinotecan etc. Plant based drugs provide outstanding contribution to modern therapeutics; for example: serpentine isolated from the root of Indian plant *Rauwolfia serpentina* in 1953, was a revolutionary event in the treatment of hypertension and lowering of blood pressure. Vinblastine isolated from the *Catharanthus rosesus* (Farnsworth and Blowster, 1967) is used for the treatment of Hodgkins, choriocarcinoma, non-hodgkins lymphomas, leukemia in children, testicular and neck cancer.

Vincristine is recommended for acute lymphocytic leukemia in childhood advanced stages of hodgkins, lymophosarcoma, small cell lung, cervical and breastcancer (Farnsworth and Bingel, 1977). Phophyllotoxin is a constituent of Phodophyllum emodi currently used against testicular, small cell lung cancer and lymphomas. Indian indigenous tree of *Nothapodytes nimmoniana* (Mappia foetida) are mostly used in Japan for the treatment of cervical cancer. Plant derived drugs are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer. Medicinal plants play an important role in the development of potent therapeutic agents. Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine. More than 64 plants have been found to possess significant antibacterial properties; and more than 24 plants have been found to possess antidiabetic properties, antimicrobial studies of plants (Samy P and Ignacimuthu, 1998; Samy P *et al*, 2006), plant for antiodotes activity- *Daboia russellii* and *Naja kaouthia* venom neutralization by lupeol acetate isolated from the root extract of Indian sarsaparilla *Hemidesmus indicus* R.Br (Chatterjee *et al.*, 2006). Which effectively neutralized *Daboia russellii* venom induced pathophysiological changes (Alam *et al*., 1994).

Before the introduction of modern medicines, disease treatment was entirely managed by herbal remedies. It is estimated that about 80% of the world population residing in the vast rural areas of the developing and under developed countries still rely mainly on medicinal plants. Studies reveal that there are more traditional medicine providers than the allopathic providers especially in the rural areas (WHO 2002).

Increasing interest by multinational pharmaceutical companies and domestic manufacturers of herbal-based medicines is contributing to a significant economic growth of the global medicinal plants sector. However, a large proportion of medicinal plant research is focused on nutraceuticals, chronic and metabolic disorders (diabetes, cardiovascular, etc.) and other

diseases like HIV/AIDS, malaria, etc. Whereas, the common diseases of resource poor communities such as diarrhoeal diseases and acute respiratory tract infections (ARI) are often not addressed.

Moreover, unlike the rural communities who use fresh/dried plant material or their crude extracts, the industry lays importance on isolation of active principles or standardized fractions since crude extracts are not patentable. However, it is often seen that a crude extract is more active compared to the isolated active fractions e.g. *Cirriformia tentaculata* loses its activity upon fractionation with hexane (Kicklighter *et al*., 2003). It is generally believed that standardization of the plant material is not required when used by the rural communities for their primary health care. But, regardless of whether the medicinal plant is to be used by local communities or by industry, a systematic approach is required for a plant identified from traditional medicine, as is done in modern medicine. It is necessary to focus on all aspects of medicinal plant research: from cultivation, ethno-pharmacology, utilization, isolation and identification of active constituents to efficacy evaluation, pharmacology, safety, standardization, formulation and clinical evaluation. Artuso (1997) has outlined the entire process which includes formulating an appropriate strategy and he estimates that the entire process would take more than 10–20 years. This approach is very demanding since there is an estimated 250,000 species of higher plants present on this earth (Ayensu and DeFilipps, 1978). However, this scenario would change with use of the high throughput advanced screening methods that are available today. Another approach than can prove to be a highly productive and cost effective in development of safe, effective and acceptable therapeutic agents is reverse pharmacology which is based on the documented therapeutic effects of plants in ancient texts. This paper will discuss the approaches that need to be considered while studying medicinal plants. It focuses on aspects of the medicinal plant research: from

collection of plant material, to efficacy and safety evaluation through preclinical studies and phytochemical standardization.

1.2 Plant Profile

Jatropha gossypifolia L is a traditional medicinal plant of the Euphorbiaceae family.

1.2.1 Common name

Bengali/vernacular name: Lal Bheranda, Laljeol, Erenda, Aar kocha.

Tribal name: Karachuni (Marma); Kander (Garo).

English name: Bellyache-bush (Servin SC, 2006).

1.2.2 The plant family

Kingdom	Plantae
Phyllum	Tracheophyta
Class	Magnoliopsida
Order	Malpighiales
Family	Euphorbiaceae
Subfamily	Crotonoideae
Genus	*Jatropha*
Species	*Jatropha gossypifolia*

1.2.3 Plant description

1.2.3.1 *Habit*

Bellyache bush is an opportunistic colonizer of disturbed sites. It can form pure stands in open areas where the natural vegetation has been damaged or removed by cattle, man or floodwaters

J. gossypifolia (Linn) is a perennial, erect shrub or small tree (Figure 1.1) usually about 2.5 m tall but which can exceed 4 m in some areas (Peirce, 1998). Some specimens have a single

stem, whereas others can have two or more stems. The stems are thick, rather soft, coarsely hairy, 1-2 m long and exude a watery sap when damaged (Parsons and Cuthberston, 1992). Stems rise from a herbaceous crown.

Figure 1.1: *Jatropha gossypifolia* plant

1.2.3.2 *Leaves*

The plant's leaves are arranged alternately along the stem (Figure 1.2). Leaf petioles are 2-7 cm long and the leaf blades are palmately 3-5-lobed, 45-90 x 50-130 mm; the lobes are more or less elliptic (Wheeler, 1992). Immature leaves are deep purple and sticky. Older leaves are generally glossy green although some may have a purple colouration (Pitt and Miller 1991). Petioles and leaf margins are covered with coarse, gland-tipped, sticky brown hairs.

Figure 1.2: *J. gossypifolia* leaf

1.2.3.3 *Inflorescence/Flowers*

The flowers are purple/red with yellow centers and are produced in clusters on branched stalks in the upper leaf axils (Figure 1.3). Both male and female flowers are in the same inflorescence, with 2-8 females and 27-54 males per inflorescence. The average male-to-female ratio is 11:1. Each inflorescence may bloom for 18 days (Reddi and Reddi, 1983). Male flowers have 8-12 stamens with filaments that are connate towards the base. Anthers are 2- celled, opening by longitudinal slits. Female flowers, which are larger than the male flowers, have 3 styles and a 2-5-celled ovary with one ovule per cell.

1.2.3.4 *Flowering*

Flowering occurs in February to May. Flowering has been observed to occur in plants that are only 4-6 weeks old and 15-30 cm tall (Vitell J, 1998), however, most plants start to flower at about two years of age (APB Infonote, 1994).

Figure 1.3: *J. gossypifolia* flower

1.2.3.5 *Fruits and seed*

The fruit capsule (seed pod) is sub-globular (round to oblong), about 12 mm long and 10 mm wide, separated into mericarps (i.e. a 3-lobed capsule). Each pod contains 3-4 orange-brown, ovoid seeds (7-8 mm long and 4 mm wide) with car uncles (Wheeler, 1992; Anon, 1996b).

1.2.3.6 *Distinguishing characters*

Bellyache bush is often mistaken for castor oil plant (*Ricinus communis*) which also colonizes riverbanks and freshly deposited alluvial soils and gravel. It is easily distinguished from castor oil plant by the shape of the leaves (bellyache bush leaves have 3-5 lobes, whereas the leaves of castor oil plant are divided into 7-9 lobes).

Figure 1.4: *J. gossypifolia* fruit

1.2.3.7 *Distributional Range*

Bellyache bush is native to tropical America, from the Caribbean region and Mexico to southern Brazil and Paraguay (Gardner and Bennetts, 1956). Within this region, it is widespread (Palmer unpubl.). The Missouri botanical garden's 'TROPIC and icar OS' database holds 84 records of the plant from Central America (Costa Rica, Honduras agua), South America (Bolivia, Colombia, Ecuador, Paraguay, Peru and Venezuela), the Caribbean (Dominican Republic, Puerto Rico and Leeward Islands) and Africa (Cameroon and Ghana). In Bangladesh *J. gossypifolia*, is distributed in Chittagong, Dhaka and other districts, by the road sides and fallow lands.

1.2.4 Uses of *Jatropha gossypifolia*

Different parts of *J. gossypifolia* are used in different countries in many ways.

1.2.4.1 *Leaves*

The leaves of *J. gossypifolia* are used for intermittent fevers, carbuncles, eczema, itches, sores on the tongues of babies, swollen mammae, stomachache, and venereal disease. The leaf decoction is used for bathing wounds.

1.2.4.2 *Bark*

The bark contains the alkaloid jatrophine and a lignan (jatroiden) is found in its stem (Robineau, L, 1991).

1.2.4.3 *Seed*

Seeds are emetic, purgative, and used for body pain (Horsten S *et al*., 1996). Ethno botanical uses of *J. gossypifolia* reported for cancer, diarrhea, dysentery, skin diseases (leprosy), arthritis, ulcer, gum infections and wound healing (Hussain *et al*. 1992; Dash and Padhy 2006; Rajesh *et al*. 2007). Seed oil of *J. gossypifolia* is used in rheumatism and paralytic affections (Annon, 1965).

1.2.4.4 *Stem latex*

In southern Nigeria, the stem latex of *J. gossypifolia* is routinely used by herbalists, rural dwellers and some people in urban centers to stop bleeding from nose, gum and skin without consideration for its safety. The stem latex is usually applied on the injured skin, bleeding gum or nose before it stops the bleeding, it may get into the body system and cause adverse reaction if it possess any. The therapeutic effects and the mechanism of action of the stem latex of *J. gossypifolia* as a haemostatic agent has been documented (Oduola *et al.*, 2005).

Plant is antibiotic, insecticidal and used for toothache and as blood purifier (Oduola T *et al*., 2005). The normal haemostatic mechanism involves normal functions of blood vessels, platelets and the blood coagulation. Vessels with muscular coats contract following injury

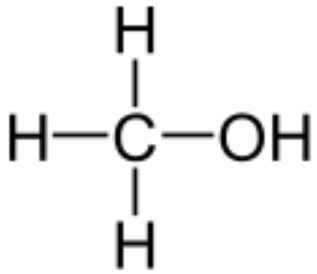
thus helping to arrest blood loss; the contraction is aided by the release of vasoconstrictors such as angiotensin II (Oduola T *et al.*, 2005).

1.2.5 Chemical constituents

Leaves contain flavonoids, a saponin, a resin, tannin and triterpenes. They also contain flavonoids, vitexin, isovitexin and apigenin. Seeds contain fatty oil. Roots contain antileukemic and tumour-inhibitor macrocyclic diterpene, jatrophone and jatropholones A and B. Bark contain β-sitosterol. Roots, stems and seeds contain arylnaphthalene lignan and the lignan prasanthaline. Cyclogossine, a cyclic heptapeptide, had been isolated from the latex of the plant. Stem contains a novel lignan, jatrodien (Ghani, 2003; Rastogi & Mehrotra, 1993).

1.3 Solvent System

1.3.1 Methanol



Chemical structure of Methanol

1.3.1.1 *Applications*

- Methanol, a common laboratory solvent, is especially useful for HPLC, UV/VIS spectroscopy.
- The largest use of methanol by far is in making other chemicals. About 40% of methanol is converted to formaldehyde, and from there into products as diverse as plastics, plywood, paints, explosives, and permanent press textiles.

• Other chemical derivatives of methanol in clude dimethyl ether, which has replaced chlorofluorocarbons as an aerosol spray and propellant, acetic acid. Dimethyl ether (DME) also can be blended with liquefied petroleum gas (LPG) for home heating and cooking, and can be used as a diesel replacement for transportation fuel (Blum, 2010).

Table 1.1: Physical and chemical properties of methanol

Physical state	**Colorless liquid**
Molecular formula	CH_3OH
Molecular weight	32.0419 g /mol
Boiling point	65°C, 338 K, 149°F
Melting point	− 98--97°C, 175-176 K, -144--143°F
Vapor pressure	13.02 kPa at 20°C
Solubility	Miscible in water
Odor	Pungent
Reactivity	Flammable; may explode when be xposed to flame
Lethal dosage LD50	5628 mg/kg (oral dose for rats) (Verschueren, 1983)

1.4 Acetic Acid Induced Writhing Test

Pain is an unpleasant feeling often caused by intense or damaging stimuli, such as stubbing a toe, burning a finger, putting alcohol on a cut, and bumping the “funny bone”. The International Association for the Study of Pain's widely used definition states: "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage".

In clinical settings it may be useful to identify several broad processes as being associated with pain: nociception, pain perception and a number of secondary consequences including suffering and pain behaviour. Under this schema, nociception may be defined as the detection of noxious stimuli and the subsequent transmission of encoded information to the brain. In

contrast, pain is essentially a perceptual process that arises in response to such activity. In contrast, pain arising from inflamed or injured tissues may arise spontaneously in the absence of an external trigger. Alternatively, responses to noxious stimuli may be enhanced (hyperalgesia) or normally innocuous stimuli may produce pain (allodynia). These features are not specific and do not, in themselves, allow recognition of distinct pathophysiological mechanisms (Loeser JD and Melzack R, 1999).

To study the analgesic activity at present there are some methods which are described by several researchers. The tests include acetic acid induced writhing test, formalin test, hargreaves test, Von Frey Fibers (Handerwerker HO and Brune K, 1987). Among them Acetic acid induced writhing test is much more popular because of its ease of use. Again this method is an established method to determine the analgesic activity of a compound. It is less time consuming and cost effective.

1.5 Brine Shrimp Lethality Bioassay

The *in vivo* lethality tests available for screening of plant extract are either base on the effect of extract on *Artimia sailna* Leach or the inhibition of hatching of the cyst (encased embryos that are metabolically inactive (Migliore *et al*., 1997). But most researchers have preferred the use of brine shrimp lethality test for the primary screening purposes.

The brine shrimp cytotoxicity assay is considered as a convenient probe for preliminary assessment of toxicity (Solís *et al.*, 1993), and it has been used for detection of plant extract toxicity (McLauglin *et al.*, 1991). The brine shrimp lethality bioassay is rapid (24 h), simple (e.g., no aseptic techniques are required), safe, easily mastered, inexpensive, and requires small amounts of test material (2-20 mg or less) (Ghisalberti, 1993).

1.6 Anti Diarrheal Test

Gastrointestinal diseases particularly constipation and diarrhea are affecting 70% of the population worldwide (Ouyang and Chen, 2004). Diarrhea is characterized by increased frequency of bowel movement, wet stool and abdominal pain (Ezekwesili *et al.*, 2004). It is one of the leading causes of malnutrition and death in developing countries and prevails as an ailment underlying 1.5–2 million deaths among children fewer than 5 years of age (WHO, 2009). Many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for the treatment of diarrhea but they have some side effects (Singh *et al.*, 2005). So, medicinal plants are usually preferred to treat gastrointestinal disorders, for example, constipation and diarrhea, because they contain multiple constituents with effect-enhancing and/or side effect-neutralizing potential, and, hence are considered relatively safe in prolonged use (Gilani and Rahman, 2005). A range of medicinal plants with anti-diarrheal properties is widely used by traditional healers. However, the effectiveness of many of these antidiarrhoeal traditional medicines has not been scientifically evaluated. The plant *J. gossypifolia* is a typical example of a plant-based remedy used to treat diarrhea (Ferro *et al.*, 2005).

1.7 Neuropharmacological Test

To check the neuropharmacological effects or side-effects of drug, three types of experiment is carried out which are hole cross, hole board and elevated plus maze test.

1.7.1 The hole cross test

The purpose of the hole cross test is to determine the stimulatory or depressive effect of test drug. Increased movement indicates stimulatory activity and decreased movement indicate depressive activity. As spontaneous movements of the animals include, by definition, both the propulsive and non-propulsive movements of the animal, and as the fluctuating and multifarious nature of many overt movements patterns impossible, to accurately measure the

effect of a drug on the spontaneous motor activity of animals by using a single experimental procedure, the hole cross test was performed (Robbins *et al.*, 1977 and Takagi *et al*., 1971).

1.7.2 The hole board test

The hole board experiment is a measure of exploratory behavior in animals and is an accepted parameter for evaluating anxiety conditions in animals. The hole board test enables the initial exploratory activity of the animal and its variations brought about by psychotropic elements of a drug to be unmistakably assessed. The hole board test is carried out to investigate the effect of the test drug on the exploratory behavior of the laboratory animal model (*Swiss albino*). Exploration can be defined as a broad category of behavior, the consequences of which are to provide the organism with information about the exteroceptive environment.

1.7.3 Elevated plus maze test

The regulation of anxiety is associated with function of the $GABA_A$ receptor system. Available evidence points to a major role for α2-containing $GABA_A$ receptors in modulating anxiety (Griebel *et al.*, 2003), although a recent study also suggests a possible implication for α3 and α5 subunit (Navarro *et al.*, 2002). Standard drug diazepam act selectively on $GABA_A$ receptors which mediate fast inhibitory synaptic transmission throughout the CNS. Benzodiazepines (BDZs) bind to the γ sub-unit of the $GABA_A$ receptor that causes an allosteric (structural) modification of the receptor results with an increase in $GABA_A$ receptor activity (Rang *et al.*, 2003).

Although original validation of the EPM was performed in rats (Broadhurst *et al*., 1987), but it has also been found to be selectively sensitive to the effects of anxiolytic and anxiogenic drugs in mice (Lister, 1987). The important variables of the elevated plus-maze test are: time spends in open arm as well as the number of entries in to these arms.

1.8 Objective of the Study

The main objective of this study was to characterize the effect of methanolic fruit extract of *J. gossypifolia* on different physiological systems of animal model. It includes find out the analgesic effect by acetic acid induced writhing test, cytotoxic effects of *J. gossypifolia* by brine shrimp lethality bio assay. To find out the anti diarrheal effect induced by castor oil was another objective. To determine the Neuropharmacological effects of the methanolic fruit extract of *J. gossypifolia*, Hole cross test, Hole board test and Elevated plus maze test were done.

Jatropha gossypifolia is a very common plant which is used in our country as well as in world by a lot of people for several purposes. All the parts of this plant are used for medicinal activity. All these studies were performed in an effort to ensure the safety of the general users of the country as a whole.

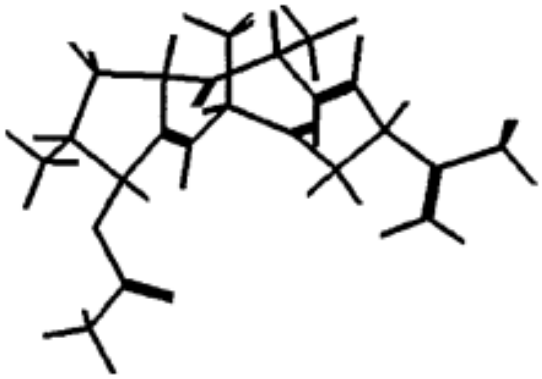
Chapter 2

Literature Review

LITERATURE REVIEW

2.1 Phytochemical Studies

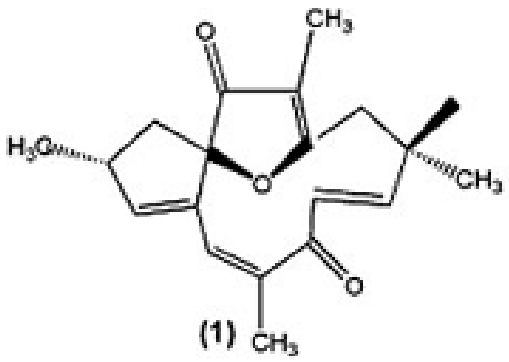
Jatrophenone, a Novel Macrocyclic Bioactive Diterpene from *Jatropha gossypifolia*



Jatrophenone

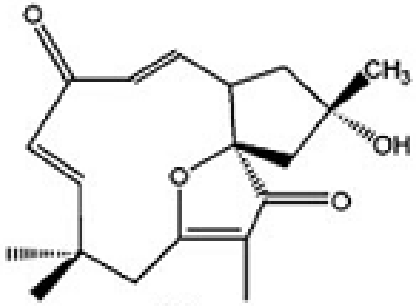
Jatrophenone, a novel macrocyclic diterpene was isolated from the whole plant of *Jatropha gossypifolia*. By using detailed studies of one- and two-dimensional (1D and 2D) NMR spectra the structure of the compound was established. Jatrophenone possesses significant antibacterial activity (Ravindranath *et al*., 2003).

Jatrophone from *Jatropha gossypifolia*

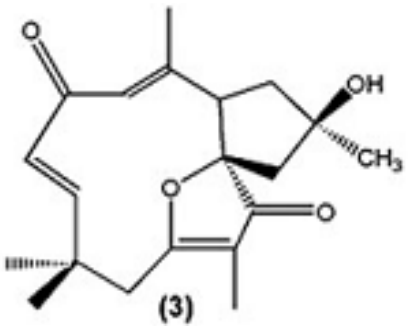


Jatrophone

Jatrophone is a macrocyclic diterpene isolated from the roots of *Jatropha gossypifolia*. Several multiple biological activities including cytotoxicity, inhibition of insulin release, relaxation effect of induced muscle contraction, relaxant action in rat portal vein, inhibition of lymphocytes activation, anti-protozoal activity, inhibition of tumor cells, molluscicidal activity and gastroprotective effects are possessed by jatrophone. There are two natural derivatives of jatrophone, named as hydroxyl jatrophone (2α-OH Jatrophone, 2β-OH Jatrophone) and 2β-OH-5,6- isojatrophone which were isolated from the root of *J. gossypifolia* (Tylor, 1983). Jatrophone has cytotoxic activity ED_{50}, 0.01 lg/ml *in vitro* against the P-388 lymphocytic leukemia test (Yang, 2003). At dose 25mg/kg body weight, jatrophone possess strong gastroprotective effect (88%). Jatrophone exhibited anti-proliferative effects (IC_{50} in l M) against fibroblasts CCL-171, AGS CRL-1739, lung HTB-58, bladder HTB-1, leukemia CCL-240: 0.29, 0.51, 1.8, 1.7 and 5.1 respectively (Theoduloz, 2009). Menezes *et al*. reported that there is significant effect of jatrophone on insulin secretion. Glucose-induced insulin release was inhibited with an ID_{50} in the presence of jatrophone close to 8l M/l (Menzes, 1992). Silva *et al*. reported that jatrophone, exhibited a vasorelaxant effect in rat portal vein contractions induced by phorbol 12-myristate 13-acetate (PMA,0.1–3 lM)), noradrenaline (NA, 0.01–100lM), endothe-lin-1 (ET, 0.01 -10 nM) or KCI (4–128 mM) with IC_{50} of 86 nM, 13, 11 and 9 lM respectively (Silva *et al.*, 1995). In rat uterine muscle jatrophone (1–300 lM) exhibited a concentration-dependent relaxant effect on sustained contraction that was induced by spasmogenic compounds acetylcholine (Ach, 100 lM), oxytocin (Ot, 30 mlU/ml) and KCI (80 mM)). Theoduloz suggested that Jatrophone possess a relaxant effect with an IC 50 (l M) in the order of following potency, Acetylcholine (14.2)> Oxitocin (19.0) > KCI (48.3) (Theoduloz, 2009).



2α-OH Jatrophone



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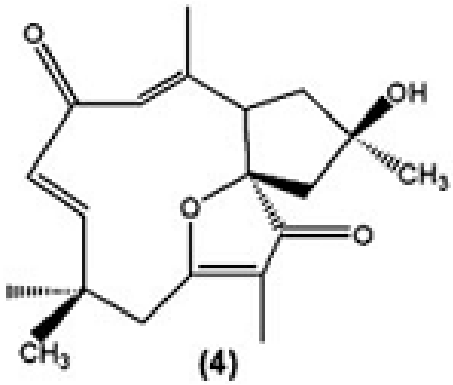
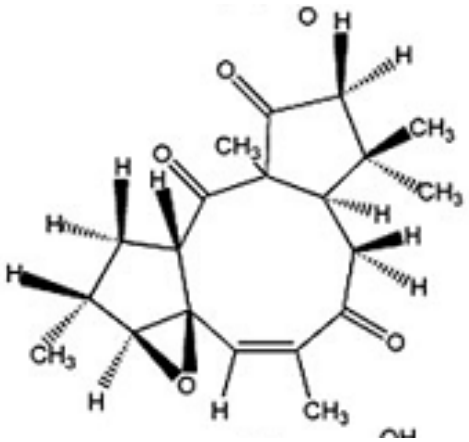
2β-OH Jatrophone



(4)

2β-OH-5,6- isojatrophone

Isolation of Citlalitrione



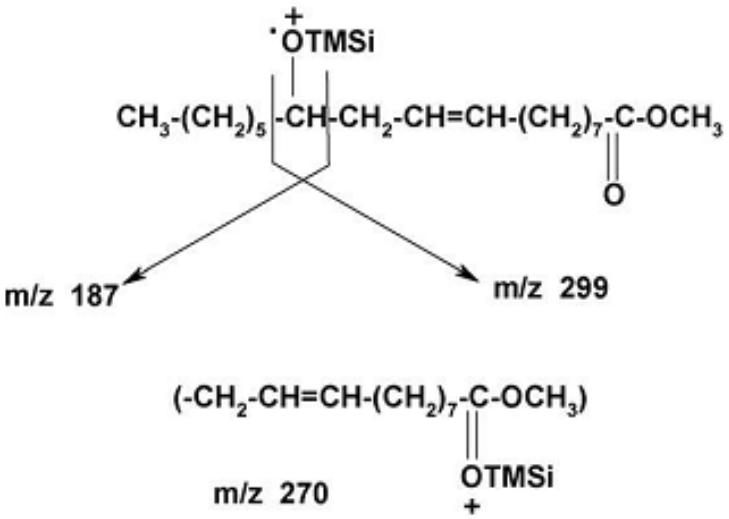
Citlalitrione

Villarreal et al. isolated citlalitrione, an epoxytrione diterpine from the dried plant of *Jatropha gossypifolia* in the year of 1988. There is structural similarity of citlatrione and jatrophenone. Yang *et al* reported anticancer activity of this compound (Yang, 2003).

Physicochemical studies of *Jatropha gossypifolia*

Rani B. Bhagat & D. K. Kulkarni studied jatropha species including *Jatropha gossypifolia*. The seed oil of the plant was studied for various physicochemical properties including oil content, ash content, moisture content, acid value (AV), saponification value (SV), iodine value (IV), free fatty acids (FFA), specific gravity (SG), mean molecular mass (MMM), Refractive Index (RI) as per standard methods. Tezara *et al*. studied that photo inhibition during drought in *J. gossypifolia* which might impose a limitation on carbon assimilation during drought (Tezara *et al*., 2005). This study showed that the plant can recover rapidly from "mid day depression" of photosynthesis or the photo inhibition without any significant change in leaf pigment contents (Das *et al*., 2010).

Characterization and structure elucidation of 12-hydroxyoctadec-cis-9-enoicacidin *Jatropha gossypifolia*

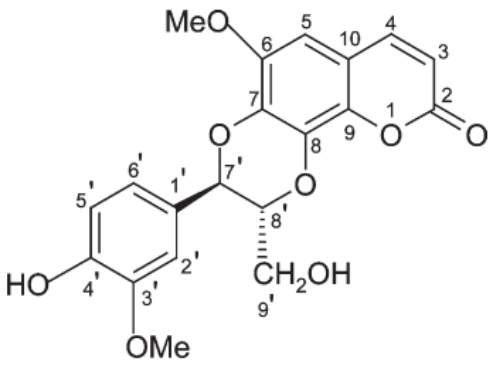


12-hydroxyoctadec-cis-9-enoic acid

In the year of 2007, Hosamani and Katagi run a study on *J. gossypifolia*. For the detection of hydroxy, cyclopropenoid, keto and epoxy functional groups various tests like direct thin layer chro-matography test, Halphen test, 2,4-dinitrophenylhydrazine, thin layer chromatography test, and picric acid thin layer chromatography test, have been employed respectively. The *Jatropha gossypifolia* seed oil responded to the direct thin layer chromatography test that ensured the presence of hydroxy fatty acid. The seed oils did not respond due to indicating the absence of cyclopropenoid, keto and epoxy fatty acids to the Halphentest,2,4-dinitrophenyl hydrazine (2,4-DNPH) thin layer chromatography test and the picric acid thin layer chromatography test, respectively.

The structure of 12-hydroxyoctadec-cis-9-enoic acid was isolated from the seed oil of *Jatropha gossypifolia*. It is a fatty acid resulted from the gas chromatography of the seed oil (Hosamani & Katagi, 2007).

Cleomiscosin A, a coumarino-lignoid from *Jatropha gossypifolia*

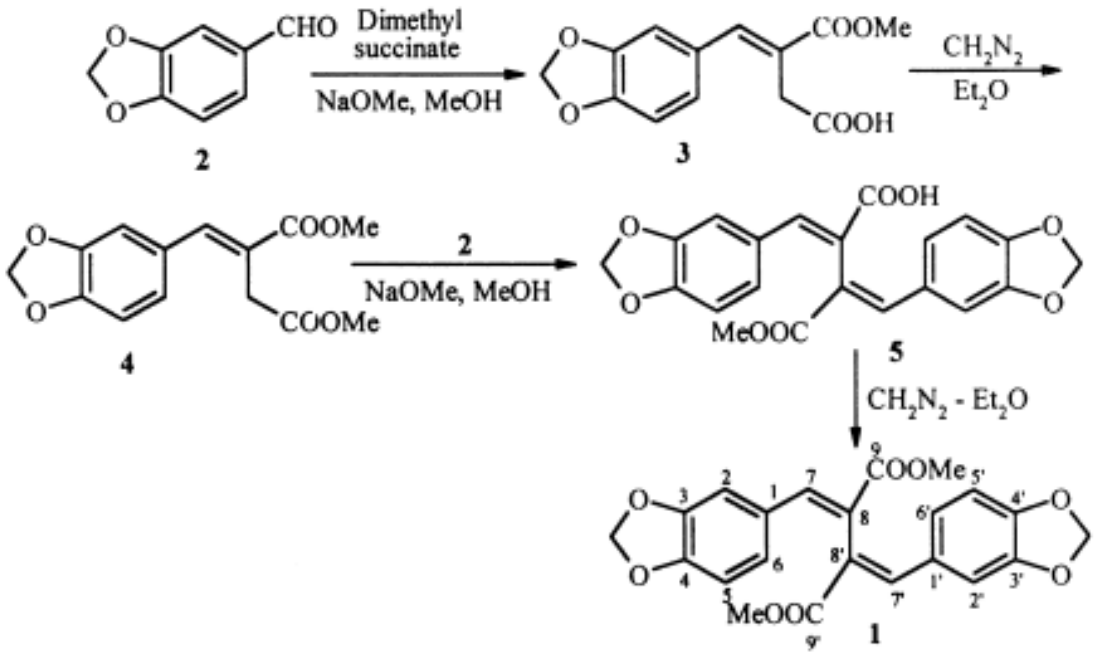


Cleomiscosin

The objective of this investigation on the stems of the plant has resulted in the isolation of a coumarino-lignoid, cleomiscosin A. The structure of the compound has presently been settled from its physical and spectral (IR, 1H-NMR and MS) data as well as by direct com-parison with authentic sample (Das et al., 2003).

Gossypidien, a lignan from stems of *Jatropha gossypifollia*

Gossypidien was isolated from stems of *Jatropha gossypifollia* by Biswanath das and G. Anjani in the year of 1998. It has no optical activity. Starting material to gossypidien(1) was piperanol.In presence of methoxide stobbe condensation of piperanol(2) with dimethyl succinate a compound(3) is afforded. It is then methylated with diazomethane to form the diester (4). A second stobbe condensation of 4 with piperonal produced compound (5) (Das and Anjani, 1998).

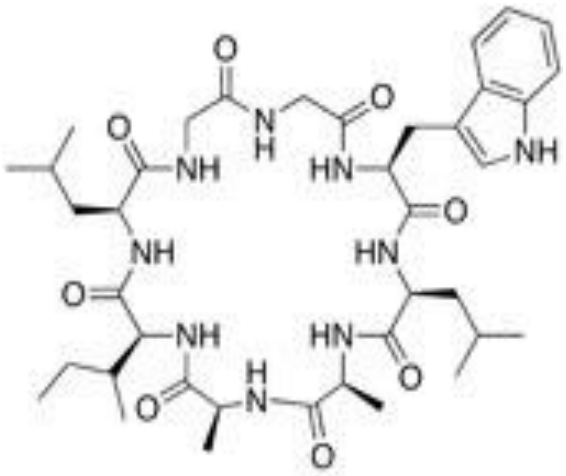


Synthesis of gossypidien

Cuyclogossine A, a novel heptapeptide isolated from the latex of *Jatropha gossypifolia*

Cyclogossine A which is a novel cyclic heptapeptide was isolated from the latex of *Jatropha gossypifolia* in the year of 1996. To determine the structure of cylogossine A, researchers used several amino acid analysis including mass spectroscopy, and two dimensional ^{1}H-NMR spectroscopy (Horsten *et al*., 1996).

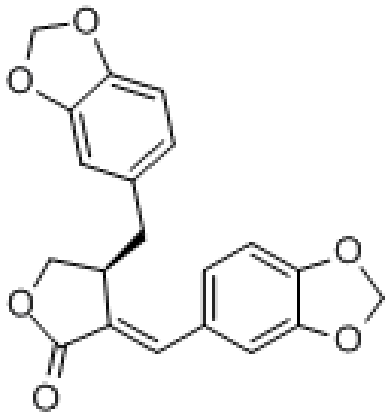
Cyclogossine B, a Cyclic Octapeptide from *Jatropha gossypifolia*



cyclogossine B

In 1997, Auvin-Guette et al. isolated Cyclogossine B from *Jatropha gossypifollia.*

Isolation of isogadain from *Jatropha gossypifolia*.



Isogadin

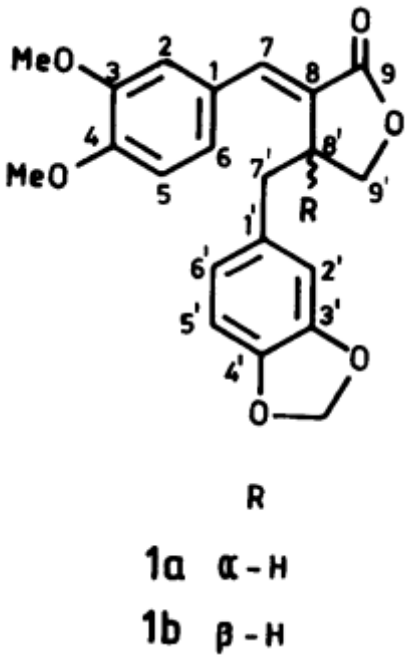
From the stem of *Jatropha gossypifollia* a lignin Isogladin was isolated in 1996. The structure was elucidated by spectral data (Das B *et al*., 1996).

Venkatasin, a new coumarino-lignoid from *Jatropha gossypifolia*

Venkatasin, A new coumarino lignoid compound was isolated from the whole plant of *Jatropha gossypifolia* . The compound was synthesized by rezioselective acetylation of a known coumarino-lignoid, cleomiscosin A (Das B *et al*., 2006).

Gossypifan, a lignan from stems of *Jatropha gossypifollia*

Gossypifan was isolated as needles. It had two optical isomers. It was observed that lignin 1a and 1b possess opposite optical direction. The Gossypifan 1a can be defined as α-(trans-3, 4-dimethoxybenzylidene)-β-S(3,4-methylenedioxybenzyl)-γ –butyrolactone by spectroscopic methods (Biswanath and Ratna, 1995).



Gossypifan

Table 2.1: Summary of the Phytochemical Studies on *Jatropha gosspyfolia*.

Plant part	Findings	Reference
Aerial	Gossypifan- A lignin isolation	Das B & Das R, 1995
Latex	Isolation of cyclogossine A	Horsten SF *et al*., 1996
Stem latex	Synthesis of tetradecyl (E)-ferulate	Kavitha J *et al*., 1999
Stem	Isolation of Gossypidien	Das B & Anjani G, 1999
Plant	Biochemical alteration	Singh D & Singh A, 2002
Leaf	Water retention and leaf anatomy of *J. gossypifolia*	E. Rengifo *et al*., 2002
Whole plant	Antibacterial activity of jatrophenone	Ravindranath N *et al*.,

		2003
Stem	Isolation of Cleomiscosin A	Das B *et al*., 2003
Whole plant	Jatrophenone, a Novel Macrocyclic Bioactive Diterpene	Avindranath NR *et al*., 2003
Leaf	Antibacterial activity of *J. gossypifolia*	Kumar Vp *et al*., 2006
Leaf	Identification of ricinoleic acid in Jatropha12-hydroxyoctadec-cis-9-enoic acid	Hosamani KM & Katagi KS, 2007
Leaf	Antibacterial activity	Dabur R *et al*., 2007
Seed	Structure elucidation of 2-hydroxyoctadec-cis-9-enoicacidin	Hosamani KM & Katagi KS, 2008
Whole plant	Molecular characterization	Senthil Kumar R *et al*., 2008
Whole plant	Comparative study of interspecific genetic divergence and phylogenic analysis	Pamidiamarri S *et al*., 2008

2.2 Pharmacological studies

Insecticidal activity of *Jatropha gossypifolia*

The insecticidal activity of *J. gossypifolia* was tested against *Spodoptera exigua*. Researchers chose this plant due to having some secondary metabolites like apigenin, cyclogossine, jatrophatrione, jatropholone of this plant. Screening showed potential larvicidal activity. This study showed significant increase in toxicity over time and the plant extract also inhibited glutathione-s-transferase activities but induced the carboxylesterase activity. Another biological property like Anti-allergic, molluscicidal and isect repellent property was recorded (Khumrungsee *et al*., 2009).

Hepatoprotective activity of *Jatropha gossypifolia*

The aerial part of *Jatropha gossypifolia* was studied to demonstrate the hepatorotective activity in carbon tetrachloride liver damaged in wister albino mice. In the study, petroleum ether, aqueous and methanolic extracts were used to determine the hepatoprotective activity.

The study confirmed t hat this plant has potent hepatoprotective activity. The assessment of the damage of liver was determined by biochemical studies (SGOT, SGPT, ALP and total bilirubin) and by histopathological examinations (Panda, 2009).

Haemostatic activity of stem latex of *Jatropha gossypifolia*

As a haemostatic agent the coagulant activity and the mechanism of action of *Jatropha gossypifolia* stem latex were investigated. screening tests were performed with the stem latex within 6 hours of collection on thirty healthy subjects. With a lancet three puncture was made and bleeding time was calculated. With bovine albumin the stem latex was diluted to several fractions. Researchers established that the stem latex with the 30% bovine albumin is a protein precipitant (Oduola, 2005).

Anticoagulant activity of *Jatropha gossypifolia*

The anticoagulant activity was demonstrated oduola *et al.* by doing some anticoagulant tests. The study showed that the pH of the plant is 7.0 and the increase of the pH of the blood plasma is between 7.35 to 7.45, therefore the increased hydrogen ion concentration (H^+) in the leaf extract was responsible for the reduced HCO_3^- (Oduola *et al.*, 2005).

Table 2.2: Summary of the Pharmacological Studies on *Jatropha gossypifolia.*

Plant part	Findings	Reference
Stem Latex	Anticoagulant activity of *J. gossypifolia*	Oduola T *et al.*, 2005
Leaf	Suitability as anticoagulant	Oduola T *et al.*, 2005
Stem latex	Safety of haemostatic activity of *J. gossypifolia*	Oduola T *et al.*, 2007
Stem latex	Acute toxicity test	Siriarcharungroj S *et al.*, 2008
Leaf	Insecticidal activity of *Jatropha gossypifolia*	Phowichit S *et al.*, 2008
Leaf	Antibacterial activity	Nair R *et al.*, 2008
Whole plant	Toxicity and its Detoxifying Enzyme Activities	Khumrungsee N *et al.*, 2009

Aerial part	Hepatoprotective activity	Panda BB *et al*., 2009
Leaf	Insecticidal activity of *Jatropha gossypifolia*	Khumrungsee N *et al*., 2010
Whole plant	Antitumour , Cytotoxic activity of Jatrophone	Rakshit K *et al*., 2011
Whole plant	Molecular identification begomovirus associated with yellow mosaic disease	Snehi SK *et al*., 2011

Chapter 3

Materials and Methods

MATERIALS AND METHODS

3.1 Collection and Identification

The whole plant was collected from Sadhuhati, Jhenidah during rainy season (July - August) in 2011. The plant was taxonomically identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka, where a Voucher specimen (DACB Accession No. 35937) has been deposited for future reference.

3.2 Materials

Table 3.1: Name of chemicals, equipments and glass apparatus for extraction

Chemicals	Equipments	Glass apparatus
n-hexane (Merck, Germany)	Balance (Shimadzu, Japan)	Beaker
Ethyl acetate (Merck, Germany)	Hot air oven (NUVE)	Conical flask
Methanol (Merck, Germany)	Soxhlet apparatus	Funnel
Dichloromethane (Sigma-Aldrich, Germany)	Rotary evaporator (IKA, Germany)	Measuring cylinder
Acetone (Merck, Germany)	Blender (Miyako, Japan)	

3.3 Method

3.3.1 Drying of the aerial parts

The plant was thoroughly washed with water. Fruits were taken and cut into small pieces and spread in thin layers in trays. The fruits were then sun dried for seven days.

3.3.2 Grinding and storage of the dried samples

The dried fruits were ground to coarse powder with a mechanical grinder (Grinding Mill). This process breaks the dried fruits to smaller pieces thus exposing internal tissues and cells to solvents thus facilitating their easy penetration into the cells to extract the constituents (Ghani, 2003). Then the powdered sample was kept in clean closed glass containers till

extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder. The weight of the total dry powder was 300 g.

3.3.3 Extraction of the dried powdered sample

The dried fruits of *J. gossypifolia* were coarsely powdered by a milling machine and soaked in 1500 ml of methanol. The container with its contents was sealed and kept for a period of 4 days accompanying occasional shaking and stirring. The whole mixture then underwent a course filtration by a piece of clean, white cotton material.

Figure3.1: Rotary Evaporator (IKA ®RV05 Basic, Biometra, Germany)

3.3.4 Condensation of the leaf extracts

The extracts were transferred to the round bottle flask of rotary evaporator. Then excess amount of solvents in the extracts were removed by rotary evaporator (Figure 3.1), with reduced pressure which was done by using a vacuum pump. The temperature of the rotary evaporator was set 50ºC. It run for 1h 10min and the RPM was set 120 for evaporation

process. After evaporation extract was transferred in a beaker. Rest of the extract was removed from the round bottle flask by using dichloromethane. Then extract was kept in hot air oven to get more dried extract. All beakers were covered with aluminum foil. The extract was then collected and stored in a cool (4˚C) dry place for further assay.

3.3.5 Experimental animal

Swiss albino mice of either sex were obtained from the Animal resource board of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions (relative humidity 55-65 %, room temperature 23.0 ± 2.0 ^{o}C and 12 h light: dark cycle). The animals were fed with standard diet and water.

3.4 Acetic Acid Induced Writhing Test

3.4.1 Materials

20 swiss albino mice	Syringe
Acetic acid	Counter
Stopwatch	Feeding needle

3.4.2 Principle

In this method (Koster *et al.*, 1959; Whittle, 1964; Vogel & Vogel, 1997; Ahmed *et al.*, 2001) acetic acid is administered intraperitoneal (Figure 3.2) to the experimental animals to create pain sensation. As a result, the animals squirms their body at regular interval out of pain. This squirm or contraction of the body is termed as “writhing”. As long as the animals feel pain, they continue to give writhing. Each writhing is counted and taken as an indication of pain sensation. Any substance that has got analgesic activity is supposed to lessen the number of writhing of animals within in a given time frame and with respect to the control group. The writhing inhibition of positive control was taken as standard and compared with test samples

and control. As positive control, any standard NSAID drug can be used. In the present study, Diclofenac at dose 10 mg/kg body weight was used to serve the purpose.

Figure 3.2: Intraperitonial injection in mice

3.4.3 Experimental design

Twenty experimental animals were randomly selected and divided into three groups denoted as group-1, group-2, group-3 and group- 4 consisting of 5 mice in each group. Each group received a particular treatment i.e. control, standard and the dose of the extract of the plant respectively. Prior to any treatment, each mouse was weighed properly and the dose of the test sample and control materials was adjusted accordingly (Koster R *et al.*, 1959).

3.4.4 Preparation of test materials

In order to administer the crude extract at dose of 200mg/kg and 400 mg/kg body weight of mice, 200 & 400 mg/kg of the extract was measured and was triturated unidirectional way by the addition of small amount of suspending agents Tween-80. After proper mixing of extract

and suspending agent, normal saline or water was slowly added. The final volume of the suspension was made 5 ml. To stabilize the suspension, it was stirred well by vortex mixture. For the preparation of Diclofenac at the dose of 10-mg/kg-body weight, 2.5mg of diclofenac was taken and a suspension of 5 ml was made.

3.4.5 Method

At zero hour group-1 and group-2 received negative control (1% Tween-80 solution in saline), and standard (Diclofenac). The test groups received methanolic extract of fruit of *Jatropha gossypifolia* at the dose of 200mg/kg and 400mg/kg body weight respectively administered orally by means of a long needle with a ball-shaped end. After 30 minutes acetic acid (0.5%) was administered intra-peritoneal to each of the animals of all the groups. The thirty minutes interval between the oral administration of test materials and intra-peritoneal administration of acetic acid was given to assure proper absorption of the administered samples. Five minutes after the administration of acetic acid, number of squirms or writhing were counted for each mouse for fifteen minutes.

3.5 Brine Shrimp Lethality Bioassay

3.5.1 Materials

Artemia salina leach (Brine shrimp eggs)	0.1N NaOH
Distilled Water	Small tank to hatch the shrimp
Vials	Pump
Lamp	NaCl salt
pH Meter	Pasteur Pipette

3.5.2 Method

3.5.2.1 *Preparation of Simulated Sea Water*

The lethality test involves the culture of brine shrimp nauplii that requires sea water to be grown up in the laboratory. In the composition of seawater 3.8% of sodium chloride is

present in 100 ml of water. So 3.8% solution of sodium chloride was made by dissolving 38g of NaCl in 1L of distilled water. The pH of the solution was adjusted to 8.4 by adding sufficient volume of 0.1N NaOH and was checked by using a pH meter.

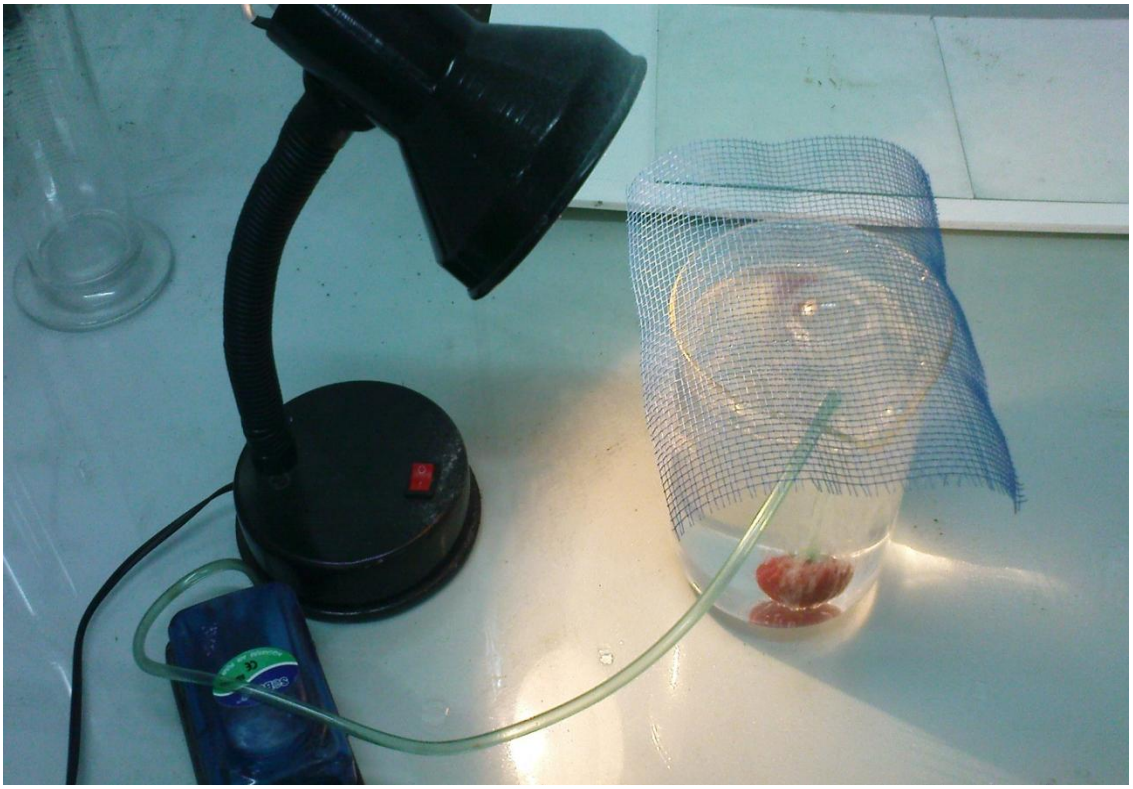


Figure 3.3: Hatching of brine shrimp eggs

3.5.2.2 *Hatching of brine shrimp eggs*

Artemia salina Leach (brine shrimp eggs) was used as the test organism which was collected from the pet shop. In the small tank 1L of simulated seawater was taken and 1.5g shrimp eggs were added in one side of the tank. An electronic pump was used to deliver continuous air with oxygen supply to the tank where eggs were added. A lamp was placed in the other side of the tank. The shrimps were allowed 24 hours to hatch and get matured as nauplii (larvae). The hatched nauplii were attracted to the lamp light on the other side through the perforated dam. These naupliis were then used for the test.

3.5.2.3 *Preparation of test sample solutions*

15 glass vials of 5ml were taken. 10 naupliis with 2.5ml sea water were added to each and every vial. 8.0 mg of Ethanolic extract of *Jatropha gossypifolia* fruit was dissolved in 400µl of Dimethyl sulfoxide (DMSO). The extract was dissolved by vigorous vibrating through a vortex. From the solution 200µl was taken to a vial and volume was adjusted to 5ml by adding 4.8ml sea water. The concentration of the stock solution was 400µg/ml. 2.5ml of sample solution was transferred to pre marked glass vials containing 10 nauplii. The volume of the sample solution again adjusted to 5mL by adding 2.5ml of sea water that makes the concentration 100 µg/ml. 2.5ml of sample solution was then again added to another vial. The same process continued to 15 times where final sample solution concentration becomes 0.0244µg/ml. So the concentration used in the test were 400µg/ml, 200µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml,1.5625 µg/ml, 0.78125 µg/ml, 0.39 µg/ml, 0.195 µg/ml, 0.097 µg/ml, 0.0488 µg/ml, 0.0244 µg/ml respectively.

3.6 Anti Diarrheal Test

3.6.1 Materials

20 Swiss albino mice	Filter paper
Syringe	Feeding Needle
Glass beaker	Tween 80
Loperamide	Castor oil

3.6.2 Method

20 mice were weighed and divided into 4 groups (n=5). Group 1 and 2 were termed as negative and positive control group respectively; whereas 3 and 4 were test group. Group 1 received only 0.5 ml of 1% Tween 80 solution, group 3and 4 received 200mg/kg and

400mg/kg extract orally by a feeding needle. Group 2 received Loperamide 2mg/kg body weight (Sunder S, 2011).

30 minutes after the above treatment each of the mice was administered 0.2ml of castor oil orally with the help of a feeding needle. Following the administration, each of the mice was placed in 1000ml glass beaker. The floor of the beaker was lined with an absorbent sheet of paper (filter paper). The diarrhea episodes were observed for 3hours. During this time the consistency of the fecal matter, onset of the stool, weight of stool and frequency of defecation were observed. Weight of paper before and after defecation was noted to determine the weight of stool. The onset was measured as the time intervals between the administration of castor oil and the appearance of the first diarrheal stool. The percent inhibition of defecation was measured by using the following formula.

$$\% \text{ Inhibition} = \frac{\text{Mean defecation (Control group} - \text{Treated group)} \times 100}{\text{Mean defecation of control group}}$$

3.7 Hole Cross Test

3.7.1 Materials

20 Swiss albino mice	Stopwatch
Hole cross apparatus	Counter
Feeding needle	

3.7.2 Method

The animals were divided into 4 groups (n=5). Group 1 and 2 were named as negative and positive control and 2 other groups were termed as treated group. The test groups received methanolic extract of fruit of *Jatropha gossypifolia* at the dose of 200 and 400mg/kg body weight respectively. Group 1 received 1% Tween 80 solution. Group 2 got administration of

Diazepam (as a standard drug) at 1mg/kg body weight. Each mouse was placed on one side of the hole cross apparatus. Hole cross apparatus is a wooden box having a dimensions of 30×20×14 cm, constructed with a partitioning wall with a hole of 3cm diameter was made at height of 7.5 cm from the floor. The spontaneous movement of the mice from one chamber to other through the hole was observed for 3 minutes. The observation was conducted at 0, 30, 60, 90 and 120 minutes after the oral administration of the drugs.

3.8 Hole Board Test

3.8.1 Materials

20 Swiss albino mice	Feeding needle
Hole board apparatus	Stopwatch

3.8.2 Method

The study was conducted using a wooden board measuring 20 cm by 40 cm with sixteen evenly spaced holes (Perez *et al*., 1998). The animals were randomly grouped into four groups each containing five mice. Group 1 served as the control group and was treated with 1% Tween 80 solution *i.p.* Group two got administration of Diazepam (as a standard drug) at 1mg/kg body weight *i.p*. Drug or vehicle was injected intraperitoneally in a volume of 0.5ml/kg. Groups three and four were treated with the methanolic extract of fruit of *J. gossypifolia* at the dose of 200 and 400mg/kg body weight respectively. Thirty minutes after treatment, the mice were placed singly on the board and the number of times the mice dipped their head into the holes at the level of their eyes during a five minute trial period was counted using a tally counter (Aiyelero OM *et al.*, 2012; Perez GRM *et al*., 1998).

3.9 Elevated Plus Maze Test

3.9.1 Materials

20 Swiss albino mice	Video Camera
Elevated Plus Maze apparatus	Feeding needle
Stopwatch	

3.9.2 Method

Animals were randomly allocated to four experimental groups (n=5). Group 1 and 2 were named as negative and positive control and 2 other groups were termed as treated group. The test groups received methanolic extract of fruit of *Jatropha gossypifolia* at the dose of 200 mg/kg and 400mg/kg body weight respectively. Group 1 received 1% Tween 80 solution. Group 2 got administration of Diazepam (as a standard drug) at 1mg/kg body weight. Drug or vehicle was injected intraperitoneally in a volume of 0.5ml/kg. Tests were performed 30 min after injections.

3.9.2.1 *Apparatus and behavioral test*

Plus-maze test consisted of two open arms (30×5 cm, surrounded by a 0.25cm high border) and two closed arms (30×5cm, surrounded by 25cm high walls) with the two pairs of identical platform, which emerged from a central platform (5×5cm), positioned opposite each other. The apparatus was elevated 40 cm above the floor. The test was initiated by placing the mouse on the central platform of the maze, facing one of the open arms, and letting it move freely. Each session lasted 5 min, being recorded by a video camera. After each test, the maze was thoroughly cleaned. Behavioral analysis was performed by watching the recorded videos later. A number of classical parameters were collected during the session: (a) Open arm duration: the total amount of time the mouse spent in the open arms; (b) Closed arm duration: the total amount of time the mouse spent in the closed arms; (c) Central platform duration: the total amount of time the mouse spent in the central platform; (d) Open arm frequency: the frequency of mouse entry with all four paws into the open, unprotected arms; (e) Closed arm

frequency: the frequency of mouse entry with all four paws into the closed, protected arms, and (f) Total number of entries in the arms. Likewise, different ethological measures were also quantified: (a) Rearings: a body stance in which the animal sets his forepaws onto the wall of a closed arm while keeping his rear legs on the floor; (b) Stretched attend posture (SAP): a body posture in which the mouse stretches forward and then retracts to its original position without moving the feet, and (c) Grooming: Itching of the face by the front legs (Navarro JF *et al.*, 2006).

Chapter 4

Results

RESULTS

4.1 Acetic Acid Induced Writhing Test

Table 4.1: Tabular presentation of the effect of methanolic extract of *J. gossypifolia* fruit on acetic acid induced writhing test in mice

Treatment	Dose, Route	Total writhing	% Inhibition	95% Confidence interval	
				Lower	Upper
Control	0.5ml/mouse, p.o.	78.6±0.29	0.00	77.79	79.40
Positive	10mg/kg, p.o.	1.5±0.15***	98.09	1.06	1.939
JGF	200mg/kg, p.o.	17.4±0.33***	77.86	16.47	18.32
JGF	400mg/kg, p.o.	22.6±0.78***	71.25	20.43	24.76

Data is presented as mean ± S.E.M.; at *($p<0.05$) = Significant, **($p<0.01$) = Highly Significant, ***($p<0.001$)= Very Highly Significant compared to control (one-way ANOVA followed by Dunnett's post-hoc test)

Treatment	Dose, Route	Mean no. of writhing	Std. Deviation	Std. Error	95% Confidence interval	
					Lower	Upper
Control	0.5ml/mouse, p.o.	78.6	0.65	0.29	77.79	79.40
Positive	10mg/kg, p.o.	1.5	0.35	0.15	1.06	1.93
JGF	200mg/kg, p.o.	17.4	0.74	0.33	16.47	18.32
JGF	400mg/kg, p.o.	22.6	1.75	0.78	20.43	24.76

In acetic acid induced writhing test, the methanolic fruit extract of *J. gossypifolia* at both doses (200 and 400 mg/kg body weight) showed very highly significant ($p<0.001$) inhibition of the writhing response induced by acetic acid. The percentage of inhibition of the writhing response by the extract at the doses 200 and 400 mg/kg body weight was 77.86% and 71.25% respectively.

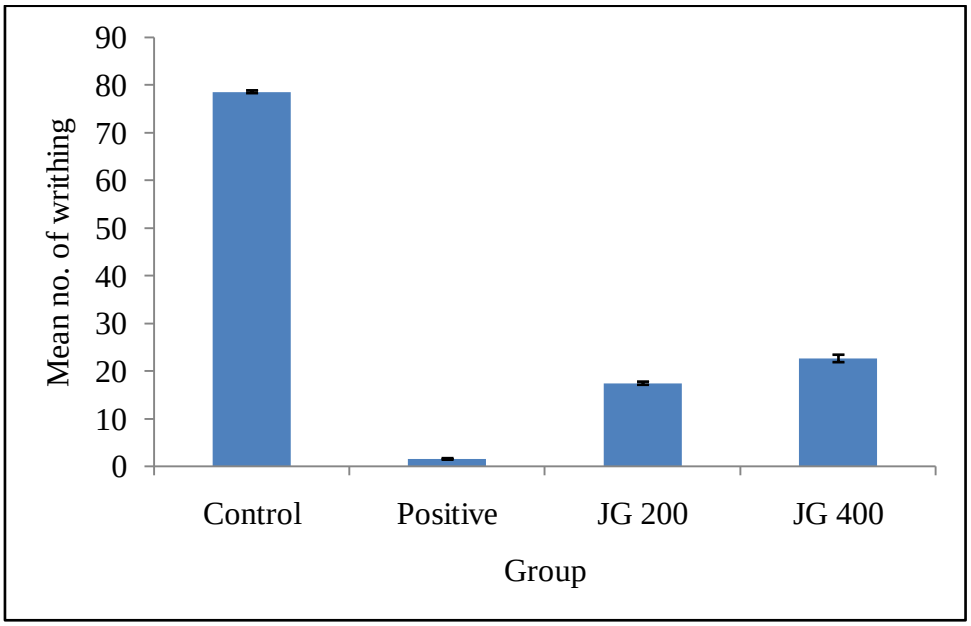


Figure 4.1: Graphical presentation Effect of *J. gossypifolia* fruit on acetic acid induced writhing test

4.2 Brine Shrimp Lethality Bioassay

Table 4.2: Tabular presentation of effect of methanolic extract of *J. gossypifolia* fruit on Brine Shrimp Lethality Test

Test Compound	**LC_{50} (µg/ml)**	**Best Fit Equation**	**R^2**
$KMnO_4$	11.88	y = 5.1977x - 0.588	0.853
JG fruit	2.84	y = 3.763x + 3.295	0.770

The obtained LC_{50} value for test sample (methanolic extract of *J. gossypifolia*) is 2.84µg/ml and for positive control test is 11.88µg/ml.

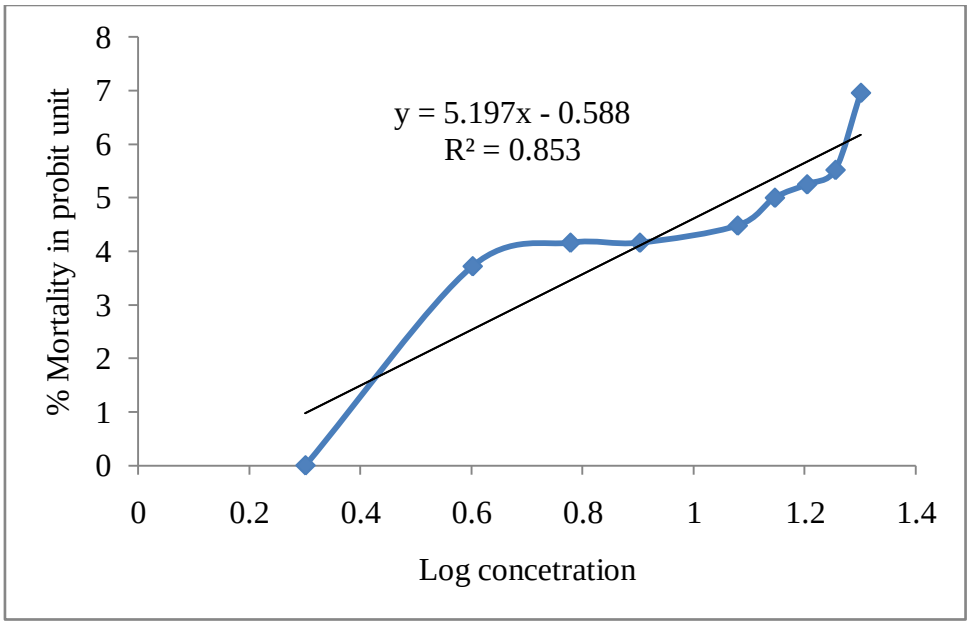


Figure 4.2.1: Effect of Potassium permanganate on brine shrimp nauplii

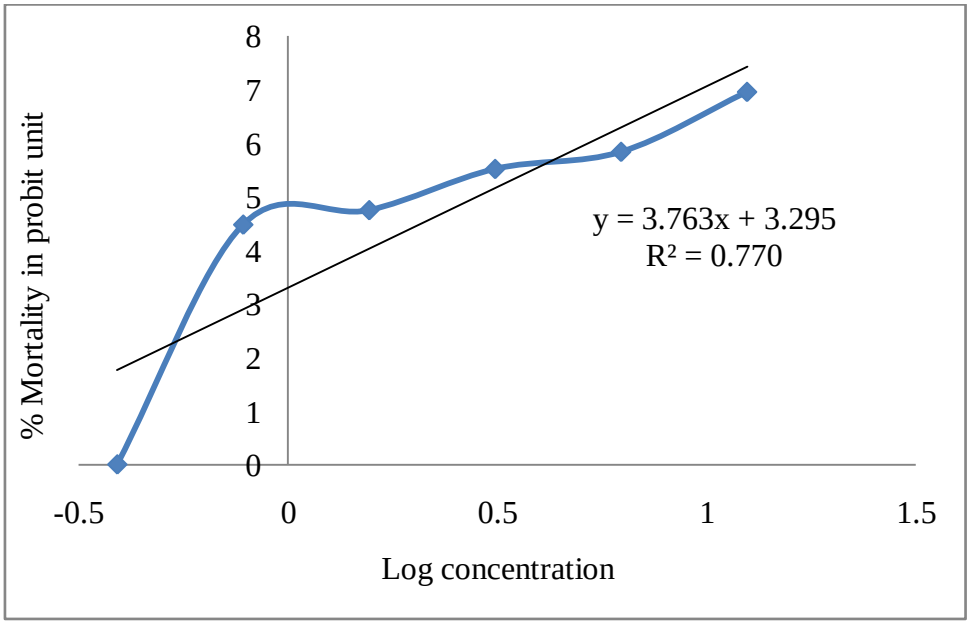


Figure 4.2.2: Effect of methanolic extract of *J. gossypifolia* fruit on brine shrimp nauplii

4.3 Anti Diarrheal Test

Table 4.3.1: Tabular presentation effect of the methanolic extract of *J. gossypifolia* fruit in castor oil induced anti diarrheal test.

Treatment	Dose, Route	Total latent period	No. of stool	Total mean weight of faecal output	% Protection
Control	0.5ml/mouse, p.o.	42.8±1.71	14.4±1.75	0.94±0.02	-
Positive	2mg/kg, p.o.	95.2±1.15***	8.6±0.74**	0.73±0.02***	40.27
JGF	200mg/kg, p.o.	32.2±0.86***	5.6±0.6***	0.25±0.03***	61.11
JGF	400mg/kg, p.o.	60.8±1.68***	5.8±0.37***	0.22±0.02***	59.72

Data is presented as mean ± S.E.M.; at *($p<0.05$) = Significant, **($p<0.01$) = Highly Significant, ***($p<0.001$)= Very Highly Significant compared to control (one-way ANOVA followed by Dunnett's post-hoc test)

Table 4.3.2: Tabular presentation effect of the methanolic extract of *J. gossypifolia* fruit on total mean latent period (min) in castor oil induced anti diarrheal test.

Treatment	Dose, Route	Total latent period (Min)	Std. Deviation	Std. Error	95% Confidence interval	
					Lower	Upper
Control	0.5ml/mouse, p.o.	42.8±1.71	2.58	1.71	90.43	99.96
Positive	2mg/kg, p.o.	95.2±1.15***	3.83	1.15	39.58	46.01
JGF	200mg/kg, p.o.	32.2±0.86***	1.92	0.86	29.81	34.58
JGF	400mg/kg, p.o.	60.8±1.68***	3.76	1.68	56.12	65.47

In castor oil induced anti diarrheal test, the methanolic extract of *J. gossypifolia* fruit at both doses (200 and 400 mg/kg body weight) showed very highly significant ($p<0.001$) increase in total mean latency period induced by castor oil. The percentage of protection of the diarrheal stool by the extract at the doses 200 and 400 mg/kg body weight was 61.11% and 59.72% respectively.

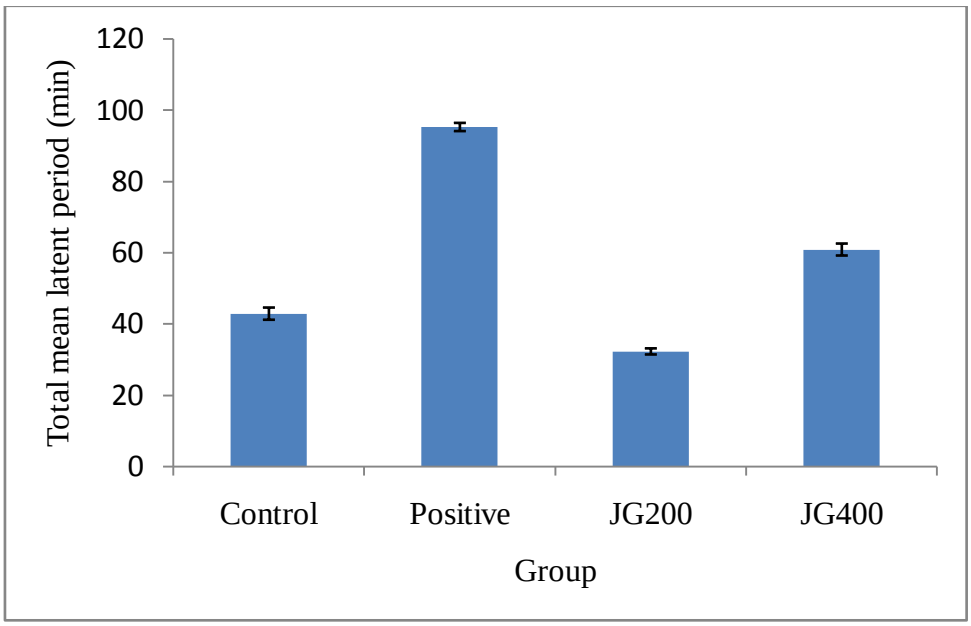


Figure 4.3.1: Graphical presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on total mean latent period in castor oil induced anti diarrheal test

Table 4.3.3: Tabular presentation effect of the methanolic extract of *J. gossypifolia* fruit on mean no. of stool in castor oil induced anti diarrheal test.

Treatment	**Dose, Route**	**Mean no. of stool**	**Std. Deviation**	**Std. Error**	**95% Confidence interval**	
					Lower	**Upper**
Control	0.5ml/mouse, p.o.	14.4±1.75	3.912	1.75	9.54	19.25
Positive	2mg/kg, p.o.	8.6±0.74**	1.673	0.74	6.52	10.67
JGF	200mg/kg, p.o.	5.6±0.6***	1.342	0.60	3.93	7.26
JGF	400mg/kg, p.o.	5.8±0.37***	0.837	0.37	4.76	6.83

Data is presented as mean ± S.E.M.; at $^{*}(p<0.05)$ = Significant, $^{**}(p<0.01)$ = Highly Significant, $^{***}(p<0.001)$= Very Highly Significant compared to control (one-way ANOVA followed by Dunnett's post-hoc test)

In castor oil induced anti diarrheal test, the methanolic extract of *J. gossypifolia* fruit at both doses (200 and 400 mg/kg body weight) showed very highly significant ($p<0.001$) decrease in mean no. of stool induced by castor oil. Methanolic extract of *J. gossypifolia* fruit decreases the mean no. of stool to 5.6 and 5.8 for the dose of 200 and 400 mg/kg respectively where as positive control decreases it to 8.6 only.

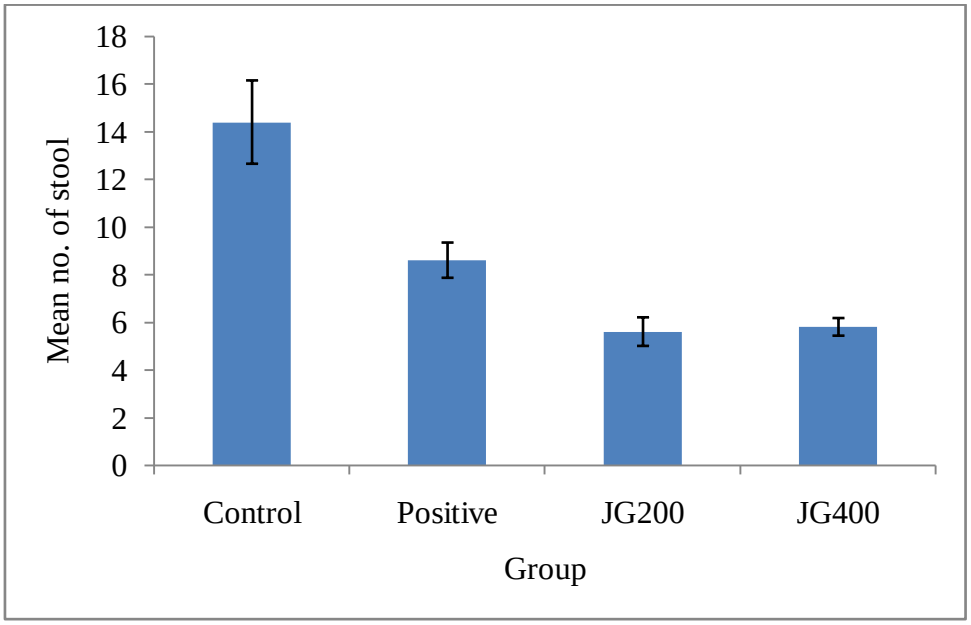


Figure 4.3.2: Graphical presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean no. of stool in castor oil induced anti diarrheal test

Table 4.3.4: Tabular presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on total mean weight of faecal output in castor oil induced anti diarrheal test.

Treatment	Dose, Route	Total mean weight of faecal	Std. Deviation	Std. Error	95% Confidence interval

		output			Lower	Upper
Control	0.5ml/mouse, p.o.	0.94±0.02	0.04	.02	.88	.98
Positive	50mg/kg, p.o.	0.73±0.02***	0.06	.02	.65	.81
JGF	200mg/kg, p.o.	0.25±0.03***	0.06	.03	.16	.32
JGF	400mg/kg, p.o.	0.22±0.02***	0.04	.02	.17	.27

Data is presented as mean ± S.E.M.; at *(p<0.05) = Significant, **(p<0.01) = Highly Significant, ***(p<0.001)= Very Highly Significant compared to control (one-way ANOVA followed by Dunnett's post-hoc test)

In castor oil induced anti diarrheal test, the methanolic extract of *J. gossypifolia* fruit at both doses (200 and 400 mg/kg body weight) showed very highly significant (p<0.001) decrease in total mean weight of faecal output induced by castor oil. Methanolic extract of *J. gossypifolia* fruit decreases the total weight of faecal output to 0.25 and 0.22 for the dose of 200 and 400 mg/kg respectively where as positive control decreases it to 0.73 only.

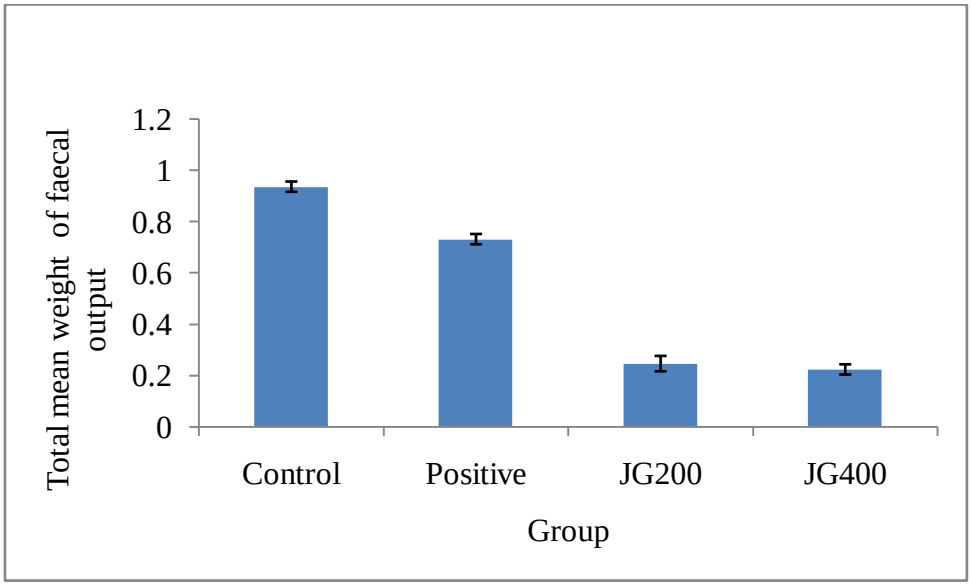


Figure 4.3.3: Graphical presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on total mean weight of faecal output in castor oil induced antidiarrheal test.

4.4 Hole Cross Test

Table 4.4.1: Tabular presentation of the effect of methanolic extract of *J. gossypifolia* fruit on mean number of movements in 0, 30, 60, 90 and 120 minute in hole cross test in mice

Treatment	Dose, route	Mean umber of movements				
		0 min	30 min	60 min	90 min	120 min
Control	0.5ml/mouse, p.o.	10±0.71	8.6±0.6	7.2±0.58	7±0.54	6.2±0.374
Positive	1mg/kg, p.o.	7.8±0.75*	6.2±0.8	5.8±0.7	4.2±1.2	3.6±0.51**
JGF	200mg/kg, p.o.	5.4±0.40**	7.2±0.73	5.4±0.40	4.8±0.8	5.2±0.58
JGF	400mg/kg, p.o.	5±0.44***	6.2±0.58	4±0.31**	2.2±0.37**	2±0.54***

Data is presented as mean ± S.E.M.; at *($p<0.05$) = Significant, **($p<0.01$) = Highly Significant, ***($p<0.001$)= Very Highly Significant compared to control (one-way ANOVA followed by Dunnett's post-hoc test)

In the hole cross test, the methanolic extract of *J. gossypifolia* fruit at both doses (200 and 400 mg/kg body weight) showed very highly significant ($p<0.001$) decrease in mean no. of movements at 0 minute. At 60 and 90 minute the sample showed highly significant ($p<0.01$) and at 120 minute it showed very highly significant decrease in mean no. of movement at dose 400 mg/kg body weight.

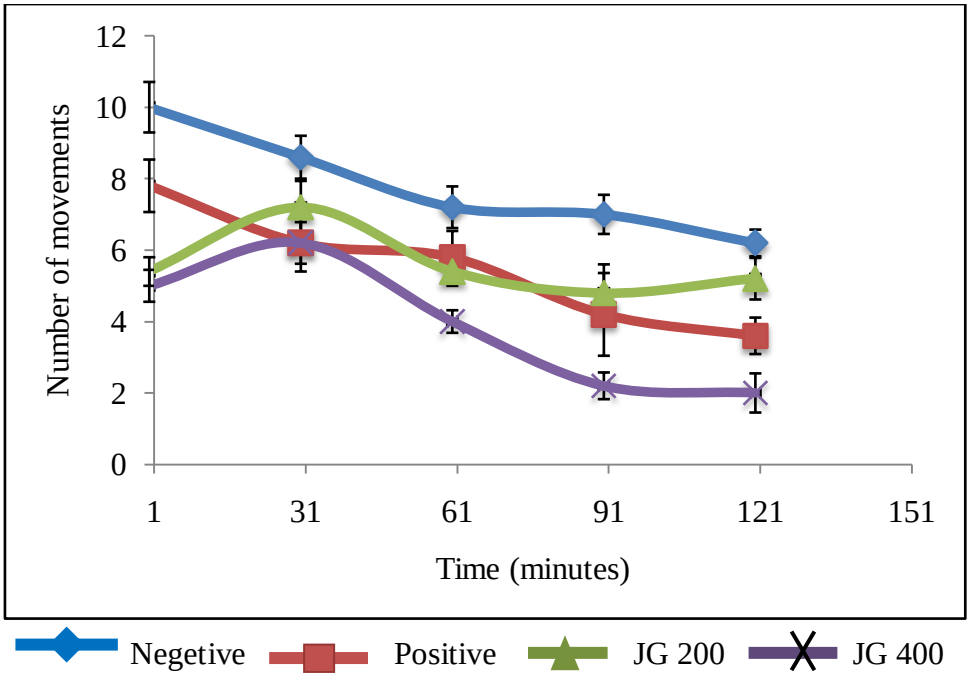


Figure 4.4.1: Graphical presentation of the effect of methanolic extract of *J. gossypifolia* fruit on mean number of movements in 0, 30, 60, 90 and 120 minute in hole cross test in mice

4.5 Hole Board Test

Table 4.5.1: Tabular presentation of the effect of methanolic extract of *J. gossypifolia fruit* in hole board test in mice

Treatment	Dose, Route	Mean no. of head dipping	Mean no. of latency until the first entry
Control	0.5ml/mouse, p.o.	44.8±1.24	14.8±0.37
Positive	1mg/kg, p.o.	29.6± .93***	2±0.45***
JGF	200mg/kg, p.o.	55±2.05***	17.4±1.77***
JGF	400mg/kg, p.o.	44.8±1.46	4.2±0.37***

Data is presented as mean ± S.E.M.; at *($p<0.05$) = Significant, **($p<0.01$) = Highly Significant, ***($p<0.001$)= Very Highly Significant compared to control (one-way ANOVA followed by Dunnett's post-hoc test)

In the board test, the methanolic extract of *J. gossypifolia* fruit at doses of 200 mg/kg body weight showed very highly significant ($p<0.001$) increase in mean no. of head dipping. The sample showed very highly significant ($p<0.001$) increase in mean no. of latency until the first entry at both (200 and 400 mg/kg body weight) doses.

Table 4.5.2: Tabular presentation of the effect of methanolic extract of *J. gossypifolia* fruit on mean no. of head dipping in hole board test in mice

Treatment	**Dose, Route**	**Mean no. of head dipping**	**Std. Deviation**	**Std. Error**	**95% Confidence interval**	
					Lower	**Upper**
Control	0.5ml/mouse, p.o.	44.8	2.77	1.24	41.35	48.24
Positive	1mg/kg, p.o.	29.6	2.07	0.93	27.03	32.17
JGF	200mg/kg, p.o.	55	4.58	2.05	49.31	60.69
JGF	400mg/kg, p.o.	44.8	3.27	1.46	40.73	48.86

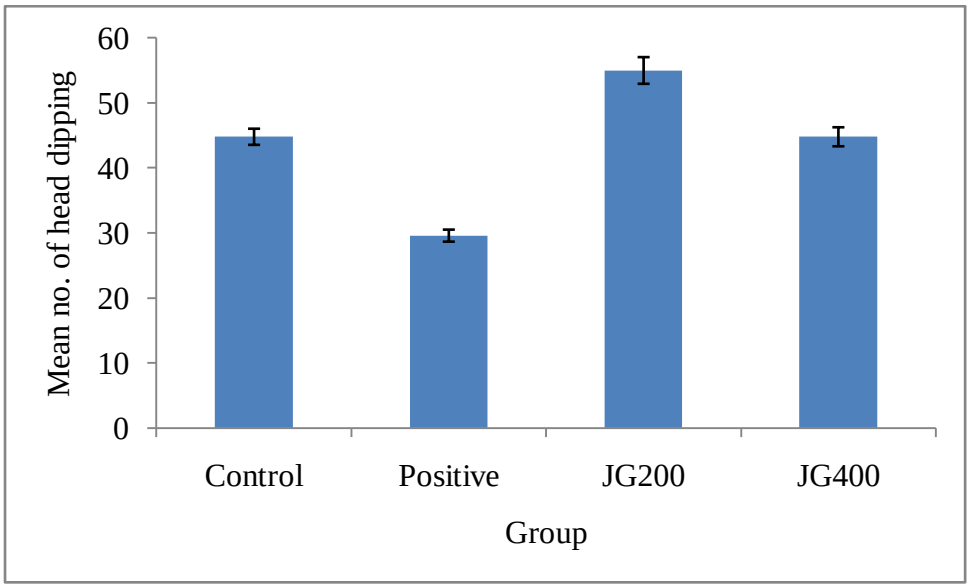


Figure 4.5.1 Graphical presentation of the effect of methanolic extract of *J. gossypifolia* fruit on mean no. of head dipping in hole board test in mice

Table 4.5.3: Tabular presentation of the effect of methanolic extract of *J. gossypifolia* fruit on mean no. of latency until first entry in hole board test in mice

Treatment	Dose, Route	Mean no. of latency until first entry	Std. Deviation	Std. Error	95% Confidence interval	
					Lower	Upper
Control	0.5ml/mouse, p.o.	14.8	0.83	0.37	13.76	15.84
Positive	1mg/kg, p.o.	2	1	0.45	0.76	3.34
JGF	200mg/kg, p.o.	17.4	1.67	1.77	14.46	24.33
JGF	400mg/kg, p.o.	4.2	0.83	.37	3.16	5.23

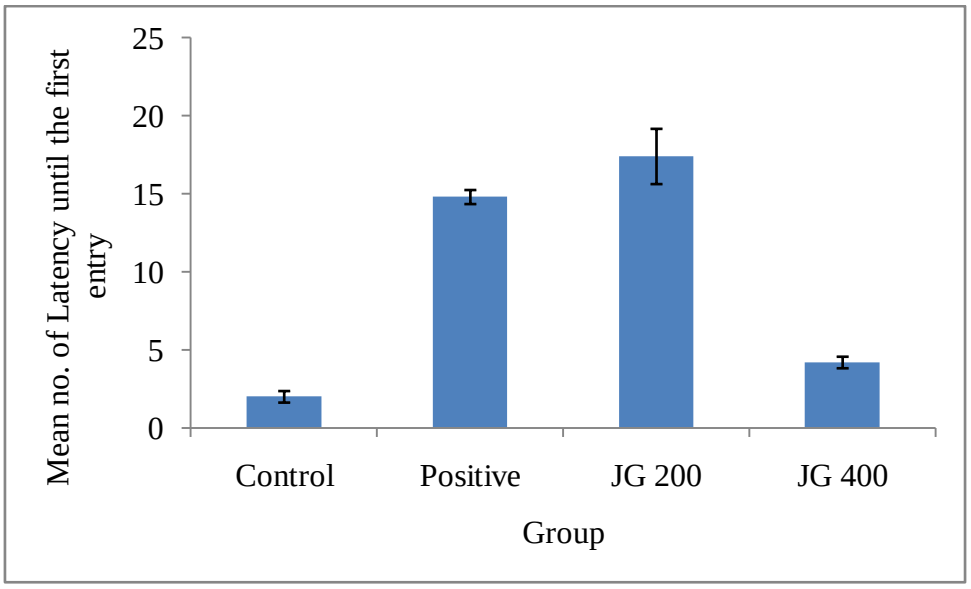


Figure 4.5.2: Graphical presentation of the effect of methanolic extract of *J. gossypifolia* fruit on mean no. of latency until first entry in hole board test in mice

4.6 Elevated Plus Maze Test

Table 4.6.1: Tabular presentation of the effect of the methanolic extract of *J. gossypifolia fruit* on mean no. of rearing in elevated plus maze test.

Treatment	Dose, Route	Mean no. of rearing
Control	0.5ml/mouse, p.o.	14.2±1.65
Positive	1mg/kg, p.o.	9.4±3.37
JGF	200mg/kg, p.o.	8.2±1.68
JGF	400mg/kg, p.o.	16.2±2.74

Data is presented as mean ± S.E.M.; at $^{*}(p<0.05)$ = Significant, $^{**}(p<0.01)$ = Highly Significant, $^{***}(p<0.001)$= Very Highly Significant compared to control (one-way ANOVA followed by Dunnett's post-hoc test)

In the elevated plus maze test, the methanolic extract of *J. gossypifolia* fruit at both (200 and 400 mg/kg body weight) doses showed no significant ($p<0.05$) difference in mean no. of rearing.

Treatment	Dose, Route	Mean no of rearing	Std. Deviation	Std. Error	95% Confidence interval	
					Lower	Upper
Control	0.5ml/mouse, p.o.	14.2	3.70	1.65	9.60	18.79
Positive	1mg/kg, p.o.	9.4	7.53	3.37	.01	18.75
JGF	200mg/kg, p.o.	8.2	3.76	1.63	3.52	12.87
JGF	400mg/kg, p.o.	16.2	6.14	2.74	8.57	23.82

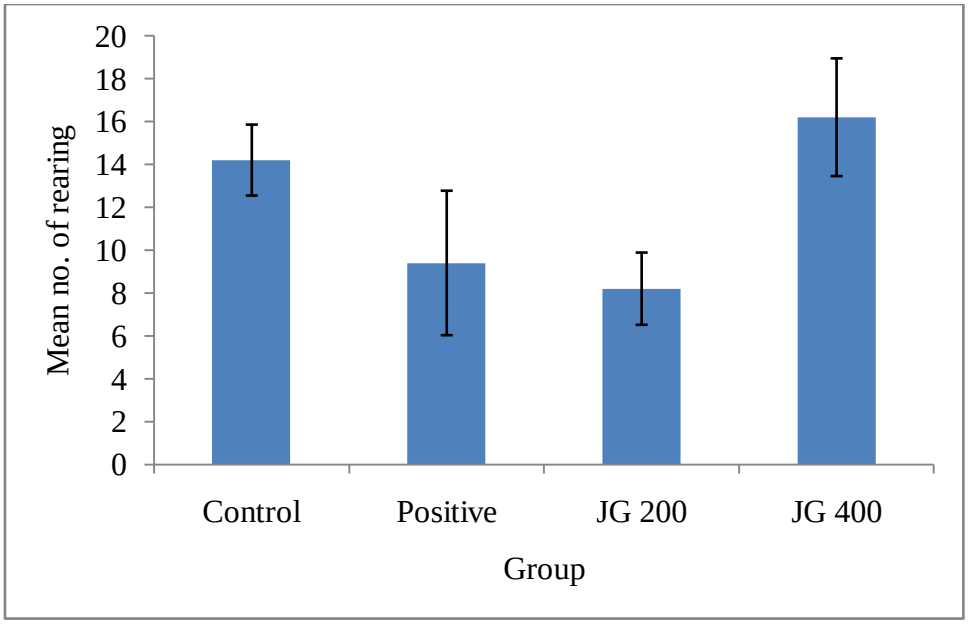


Figure 4.6.1: Graphical presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean no. of rearing in elevated plus maze test.

Table 4.6.2: Tabular presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean no. of grooming in elevated plus maze test.

Treatment	**Dose, Route**	**Mean no. of grooming**
Control	0.5ml/mouse, p.o.	3.8±.58
Positive	1mg/kg, p.o.	6.2±.80
JGF	200mg/kg, p.o.	4.8±.86
JGF	400mg/kg, p.o.	1.0±.77*

Data is presented as mean ± S.E.M.; at *($p<0.05$) = Significant, **($p<0.01$) = Highly Significant, ***($p<0.001$)= Very Highly Significant compared to control (one-way ANOVA followed by Dunnett's post-hoc test)

In the elevated plus maze test, the methanolic extract of *J. gossypifolia* fruit at 400 mg/kg body weight showed significant ($p<0.05$) decrease in mean no. of grooming where as positive control increased mean no. of grooming.

Treatment	Dose, Route	Mean no of grooming	Std. Deviation	Std. Error	95% Confidence interval	
					Lower	Upper
Control	0.5ml/mouse, p.o.	3.8	1.30	.58	2.18	5.41
Positive	1mg/kg, p.o.	6.2	1.78	.80	3.97	8.42
JGF	200mg/kg, p.o.	4.8	1.92	.86	2.41	7.18
JGF	400mg/kg, p.o.	1.0	1.73	.77	-1.15	3.15

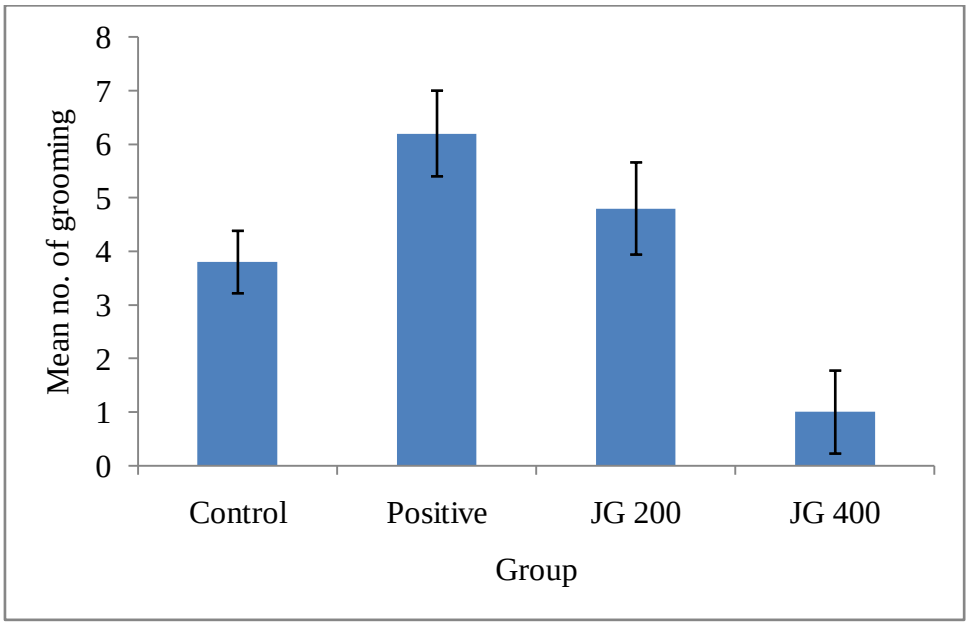


Figure 4.6.2: Graphical presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean no. of grooming in elevated plus maze test.

Table 4.6.3: Tabular presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean no. of stretch attend postures in elevated plus maze test.

Treatment	Dose, Route	Mean no. of stretch attend postures
Control	0.5ml/mouse, p.o.	9.20±1.43
Positive	1mg/kg, p.o.	8.60±2.29
JGF	200mg/kg, p.o.	13.80±2.08
JGF	400mg/kg, p.o.	8.80±1.46

Data is presented as mean ± S.E.M.; at $^{*}(p<0.05)$ = Significant, $^{**}(p<0.01)$ = Highly Significant, $^{***}(p<0.001)$= Very Highly Significant compared to control (one-way ANOVA followed by Dunnett's post-hoc test)

In the elevated plus maze test, the methanolic extract of *J. gossypifolia* fruit at both (200 and 400 mg/kg body weight) doses showed no significant difference in mean no. of stretch attend postures.

Treatment	Dose, Route	Mean no. of stretch attend postures	Std. Deviation	Std. Error	95% Confidence interval	
					Lower	Upper
Control	0.5ml/mouse, p.o.	9.20	3.19	1.42	5.23	13.16
Positive	1mg/kg, p.o.	8.60	5.12	2.29	2.23	14.96
JGF	200mg/kg, p.o.	13.80	4.65	2.08	8.09	19.58
JGF	400mg/kg, p.o.	8.80	3.27	1.46	4.73	12.86

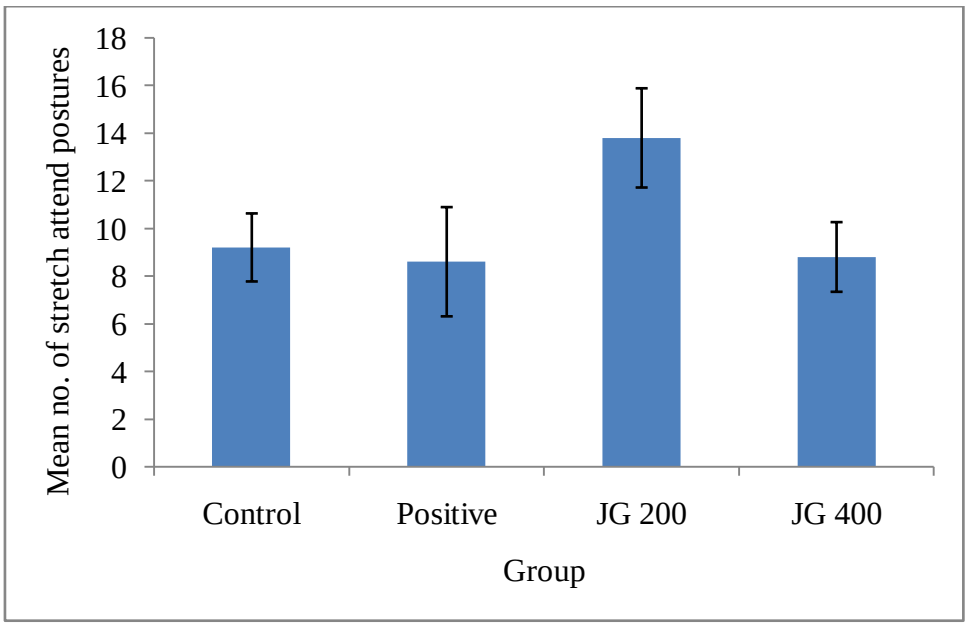


Figure 4.6.3: Graphical presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean no. of stretch attend postures in elevated plus maze test.

Table 4.6.4: Tabular presentation of the effect of the methanolic extract of *J. gossypifolia* fruit in elevated plus maze test.

Treatment	Dose, Route	Mean no of entries in		
		Open arm	Close arm	Center
Control	0.5ml/mouse, p.o.	1.20±0.49	9.40±1.91	4.20±0.20
Positive	1mg/kg, p.o.	1.60±0.81	12.00±3.03	4.60±0.75
JGF	200mg/kg, p.o.	1.60±1.03	7.80±1.83	3.00±1.09
JGF	400mg/kg, p.o.	2.40±1.17	10.80±0.97	7.00±1.64

Data is presented as mean ± S.E.M.; at $^{*}(p<0.05)$ = Significant, $^{**}(p<0.01)$ = Highly Significant, $^{***}(p<0.001)$= Very Highly Significant compared to control (one-way ANOVA followed by Dunnett's post-hoc test)

In the elevated plus maze test, the methanolic extract of *J. gossypifolia* fruit at 400mg/kg body weight increased the mean no. of entries in open arm and in center. But this difference is not statistically significant ($p<0.05$).

Table 4.6.5: Tabular presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean no. of entries in open arm in elevated plus maze test

Treatment	Dose, Route	Mean no. of entries in open arm	Std. Deviation	Std. Error	95% Confidence interval	
					Lower	Upper
Control	0.5ml/mouse, p.o.	1.20	1.09	.48	-0.16	2.56
Positive	1mg/kg, p.o.	1.60	1.81	.81	-0.65	3.85
JGF	200mg/kg, p.o.	1.60	2.17	1.02	-1.25	4.45
JGF	400mg/kg, p.o.	2.40	2.60	1.16	-.83	5.69

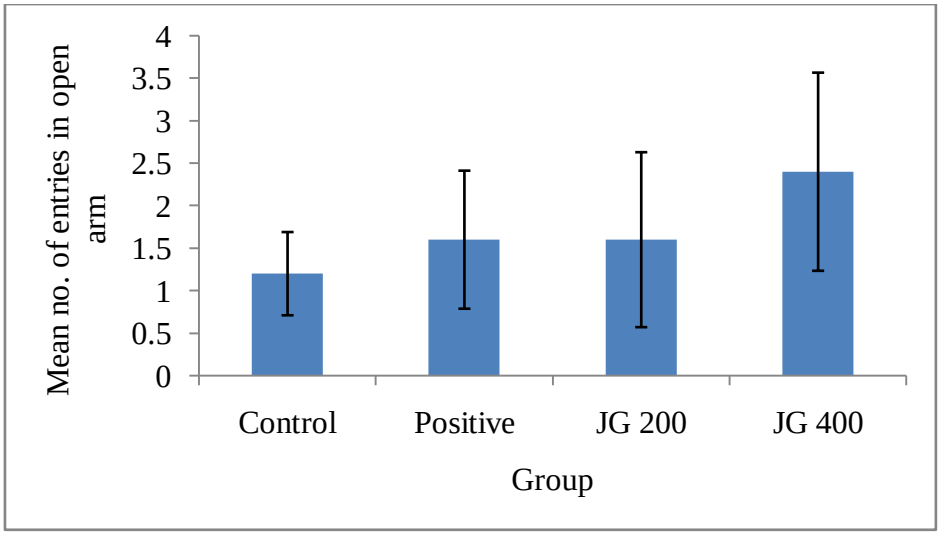


Figure 4.6.4: Graphical presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean no. of entries in open arm in elevated plus maze test.

Table 4.6.6: Tabular presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean no. of entries in close arm in elevated plus maze test

Treatment	**Dose, Route**	**Mean no of entries in close arm**	**Std. Deviation**	**Std. Error**	**95% Confidence interval**	
					Lower	**Upper**
Control	0.5ml/mouse, p.o.	9.40	4.27	1.91	4.08	14.77
Positive	1mg/kg, p.o.	12.00	6.73	3.03	3.57	20.42
JGF	200mg/kg, p.o.	7.80	4.08	1.82	2.72	12.87
JGF	400mg/kg, p.o.	10.80	2.16	.96	8.11	13.49

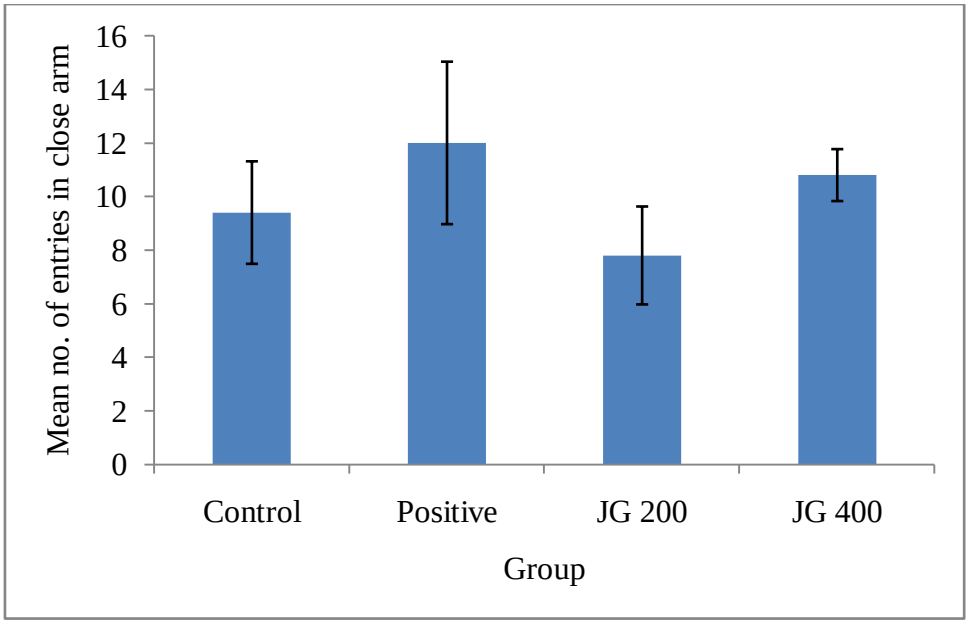


Figure 4.6.5: Graphical presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean no. of entries in close arm in elevated plus maze test

Table 4.6.7: Tabular presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean no. of entries in center arm in elevated plus maze test

Treatment	**Dose, Route**	**Mean no of entries in center**	**Std. Deviation**	**Std. Error**	**95% Confidence interval**	
					Lower	**Upper**
Control	0.5ml/mouse, p.o.	4.20	0.44	0.25	3.64	4.77
Positive	1mg/kg, p.o.	4.60	1.67	0.74	2.52	6.67
JGF	200mg/kg, p.o.	3.00	2.44	1.09	-0.04	6.04
JGF	400mg/kg, p.o.	7.00	3.67	1.64	2.43	11.56

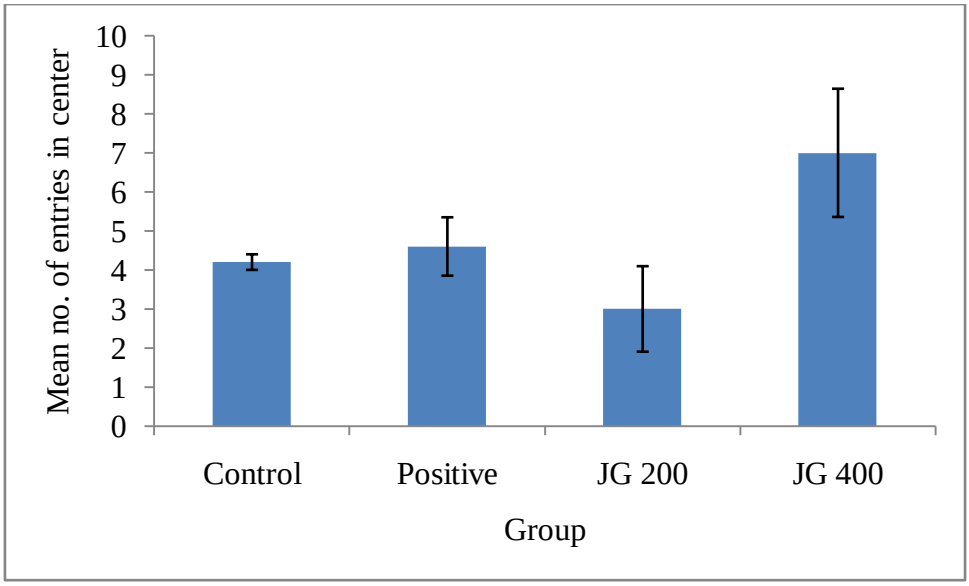


Figure 4.6.6: Graphical presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean no. of entries in center arm in elevated plus maze test

Table 4.6.8: Tabular presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean time spent in open and close arms in elevated plus maze test.

Treatment	**Dose, Route**	**Mean time (seconds) spent in**	
		Open arm	**Close arm**
Control	0.5ml/mouse, p.o.	7.60±3.20	215.00±19.76
Positive	1mg/kg, p.o.	6.80±2.81	260.00±12.95
JGF	200mg/kg, p.o.	8.40±5.23	202.00±25.12
JGF	400mg/kg, p.o.	24.20±5.34*	204.20±19.58

Data is presented as mean ± S.E.M.; at *($p<0.05$) = Significant, **($p<0.01$) = Highly Significant, ***($p<0.001$)= Very Highly Significant compared to control (one-way ANOVA followed by Dunnett's post-hoc test)

In the elevated plus maze test, the methanolic extract of *J. gossypifolia* fruit at the dose of 400 mg/kg body weight showed significant ($p<0.05$) increase in mean time spent in open arm.

Table 4.6.9: Tabular presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean time spent in open arm in elevated plus maze test.

Treatment	Dose, Route	Mean time (seconds) spent in open arm	Std. Deviation	Std. Error	95% Confidence interval	
					Lower	Upper
Control	0.5ml/mouse, p.o.	7.60	7.16	3.20	-1.29	16.49
Positive	1mg/kg, p.o.	6.80	6.30	2.81	-1.02	14.62
JGF	200mg/kg, p.o.	8.40	11.69	5.23	-6.12	22.92
JGF	400mg/kg, p.o.	24.20	11.94	5.34	9.36	39.03

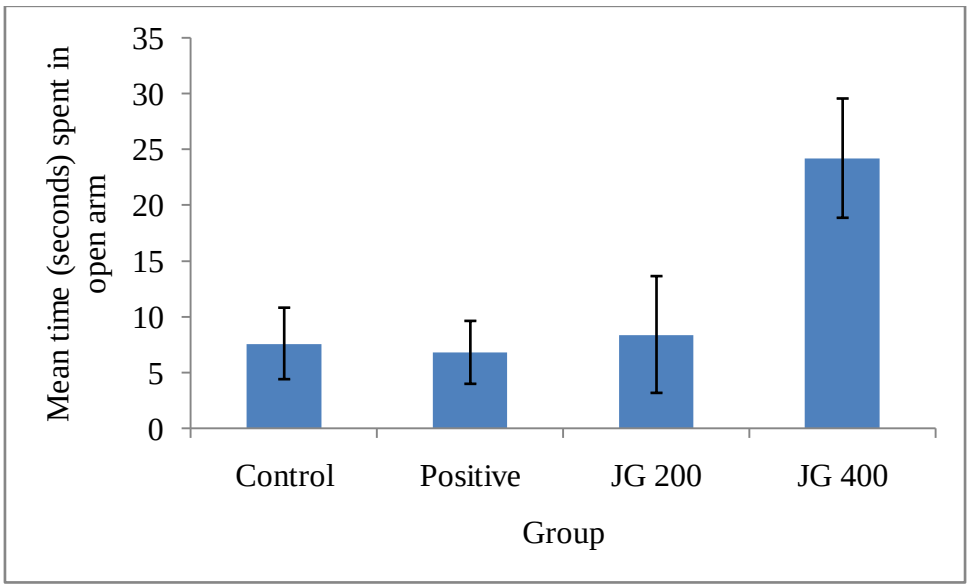


Figure 4.6.7: Graphical presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean time (seconds) spent in open arm in elevated plus maze test

Table 4.6.10: Tabular presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean time spent in open and close arms in elevated plus maze test.

Treatment	Dose, Route	Mean time (seconds) spent in close arm	Std. Deviation	Std. Error	95% Confidence interval	
					Lower	Upper
Control	0.5ml/mouse, p.o.	215.00	44.17	19.76	160.13	269.86
Positive	1mg/kg, p.o.	260.00	28.97	12.95	224.02	295.97
JGF	200mg/kg, p.o.	202.00	56.17	25.12	132.24	271.75
JGF	400mg/kg, p.o.	204.20	43.80	19.58	149.81	258.58

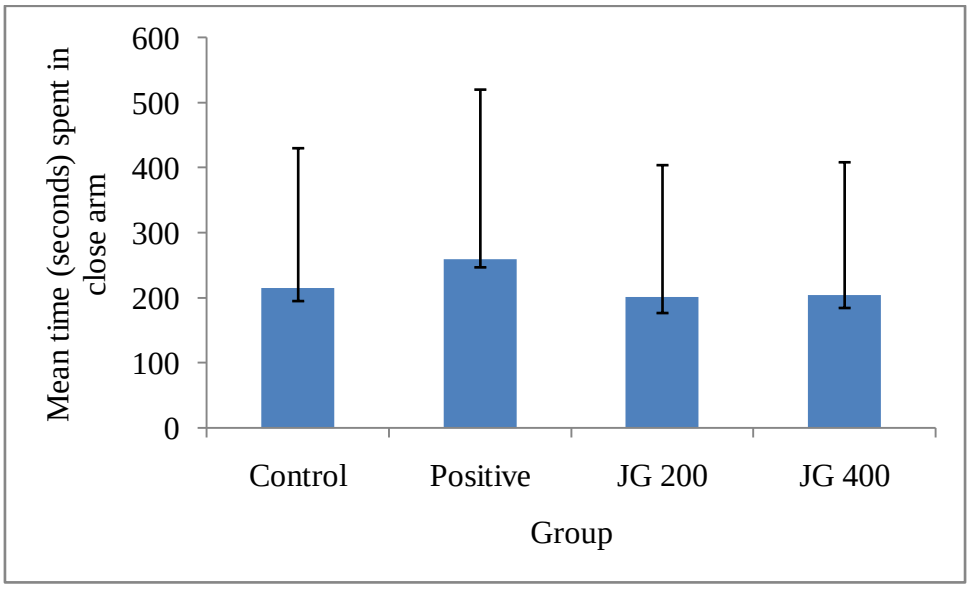


Figure 4.6.8: Graphical presentation of the effect of the methanolic extract of *J. gossypifolia* fruit on mean time (seconds) spent in close arm in elevated plus maze test

Chapter 5

Discussion

DISCUSSION

5.1 Acetic Acid Induced Writhing Test

The methanolic fruit extract of *J. gossypifolia* exerted a very highly significant ($p<0.001$) decrease in writhing response compared to control in a dose dependent manner. The results obtained in the analgesic test experiments showed that the methanolic fruit extract of *J. gossypifolia* inhibited the writhing response induced by acetic acid, which suggests that sample possesses both centrally and peripherally mediated analgesic properties. The central analgesic action may be mediated via inhibition of central pain receptors, while the peripheral analgesic effect may be mediated through inhibition of cyclooxygenase and or lipoxygenase and other inflammatory mediators (Koster R *et al.,* 1959). This hypothesis is postulated by the previous reports that acetic acid writhing methods are useful techniques for the evaluation of centrally and peripherally acting analgesic drugs. The writhing responses were inhibited to 77.86% and 71.25% at the doses 200 and 400 mg/kg body weigh respectively.

5.2 Brine Shrimp Lethality Bioassay

The lowest LC_{50} values of the methanolic fruit extract of *J. gossypifolia* 2.84 µg/ml (Table 4.2). This significant lethality of the crude plant extracts (LC_{50}<100 PPM or µg/ml) to brine shrimp is indicative of the presence of potent cytotoxic compounds indicates the presence of potential candidates of cytotoxic compounds (Peteros NP and Uy MM., 2010). From the literature we found that *J. gossypifolia* contains a compound jatrophone which has cytotoxic activity ED_{50} value is 0.01µg/ml *in vitro* against the P-388 lymphocytic leukemia test (Yang, 2003). So this noticeable lethality is probably due to presence of jatrophone. Comparison of cytotoxicity exhibited by the synthesized compounds with positive control signifies that further bioactivity guided investigation can be done to find out potent antitumor (Montanher *et al*, 2002) and pesticidal (Pisutthanan *et al*, 2004) compounds. These

compounds should be submitted to as wide a range of bioassays as possible. However, further bioassays can be conducted to get cytotoxicity on cancer cell lines.

5.3 Anti-Diarrheal Activity Test

The crude methanolic extract of *J. gossypifolia* fruit showed very highly significant (p<0.001) antidiarrheal activity compared to the negative control at both the doses (200 & 400 mg/kg body weight) in a dose dependent manner throughout the observation period.

Preliminary qualitative phytochemical screening reveals the presence of saponins, flavonoids, steroids resins and tannins in *J. gossypifolia* (Ghani, 2003; Rastogi and Mehrotra, 1993). Tannins can evoke an antidiarrhoeal effect since these substances may precipitate proteins of the enterocytes; reduce peristaltic movement and intestinal secretions (Okudo *et al.*, 1989).

On the basis of the result of castor oil induced diarrhea, it can be concluded that the crude methanolic fruit extract of *J. gossypifolia* possesses very highly significant (p<0.001) anti-diarrheal activity.

5.4 Hole Cross Test

As shown in the data the extract at both the doses produced a reduction in spontaneous motor activity and this effect may be attributed to CNS depression. The locomotor activity is a test to appraise the level of excitability of the CNS and any decrease of this activity may be narrowly related to sedation resulting from depression of the central nervous system. The sedative effect recorded here may be related to an interaction with benzodiazepines related compounds that binds to receptors in the CNS and have already been identified in certain plant extracts. Literature review of the plant reveals that *J. gossypifolia* contains flavonoids, steroids and tannins (Ghani, 2003; Rastogi and Mehrotra, 1993). Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the CNS. Different anxiolytic, sedative

hypnotic drugs elucidate their action through GABAA, therefore it is possible that extract of *J. gossypifolia* fruit may act by potentiating GABAergic inhibition in the CNS or may be due to the activation of GABA receptor by the extracts. Earlier investigation on phytoconstituents and plants suggests that many flavanoids, and steroids were found to be the ligands for GABAA receptors in the CNS which led to the assumption that they can act like benzodiazepine-like molecules (Fernandez *et al*., 2006). So, it is probable that phytoconstituents in this extract is responsible for its CNS activity.

5.5 Hole Board Test

Studies have shown that benzodiazepine receptor agonists produce behavioural changes consistent with anxiety reduction while enhancing exploratory head-dipping (Rodgers *et al*., 1997; Johnson and Rodgers, 1996). In agreement with this assertion, in this study, the methanolic fruit extract of *J. gossypifolia* at anxiolytic doses of 200 and 400 mg/kg increased the number of head-dips in a comparable manner to control. The effects of the sample at 200mg/kg body weight dose and the standard drug Diazepam, 1mg/kg were very highly significant ($p<0.001$). File and Wardill (1975) reported that suppression of exploratory behavior is an indication of CNS depressant activity; hence at sedative doses of 200mg/kg, the number of head-dips was increased in a very highly significant manner.

The extract produced a very highly significant ($p<0.001$) decrease in latency time period at both (200 and 400mg/kg body weight) doses levels and was more pronounced when compared to a control. This indicates a decrease in the curiosity or exploratory behavior of test animals and also provides evidence in favor of a anxiolytic action (Sonavane *et al*., 2001; Suba *et al*., 2002). Preliminary phytochemical analysis in this study revealed the presence of alkaloids, flavanoids, tannin, resin, saponin, terpene. These secondary metabolites, individually or in combination would account for the observed pharmacological effects of this plant in this study.

5.6 Elevated Plus Maze Test

In elevated plus maze test, the methanolic fruit extract of *J. gossypifolia* at doses of 200 and 400mg/kg showed anxiolytic property by increasing the cumulative time spent in the open arm. At 400mg/kg dose the effect was significantly ($p<0.05$) different compared to control from that produced by diazepam, 1 mg/kg. The open arm – closed arm approach for screening for anxiolytic effect has worked well in identifying the anxiolytic potential of benzodiazepine/$GABA_A$ receptor-related agents while not being reliable in detecting anti-anxiety effects through unrelated mechanisms (Rodgers *et al*., 1997). The regulation of anxiety is associated with function of the $GABA_A$ receptor system. Available evidence points to a major role for α2-containing $GABA_A$ receptors in modulating anxiety, although a recent study also suggests a possible implication for α3 and α5 subunit (Navarro *et al.*, 2002). Standard drug diazepam act selectively on $GABA_A$ receptors which mediate fast inhibitory synaptic transmission throughout the CNS. Benzodiazepines (BDZs) bind to the γ sub-unit of the $GABA_A$ receptor that causes an allosteric (structural) modification of the receptor results with an increase in $GABA_A$ receptor activity (Rang *et al.*, 2003).

Chapter 6

Conclusion

CONCLUSION

The presence of several phytochemical compounds in *J. gossypifolia* makes the plant pharmacologically active. The analgesic activity of the fruit extract may be responsible for its use in the pain management and treatment of various diseases. It has sedative activity that could be a better treatment in severe pain. The fruit possess very good antidiarrheal activity that could make it a potent drug for diarrheal treatment. Since there present some cytotoxic compound, the study suggests the fruit extract to be used as natural remedy in diarrhea treatment in a dose dependent manner.

References

REFERENCES

Abayomi S. Historical review of traditional medicine in Africa, Spectrum Book ltd. 1993. p. 9-25.

Aiyelero OM., Abdu-Aguye SN, Yaro AH, Magaji MG. Behavioural studies on the methanol leaf extract of *Securinega virosa* (Euphorbiaceae) in mice. J. Pharmacogn. Phytother. 2012; 4(2): 12-15.

Alam MI, Auddy B, Gomes A. Isolation and partial characterization of viper venom inhibiting factor from the root extract of the Indian medicinal plant sarsaparilla (*Hemidesmus indicus* R.Br.). 1994; 32: 1551–1557.

Artuso A. Drugs of Natural Origin: Economic and policy aspects of discovery, development, and marketing. Pharmaceutical Products Press, New York. 1997.

Avindranath NR, Enkataiah BV, Amesh CR, Ayaprakash PJ, and Da B. Jatrophenone, a Novel Macrocyclic Bioactive Diterpene from *Jatropha gossypifolia*. Chem. Pharm. Bull. 2003; 51(7): 870-871.

Bandow JE, Brotz H, Leichert LIO, Labischinski H and Hecker M. Proteomic approach to understanding antibiotic action, Antimicrobial agents. Chemotherapy. 2003; 47: 948-955.

Chatterjee A, Das B, Adityachaudhury N, and Debkirtaniya S. Note on the insecticidal properties of the seeds of *Jatropha gossypifolia* Linn. Indian J. Agric. Sci. 1980; 50(8): 637-638.

Chatterjee I, Chakravarty AK, Gomesa A, Daboia russellii and Naja kaouthia venom neutralization by lupeol acetate isolated from the root extract of Indian sarsaparilla *Hemidesmus indicus* R.Br . J. Ethnopharmacol. 2006; 106(1), 38-43.

Chaudhary SA, Gadhvi KV, Chaudhary AB. Comprehensive review on world herb trade and most utilized medicinal plant. Int. J. Ap Bio. Pharma. Tech. 2010; 1(2): 510-517.

Chopra RN, Nayar SL and ChopraLC. In Glossary of Indian medicinal plants. Council of Scientific and Industrial Research, New Delhi. 1956; 1: 197.

Colombo ML and Bosisio E. Pharmacological activites of chelidonium majus L (papveraceae), Pharmacol. Res.1996; 33: 127-134.

Das B and Anjani G. Gossypidien- a lignan from stems of *Jatropha gossypifollia.* Phytochemistry. 1999; 51: 115-117.

Das B and Das R. Gossypifan- A lignin from *Jatropha gossypifolia.* Phytochemistry. 1995; 40(3): 931-932.

Das B, Kashinatham A, Venkataiah AB, Srinivas KVNS, Mahender G, and Reddy MR. Cleomiscosin A, a coumarino-lignoid from *Jatropha gossypifolia.* Biochem. Syst. Ecol. 2003; 31: 1189–1191.

Das B, Rao P. Srinivas KVNS. Das R. Jatordien a lignin from stem of *Jatropha gossypifolia.* Phytochemistry. 1996; 41(3): 985-987.

Das B, Venkataiaha B, and Kashinathama A. Venkatasin, A New Coumarino-Lignoid From *Jatropha gossypifollia.* Nat. Prod. Lett. 1999; 13(4): 293-297.

Devappa RK, Makkar HPS, and Becker K. Jatropha diterpenes: A review. J Am Oil Chem Soc. 2011; 88: 301–322.

El-seedi HR, Ohara T, Sata N and Nishiyama S. Antimicrobial terpenoids from Eupatorium glutinosum (Asteraceae), J. Ethnopharmacol. 2002; 81:293-296.

Farnsworth NR, Bingel AS. Problems and prospects of discovery new drugs from higher plants by pharmacological screening. In: H.Wagner and P.Wolff (eds.), New Natural products and plant drugs with pharmacological, biological and therapeutical activity, Springer Verlag, Berlin 1997: 1-22.

Farnsworth NR, Blowster RN, Darmratoski D, Meer WA, Cammarat LV. Studies on Catharanthus alkaloids IV Evaluation by means of TLC and ammonium sulphate spray reagent, Lloydia. 1967; 27: 302-314.

Fernandez SP, Wasowski C, Loscalzo LM, Granger RE, Johnston GAR, Paladini AC, Marder M. Central nervous system depressant action of flavonoid glycosides. Eur. J. Pharmacol. 2006; 539: 168-176.

File S, Pellow S. The effect of triazolobenzodiazepines in two animal tests of anxiety and on the hole–board. Br. J. Pharmacol. 1985; 86: 729-735.

Guette CA, Baraguey C, Blond A, Pousset JL, and Bodo B. Cyclogossine B, a Cyclic Octapeptide from *Jatropha gossypifolia*. J. Nat. Prod. 1997; 60 (11): 1155–1157.

Hargreaves K, Dubner R, Brown F, Flores C and Joris J . A New and Sensitive Method for Measuring Thermal Nociception in Cutaneous Hyper alagesia. Pain. 1988; 32: 77-88.

Handerwerker HO and Brune K. Classical German Contributions to Pain Research. 1987.

Herborn JB. Phytochemical methods, A guide to modern techniques of plant analysis, 1998. 2nd ed. p. 5-11.

Hosamani KM and Katagi KS. Characterization and structure elucidation of 2-hydroxyoctadec-cis-9-enoicacidin *Jatropha gossypifolia* and *Hevea brasiliensis* seed oils: a rich source of hydroxy fatty acid. Chem. Phys. Lipids. 2008; 112: 9-12.

Horsten SFAJ, van den Berg AJJ, Kettenes JJ, Leeflang BR, and Labadie RP. Cyclogossine A: A Novel Cyclic Heptapeptide Isolated from the Latex of *Jatropha gossypifolia*. Planta Med. 1996; 62(1): 46-50.

Johnson NJT, Rodgers RJ. Ethological analysis of cholecystokinin (CCKA and CCKB) receptor ligands in the elevated plus-maze test of anxiety in mice. Psychopharmacology. 1996; 124: 355-364.

Jourdan D, Ardid D, Bardin L, Bardin M, Neuzeret D and Lanphouthacoul L. A New Method of Pain Scoring in the Formalin Test in Rats. Pain. 1997; 71: 265-270.

Kamboj V. Herbal medicine. Cur. Sc. 2000; 78(1): 35-39.

Kavithaa J, Rajasekhara D, and Subbaraju GV. Synthesis of Tetradecyl (E)-Ferulate, A Metabolite of *Jatropha gossypifolia*. J. Asian Nat. Prod. Res. 1999; 2(1): 51-54.

Khumrungsee N, Bullangpoti V, and Pluempanupat W. Efficiency of *Jatropha gossypifolia L*. (Euphorbiaceae) Against Spodoptera exigua HÜbner (Lepidoptera: Noctuidae): Toxicity and its Detoxifying Enzyme Activities. KKU Sci. J. 2009; 37: 50-55.

Kicklighter CE, Kubanek J, Barsby T, Hay ME. Palatability and defense of some tropical infaunal worms: alkylpyrrole sulfamates as deterrents to fish feeding. Mar Ecol Prog Ser. 2003; 263: 299-306.

Koster R, Anderson M and Beer EJ. Acetic acid for analgesic screening. *Fed. Proc*. 1959; 18: 412.

Levetin and Mahon M. Plants and Society. 3rd ed.; 2003.

Loeser JD, Melzack R. Pain: an overview. CrossRefMedlineWeb of Science. 1999; 353: 1607–9.

Misra MM and Amarendra MN. Jatropha: The Biodiesel Plant Biology, Tissue Culture and Genetic Transformation – A Review. Int. J. Pure Appl. Sci. Technol.2010; 1(1): 11-24.

Montanher ABP, Pizolatti MG, Brighente IMC. An application of the brine shrimp bioassay for general screening of brazilian medicinal plants. Acta Farm Bonaer 2002; 1: 175-178.

Navarro JS, Buron E, Lopez MM. Effect of SB-205384, a positive modulator of α3-subunit containing $GABA_A$ receptors on isolation induced aggression in male mice. Psicothema. 2002; 20: 144-147.

Newman DJ, Cragg GM, Snader KM. Natural products of new drugs over the period 1981–2002. J Nat Prod. 2003; 66: 1022–1037.

Oduola T, Adeosun GO, Oduola TA, Avwioro AG, and Oyeniyi MA. Mechanism of action of jatropha gossypifolia stems latex as a haemostatic agent. Eur J Gen Med. 2005; 2(4): 140-143.

Oduola T, Avwioro OG, and Ayanniy TB. Suitability of the leaf extract of Jatropha gossypifolia as an anticoagulant for biochemical and haematological analyses. Afr. J. Biotechnol. 2005; 4 (7): 679-681.

Oduola T, Popoola GB, Avwioro OG, Oduola, TA, Ademosun AA, and Lawal MO. Use of Jatropha gossypifolia stem latex as a haemostatic agent: how safe is it? J. Med. Plants Res. 2007; 1 (1): 014 – 017.

Okuda T, Yoshida T and Hatano T. Hydrolyzable tannins and related polyphenols. Progress in the chemistry og organic natural products. 1995; 66: 1-117.

Panda Bb, Gaur K, Nema Rk, Sharma Cs, Jain Ak, and Jai Cp. Hepatoprotective Activity Of Jatropha Gossypifolia Against Carbon Tetrachloride- Induced Hepatic Injury In Rats. Asian J. Pharm. Clin. Res. 2009; 2(1): 50-54.

Parsons WT and Cuthbertson EG. Noxious weeds of Australia. Inkata press, Melbourne; 1992.

Peirce J. Declared plant control handbook. 5th ed. Miscellaneous publication 4/98; 1998.

Perez GRM, Perez IJA, Garcia D, Sossa MH. Neuropharmacological activity of *Solanum nigrum* fruit. *J. Ethnopharmacol.*1998; 62: 43-48.

Perumal SR, Ignacimuthu S. Screening of 34 Indian medicinal plants for antibacterial properties. J. Ethnopharmacol. 1998; 62: 173-182.

Peteros NP, Uy MM. Antioxidant and cytotoxic activities and phytochemical screening of our Philippine medicinal plants. *J. Med. Plants Res*. 2010; 4(5): 407-414.

Pitt JL and Miller IL. The pest status and chemical control of *Jatropha gossypifolia* in the Northern Territory, Australia. Proceedings of the 13th Asian-Pacific Weed Science Society Conference, Jakarta; 1991.

Ravindranath N, Venkataiah B, Amesh CR, J Ayaprakash PJ, DAS B. Jatrophenone, a novel macrocyclic bioactive diterpene from *Jatropha gossypifolia*. Chem. Pharm. Bull. 2003; 51(7): 870—871

Reddi EUB and Reddi CS. Pollination ecology of *Jatropha gossypifolia* (Euphorbiaceae).

Pisutthanan S, Plianbangchang P, Pisutthanan N, Ruanruay S, Muanrit O. Brine shrimp Lethality Activity of Thai Medicinal Plants in the Family Meliaceae. Naresuan University Journal 2004; 12(2): 13-18.

Proceedings of the Indian Academy of Sciences (Plant Sciences). 1983; 92: 215- 231.

Robineau L. Towards a Carribbean pharmacopoeia, TRAMIL 4 Workshop: Scientific Research and Popular Use of Medical plants in the Caribbean. Santo Damingo, DO: Enda-caribe UNAH, 1991

Rodgers RJ, Cao BJ, Dalvi A, Holmes A. Animal models of anxiety: an ethological perspective. *Braz J. Med. Biol. Res.*1997; 30: 289-304.

Scazzocchio F, Comets MF. Tomassini L and Palmery M. Antibacterial activity of Hydrastis Canadensis extract and its major isolated alkaloids, Plants med. 2001; 67: 561-563.

Servin SC, Torres OJ, Matias JE, Agulham MA, de Carvalho FA, Lemos R, Soares EW, Soltoski PR and de Freitas AC. Effects of *Jatropha gossypiifolia* L. (bellyache bush) extract

on the healing process of colonic anastomosis: experimental study in rats. Acta Cir Bras. 2006; 21(3): 89-96.

Snehi SK, Khan MS, Raj SK, and Prasad V. Complete nucleotide sequence of Croton yellow vein mosaic virus and DNA-β associated with yellow vein mosaic disease of Jatropha gossypifolia in India. Virus Genes. 2011; 43: 93-101

Sofowora A. Medicinal Plants and Traditional Medicine in Africa, John Wiley and Sons Ltd., Chichester. New York. Toronto.1982; p. 6,10,11,74,114,256.

Sonavane, G, Sarveeiya V, Kasture Vand Kasture SB. Behavioural actions of *Myristica fragrans* seeds. Ind. J. Pharmacol. 2001; 33: 417-424.

Suba V, Murugesan T, Rao RB, Pal M, Mandal SC and Saha BP. Neuropharmalogical profile of *Barleria lupulina* Lindl. Extract in animal models. J. Ethnopharmacol. 2002; 81: 251-255.

Taylor M, Smith A, and Furst G. New antileukemic Jatrophone derivatives from *Jatropha gossypifolia*:Structural and stereochemical assignment through nuclear magnetic resonance spectroscopy. J. Am. Chem. Soc. 1983; 105(10): 4.

The State of Queensland, Department of Employment, Economic Development and Innovation. Bellyache bush *Jatropha gossypiifolia*; 2011.

Wheeler JR. Flora of the Kimberley Region 2nd ed. Western Australia Herbarium; 1992.

Whittle BA. The use of changes in capillary permeability in mice to distinguish between narcotic and non narcotic analgesics. *Br.J. Pharmacol. Chemotherp*. 1964; 22: 246-53.