TITLE

ANTIFUNGAL PROPERTIES OF METHANOLIC EXTRACTS OF SOME MEDICINAL PLANTS IN ENUGU,

SOUTH EAST, NIGERIA

BY

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CERTIFICATION

This dissertation entitled "**Antifungal Properties of Methanolic Extracts of Some Medical Plants in Enugu, South East, Nigeria.**"

was carried out by Ndam Paul C. under my supervision.

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Prof. F.N. Onyemelukwe	**Date**
Certifier/Supervisor	

DEDICATION

This work is dedicated to my dear wife Mrs. Ndam, Chidinma Ada and my kids Gift, Victory and Blessing, for their immense contribution to my joy and encouragement at home during this program.

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September, 2014

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ABSTRACT

Antifungal activities of methanolic extracts of the leaves and fruits of some medicinal plants were evaluated by incorporating known volumes of the different extracts into molten Sabouraud Dextrose Agar (SDA) to make up for concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml respectively. Concentrations of 125 µg/ml, 250 µg/ml and 500 µg/ml of Miconazole were incorporated to serve as a positive control while 0.5 ml of DMSO served as a negative control. The plants extracts employed were from *Azadirachta indica* (*Neem*)*, Anacardium occidentale* (Cashew), *Euphorbia hirta* (Asthma weed)*, Jatropha curcas* (Physic nut)*, Acantus montanus* (Mountain thistle) *and Picralima nitida* (òsúigwe in Igbo). A 2 mm agar disc cut out from the margin of actively growing cultures of each dermatophyte (*Trichophyton soudanense, T.mentagrophytes, Cladosporium* sp*, T.rubrum* and *Fusarium* sp) were inoculated on the agar plates containing varying concentrations of the different plant extracts, the standard antifungal agent and negative control in triplicates and incubated at 28 ºC. All the extracts exhibited antifungal activities of varying degrees with radial growth inhibitions (RGIs) ranging from 0-0.45 mm radius. Fifty percent (50 %) of the plants extracts comprising of *Picralima nitida, Euphorbia hirta* and *Acantus montanus* exhibited complete inhibition at 100 mg/ml against all the clinical isolates under investigation ($p < 0.05$) . *Picralima nitida* seed was the only plant extract with complete inhibition at 25 mg/ml against *T.soudanense* and *T.rubrum*. *Picralima nitida* was the closest in activity to miconazole. *Azadirachta indica* (Neem) was the only plant extract which did not exhibit complete inhibition against any of the clinical isolates at 100 mg/ml yet exhibited a significantly low RGI at the lowest concentration of 25 mg/ml better than others. Antifungal activities of the extracts against the clinical isolates were all found to be statistically significant with reference to miconazole.

CHAPTER ONE

INTRODUCTION

The increasing incidence of growing resistance to antifungal agents despite the intensive use of antifungal drugs in the treatment of fungal infection (Svetaz *et al*., 2010) has become a great health challenge. However, there has been some claims by the traditional healers that some medicinal plants are more efficient in the treatment of infectious diseases than synthetic antibiotics. Plants such as *Euphorbia hirta* (Asthma weed), *Anacardium occidentale* (Cashew), *Picralima nitida* (Òsúigwe), *Jatropha cucas* (Physic nut), *Azadirachta indica* (Neem) and *Acantus montanus* (Mountain thistle) have been used by the people within the Enugu metropolis for the local treatment of skin diseases because of their effectiveness, availability and cost effectiveness.

Furthermore, nature has bestowed a very rich botanical wealth of vegetation, and a large number of diverse types of plants grow in different parts of the country. They constitute the richest source of drugs for traditional systems of medicine, modern medicines, food supplements (Hammer *et al*., 1999). They have been studied as an alternative treatment for diseases in order to overcome the problem of antibiotic resistance by pathogenic organisms (Phillip *et al*., 2005; Timothy *et al*., 2008). Their extracts may be an alternative to currently used fungicides for controlling pathogenic fungi because they constitute a rich source of bioactive chemicals (Wink, 1993).

Fungi are eukaryotic organisms with a cell wall like plants, but they do not have chlorophyll. They can neither ingest nor manufacture their own food the way plants do. They are ubiquitous in nature and exist as free-living saprobes that derive no obvious benefits from parasitizing humans or animals.

Since they are widespread in nature and are often cultured from diseased body surfaces, it may be difficult to assess whether a fungus found during disease is a pathogen or a transient environmental contaminant. Therefore, before a specific fungus can be confirmed as the cause of a disease, the same fungus must be isolated from serial specimens and fungal elements morphologically consistent

with the isolate must be observed in tissues taken from the lesion. Generally, most fungal diseases are as a result of accidental encounters with the agent and many fungi have developed mechanisms that facilitate their multiplication within the host.

Dermatophytes are keratinophilic fungi able to infect keratinized tissues of human or animal origin, leading to infections that are mainly restricted to the corneocytes of the skin, hair and nails (Weitzman *et al*.,1995). Dermatophytosis, is often caused by a group of closely related filamentous fungi. *Epidermophyton, Microsporum* and *Trichophyton* are the genera of dermatophytes implicated in superficial mycoses. These filamentous fungi are usually identified on the basis of clinical features and isolation patterns together with conidial morphology, and sometimes with physiological characters, such as the hair perforation and urease tests. In some cases, morphological identification can be difficult or uncertain because there is considerable variation and pleomorphism among isolates of the same species.The phylogeny of dermatophytes, however, remains unclear because their members are phylogenetically and taxonomically closely related. Their phenotypic features are sometimes poor, and many isolates from medical and veterinary samples have lost their sexual activity (Takashio, 1977).

These organisms gain entry and establish themselves in the non-living and cornified layers of traumatized or macerated skin and its integument and multiply by the production of keratinase to metabolize the insoluble, tough fibrous protein. They possess greater invasive properties but are limited to the keratinized tissues (Kobayashi, 1996). The disease is precipitated by the humid weather, over population and poor hygienic conditions.

They are spread by direct contact from person to person (anthropophilic organisms), animals (zoophilic organisms), and soil (geophilic organisms), as well as indirectly from fomites (Barry and Hainer, 2003). They have been prevalent since before 1906, at which time ringworm was treated with compounds of mercury or sometimes sulfur or iodine. Hairy areas of skin were considered too difficult to treat, so the scalp was treated with x-rays and followed up with antiparasitic medication (Sequeira, 1906).

Skin infections by fungi are a health concern worldwide. Whereas some infections are merely unsightly or annoying, others may cause significant morbidity and mortality, especially in the elderly and those with significant illness or immune compromise (Zuber and Baddam, 2001). Dermatophytes are assuming greater significance due to the excessive use of immunosuppressive drugs for controlling serious infectious as well as non serious infectious conditions. These infections are known as ótinea infectionsô and are named according to the location of the lesions on the body. On the scalp (*tinea capitis*), glabrous skin (*tinea corporis*), groin (*tinea cruris*), nail (*tinea unguium*), feet (*tinea pedis*), beard (*tinea barbae*), and hand (*tinea manuum*).

Other dermatophytoses are named for their appearance, such as *tinea favosa* (favus, or honeycomb-like due to *T. schoenleinii*) or *tinea imbricata* ñcomposed of overlapping partsò; ringworm due to *T. concentricum* (Winn, 1996). Since these infections are often confused with other skin disorders, it is therefore, necessary to make early laboratory diagnosis for better management of these conditions (Huda *et al.,* 1995) which have increasingly become an important cause of mortality in hospitalized patients. They are the major centre of superficial mycoses of man and remained a public health problem especially in tropical countries such as India (Venugopal and Venugopal, 1993).

Antifungals work by exploiting the differences between mammalian and fungal cells to kill the fungal organism with fewer adverse effects to the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus, fungal and human cells are similar at the biological level.

This makes it more difficult to discover drugs that target fungi without affecting human cells. As a consequence, many antifungal drugs cause side-effects. Some of these side-effects can be life-threatening if the drugs are not used properly. The cell membrane and cell wall of fungi are the most important targets for antifungal drugs. These physical and chemical barriers are responsible for the communication with the environment and, therefore, have a key role in metabolic processes (Richardson and Warnock, 2012; Hector, 1993). Ergosterol is the predominant sterol in fungal cell membranes, responsible for maintaining cell integrity, viability, function and normal growth.

The three major groups of antifungal agents in clinical use include azoles, polyenes and allylamines.They owe their antifungal activity to the interaction with ergosterol or to the inhibition of its synthesis (Ghannoum and Rice, 1999). Fungal cell wall is a target for antifungals action. Over the past decades a number of compounds capable of affecting fungal cell wall has been discovered, being active over the synthesis of chitin and ɓ-glucans, which are essential cell wall components, responsible for fungal structure and normal cell growth. Among them, only echinocandins are commercially available. These compounds are able to inhibit ɓ-glucans synthesis, which are unique compounds among the fungal kingdom (Hector, 1993). Topical agents in wide use for treating localized dermatophytic infections include the imidazoles (ketoconazole, econazole, and oxiconazole), the allylamines (naftifine and terbinafine hydrochloride), and the pyridone ciclopirox olamine (Rosso, 1997). Management is usually challenging due to the tendency of the fungi recurring at the same or different sites.

The increasing prevalence of serious mycoses coupled with the frequent use of the available antifungal drugs, has resulted in rising resistance of fungal pathogens to antifungal agents. However, these topical drugs are generally ineffective against fungal infections of the nails due to their inability to penetrate the entire nail unit and eradicate the infection. It was until recently, when the fungal nature of these locally invasive infections was appreciated, then have systemically active drugs been developed for their treatment Brody (1995), with recurrence reported in up to 25 to 40% of cases (Hay**,** 2001). The reasons for the failure rate may include inaccurate diagnosis; misidentification of the pathogen; and the presence of a second disorder, such as psoriasis. In some cases, the presence of a high fungal inoculum and host-related factors such as a compromised immune system (typically seen in individuals infected with HIV), diabetes mellitus, or peripheral vascular disease may also impede success (Boni, 1998). The increasing prevalence of serious mycoses coupled with the frequent use of antifungal drugs currently available, has resulted in rising resistance of fungal pathogens to antifungal agents. There is therefore a need for new broad spectrum antifungal agents that can be used empirically in immune compromised patients, organ transplant patient and other challenging situations. Use of herbal medicine in the treatment of infection with microorganism predates the

introduction of antibiotics (Owoyale *et al*., 2005). Herbs are widely exploited in the traditional medicine and their curative potentials are well documented too (Dubey *et al*., 2004). Medicinal plants represent a rich source of antimicrobial agents (Mahesh *et al.,* 2008) with many of them readily available in rural areas at relatively cheaper price (Mann *et al*., 2008). Medicinal plants extracts are promising as alternative or complementary control means because of their anti-microbial activity, nonphytotoxicity, as well as biodegradability properties (Gómez *et al*., 1990;Talibi *et al*., 2012).

They produce a great deal of secondary metabolites, many of which have antifungal activity. Well-known examples of these compounds include flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates (Bennett *et al.,* 1994; Osbourne, 1996).

Research on plants materials with less toxic materials has become necessary (Hitchcock, 1993). About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful, especially in the areas of infectious disease and cancer (Cragg *et al*., 2005).

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being (Igbinosa *et al.,* 2009). Studies conducted on medicinal plants shows that they have been used as an alternative treatment for diseases in order to overcome the problem of antibiotic resistance by pathogenic organisms. This may be the reason perhaps why a majority of the world's population in developing countries still rely on herbal medicines to meet its health needs.

Furthermore, herbal medicines are often used to provide first-line and basic health service, both to people living in remote areas where it is the only available health service, and to people living in poor areas where it offers the only affordable remedy. Moreover, natural products are biodegradable and safe for use as an alternative for disease control in a traditional production system (Wilson *et al.,* 1997; Cowan, 1999).

Considering the vast potential of plants materials in the health care and the challenges in the management of fungal infections, it is necessary to clinically investigate strategies to develop new antifungals by screening for antifungal properties of common medicinal plants in order to reformulate the existing antifungals. This is essential for improved patient management.

1.1 Aim and Objectives

1.1.1 Aim

To screen for the antifungal activity of *Euphorbia hirta* (Asthma weed), *Anacardium occidentale* (Cashew), *Picralima nitida* (Òsúigwe), *Jatropha curcas* (Physic nut), *Azadirachta indica* (Neem) and *Acantus montanus* (Mountain thistle) plants extracts.

1.1.2 Objectives

1. To determine the plant extract with the highest antifungal activity.

2. To determine the phytochemistry of the fraction of the extract with highest antifungal activity.

CHAPTER TWO

LITERATURE REVIEW

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Farnsworth, 1989).They have been an important source of medicine for thousands of years. The World Health Organisation (WHO) estimates that up to 85% of people still rely mainly on traditional remedies such as herbs for their medicine (Leena and Jaindranath, 2003). Traditional healing plays an integral role in black African culture as it provides health care needs for a large majority of the society (WHO, 2002). Presently, there is growing awareness by scientific and medicinal plants in the health care system of many developing countries (Ame and Salah, 1995). In Nigeria, there is a rich tradition in the use of herbal plant products for the treatment of several ailments.

2.1 *Euphorbia hirta L* (Asthma weed)

2.1.1 General features and distribution

Euphorbia is the largest genus in the family of Euphorbiaceae, comprising about 2000 species. More than 80 of them are distributed in China (Ma and Tseng, 1997). *Emphorbia hirta L.* is a mesophytic or xerophytic plant (Figure 1), distributed throughout the hotter part of India and Australia, often found in waste places along the road sides. In habit, *Euphorbia hirta L.* is an annual plant that grows up to 2 feet in height. Flowers are small and clustered with opposite oblong leaves and toothed margin. The inflorescence is cyathium and flower is unisexual. There is a milky latex present in all parts of the plant (Bhasker, 2011).

2.1.2 Classification

Euphorbia is one of the most diverse genera in the family Euphorbiaceae. Members of the family and genus are sometimes referred to as Australian asthma plant, garden spurge, snake weed or cats hair.

Kingdom - Plantae

Division - Magnoliophyta

Class - Magnoliopsida

Order ï Malpighiales

Family - Euphorbiaceae

Genus - *Euphorbia*

Species ï *hirta* Bhasker, 2011.

2.1.3 Phytochemistry

The leaves of *Euphorbia hirta* are found to contain triterpenoids, sterols, alkaloids, glycosides and tannins (Anozie, 1991). Other studies reveals the presence of tannins,saponins, flavonoids, cardiac glycosides, alkaloids and steroids (Okoli *et al.*, 2009) and in that carried out by Gopinath (2012), the plant sample reveals the presence of bioactive compounds such as steroids, terpenoids, saponins, tannins, phenol, quinine from different solvent extract.

2.1.4 Medicinal and traditional uses

Euphorbia hirta is used in the health care system of Edo State to manage some common ailments and infections. A decoction of the leaves is taken to induce the flow of milk and the leaf can be chewed with palm kernel for the restoration of virility. A poultice of the leaves is externally applied to abscesses to bring it to a head and for faster pain relief. Similar uses of the plant have been reported in Ghana by Dokosi (1998). The plant has been used for female disorders but is now more important in treating respiratory ailments, especially cough, coryza, bronchitis and asthma. In India, it is used to treat worm infestations in children and for dysentery, gonorrhea, jaundice, pimples, digestive problems and tumors (Kartikar and Basu, 1991). In China, the plant has been used traditionally for the treatment of gastrointestinal disorder (diarrhoea, dysentery, and intestinal parasitosis), conjunctivitis and respiratory diseases (Mhasker *et al.,* 2000; Daphne *et al*., 2009). The plant is also widely used in Angola against diarrhea and

dysentery, especially amoebic dysentery. The alcoholic extract of *Euphorbia hirta* leaves is highly effective against Gram positive bacteria and moderately effective against Gram negative bacteria. In Nigeria, extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore and promoting wound healing (Chika *et al*., 2007). An ethanolic extract of *Euphobia hirta* showed antifungal activity against plant pathogens, *Colletotrichum capsici, Fusarium pallidoroseum, Botryodiplodia theobromae, Phomopsis caricae-papayae* and *Aspergillus niger* using the paper disc diffusion technique (Mohamed *et al*.,1996). The latex of the plant is used by the local tribes and Vangujjars in the treatment of wart. After cleaning with water, external application of the latex is made three times a day up to 15 days. It is known to cure almost every type of wart on any part of the body. This has served as a basis for further studies (Blasker, 2011).

2.2 *Anacardium occidentale L.* (Cashew)

2.2.1 General features and distribution

The tree is small and evergreen, growing to 10-12 m (32 feet) tall (Figure 2), with a short, often irregularly shaped trunk. The leaves are spirally arranged, leathery textured, elliptic to abovate, 4-22 cm long and 2-15 cm broad, with smooth margin. Cashew (*Anacardium occidentale L.*) belonging to Anarcardiaceae member, native of Brazil and have great economic and medicinal value (Rajesh *et al*.,2009). *Anacardium occidentale* is commonly called cashew in English, ôKashiô in Hausa, ñOkpokpoò in Ibo and Kaju in Yoruba.

2.2.2 Classification

Kingdom ï Plantae

Order ï Sapindales

Family ï Anacardiacea

Genus ï *Anacardium*

Species ï *occidantale*

2.2.3 Phytochemistry

Phytochemical studies on the leaf of cashew by Fazali *et al*. (2011) reveal the presence of phenolics, flavonoids, steroids and triterpenes, while other constituents such as alkaloids, saponins and tannins were not detected. Other studies reported the presence of several phenolic acids in the leaves of *Anacardium occidantale*, mainly gallic acid, protocatechuic acid, p-hydroxybenzoic acid, cinnamic acid, p-coumaric acid and ferulic acid. Flavonoids and tannins are phenolic compounds, a major group of compounds that act as primary antioxidants or free radical scavengers. In another work, by Abulude *et al*. (2010), the phytochemical analysis reveals the presence of carbohydrate, alkaloids, tannins, flavonoids, glycosides, steroids and saponins in varying concentrations. Phlobatannins were absent.

2.2.4 Medicinal and traditional uses

Cashew leaves is still widely used in the tropics for the treatment of diarrhoea and colic. In Nigeria, the extract of the leaf has been used to lower blood pressure and sugar (Esimone *et al*., 2001). In Brazil, the tea of the bark is used as a douche and as an astringent to stop bleeding after tooth extraction (Mota, 1985). Anarcardic acid, one of the phytochemical constituents of cashew extract, has been shown to curb the darkening effect of aging by inhibiting tyrosinase activities and kill certain cancer cells (Mendes, 1990). The ethanolic extracts (by continuous hot percolation 75 ^{o}C for 72 hours) of *Anacardium occidentale L*. nut showed antifungal activity of 4 out of 5 pathogenic isolates. The maximum percentage of inhibition was shown on *A. flavus* (94 %) and *Fusarium* sp (95.45 %) and less against A. *fumigates* (68.96 %). *Curvalaria* sp was highly resistant and show nil percentage. The ethyl acetate extracts had less percentage of antifungal activity, inhibited three fungal isolates. *A. niger* (82.14 %) was more compared to *A. flavus* (72 %) and *A. fumigatus* (41.37 %). No activity for *Fusarium* sp and *Curvalaria* sp (Rajesh *et al*., 2009). In a work carried out by Rajesh Kannan *et al*. (2009) on elementary chemical profiling and antifungal properties of acetone, ethanolic and ethyl acetate extract of cashew (*Anacarduim occidentale*) nuts on *A. flavus, A. fumigatus, A niger, Curvalaria* sp and *Fusarium*

sp. It was discovered that *Fusarium* sp was very sensitive to ethanol extracts with percentage inhibition of more than 94 %. The effect is due to triterpenoids, phenolics and volatile oils. Phenolics are well known antifungal compounds present in plants that rapidly accumulate at the site of infection in plants to resist the fungal infection or other pathogens (Matern *et al*., 1998). In a work done by Adejumo *et al.* (2009), Ethanol extracts of the leaves of *Anacardium occidentale, Azadirachta indica, Cassia alata* and *Jatropha gossypifolia* at 2 mgl^{-1} completely inhibited the growth of *T. mentagrophytes*, while there was growth on the plates containing *Jatropha curcas.*

2.3 *Picralima nitida* (Òsúigwe)

2.3.1 General features and distribution

It is an entirely glabrous shrub of up to 35 m high (Figure 5). It bears white flowers (about 3 cm long). The fruits are ovoid and yellowish at maturity. It originates from the African forest region, spread through Ivory Coast to Zaire and Uganda (Adjanohoun *et al.,* 1996). *Picralima nitida* (Apocynaceae) is commonly known as Akuamma, Limeme in Congo, Eban Obero (Gabon), Òsúigwe in Igbo (Nigeria).

2.3.2 Classification

Kingdom ï Plantae

Order ï Gentianales

Family ï Apocynaceae

Genus ï *Picralima*

Species--*nitida*

2.3.3 Phytochemistry

Phytochemical screening/analysis of the extract of *P. nitida* seeds revealed the presence of alkaloids, cardiac glycosides, flavonoids, saponins, tannins, and terpenes (Ubulom *et al*., 2011). The stem bark, fruit and seeds of *Picralima nitida*

contain as major compounds the indole alkaloids, akuamimine, akuammicine (strychnan class), akuammidine and akuammiline (both corynanthean class), akuammigine and the very similar alstonine, pseudo-akuammigine and picraline. The seeds are particularly rich in alkaloids. Akuammine is the principal alkaloid of the mature seeds, while minor alkaloids are pseudo-akuammicine, picranitine, picratidine (N-methyl picraline), eburnamine (desacetyl picraline), and desacetyl akuammiline (rhazimol).

2.3.4 Medicinal and traditional uses

It has been shown to possess antiplasmodial, antimicrobial, anti-inflammatory, antipyretic as well as anti-trypanosomiasis properties (Francois *et al*., 1996). The bark is used to prepare remedies to treat malaria and sexual impotence, while the fruits are used for dysmenorrhoea and gastrointestinal disorders (Adjanohoun *et al*., 1996). The results of the phytochemical analysis of the extracts of the fruits of *P. nitida* are similar to those obtained by Kouitchen (2007). Several alkaloids previously isolated from this plant include akuammicine, akuammine, akuammidine, picraphylline, picraline and pseudoakuammigine (Nkere *et al*., 2005; Francois *et al*., 1996). Their antibacterial activities have not yet been demonstrated but many alkaloids are known to be active on Gram negative bacteria (Kuete, 2010). In the evaluation of the antifungal properties of *Picralima nitida* seed extracts by Okorondu (2011) using different solvents, the antifungal action was found to be in the order of methanol > ethanol > hot water. Numerous works have shown the efficacy of *Picralima nitida* plants extracts against skin conditions of *Tinea pedis* (Atheletes foot*), Tinea capitis* (ringworm of the head), *Tinea corporis* (ringworm of the body) and *Trypanosoma brucei* (Ezeamuzieji *et al*.,1994; Wosu *et al*., 1989). In a work carried out by Peace *et al*. (2011), aqueous and ethanolic extracts of *Picralima* seeds were tested for their antifungal activities using *Aspergillus flavus, Candida albicans* and *Mociosporum canis* as test organism. Phytochemical analysis revealed the presence of some plants metabolites which have been reported to have antimicrobial effects. The study reveals that the extracts hold antifungal potential, which can be further explored in the treatment and control of some fungal infections. It was observed that the fungal isolates used in the research exhibited varying degree of susceptibility to the extracts.

2.4 *Jatropha curcas* (Physic nut)

2.4.1 General features and distrubution

Jatropha curcas is a shrub of about 3 m height or a small to medium size tree commonly grown on hedges and fences around gardens (Figure 12). It finds holds in some parts of Nigeria. It finds usefulness in reclaiming eroded areas because of its drought-resistant nature. It is believed to have originated from Mexico and Central America. It was introduced to Africa and Asia during colonial times and now grown in the tropics worldwide (Jatro solutions GmbH, 2013).

2.4.2 Classification

Kingdom Plantae

Subkingdom Tracheobionta

Subdivision Spermatophyta

Division Mangnoliophyta

Class Mangnoliophyta

Subclass Rosidae

Order Euphorbiales

Family Euphorbiaceae

Genus *Jatropha L*

Species *Jatropha curcas*

Common names: Barbados nut, Physic nut, Nettle spurge.

2.4.3 Phytochemistry

Phytochemistry of the plant leaves according to Oseni *et al.* (2011), reveals the presence of reducing sugars, saponins, alkaloids, triterpenes and tanins.

2.4.4 Medicinal and traditional uses

Medicinally, it has been reported that the latex of *Jatropha curcas* contained alkaloid known as Jatrophine, which is used in the treatment of cough, skin

diseases and rheumatism. The latex is also known to heal wound and possessed antimicrobial properties (Ejelonu *et al*., 2010). The antimicrobial activities are unstable leading to loss of activity with time (Oyi *et al*., 2002). Ayanbimpe *et al.* (2005) reported antifugal activity of the methanolic extract of the seed, leaves, root and barks of *Jatropha curcas* against *Trichosporon beigeli*, *Trichophyton mentagrophytes*, *Candida albicans* and *T. verrucosum* at a concentration of 320 mg/ml, 480 mg/ml, 280 mg/ml and 400 mg/ml respectively. Furthermore, in another work conducted by Igbinosa *et al.* (2009), the antifugal activity of methanolic extract of the bark of *J. curcas* reveals zone of inhibitions of 15±1.5 mm, 18±2.0 mm, 18±1.5mm, 15±1.2 mm at 10 mg/ml against *Trichophyton longifusis, Candida glaberata, Fusarium solani* and *Microspoirum canis* respectively. *Jatropha curcas* is used in traditional folklore medicine to cure various ailments such as skin infection, gonorrhoea, jaundice and fever (Chopra *et al.,* 1956). It is used as antimicrobial agents and several works have been carried out by scientists to find out its scientific basis (Omotayo, 1998). The evaluation of the antifungal potential of the hedge plant *Jatropha curcas* on some pathogenic fungi (*Trichophyton mentagrophytes, T. verruscosum, T. biegelii, Candida albicans* and *Aspergillus fumigatus* using aqueous and methanolic extracts of the root, leaves and seeds of the plant reveals that the seed extract inhibited all the fungi tested except *A. fumigatus*. The root extract had no inhibitory effect on any of the organisms. The MIC of the extract ranges between 100 mg/ml and 800 mg/ml. The extract was most active at 10 % concentration (Ayanbimper *et al.,* 2005).

2.5 *Azadirachta indica A* (Neem)

2.5.1 General features and distribution

Neem is an attractive broad-leaved, evergreen tree which can grow up to 30 m tall and 2.5 m in girth. Its trunk usually straight is 30-80 cm in diameter. Its spreading branches form a rounded crown of deep-green leaves and honey-scented flowers as much as 20 m across. Neem is the most useful traditional medicinal plant in India with almost every part of the plant possessing one medicinal property or the other. Its importance has been recognised by the US National Academy of Sciences with publications entitled ñNeem- A Tree for

Solving Global Problemsò. Itôs a native of India, Pakistan, Thailand and Burma. Its actual origin is still being debatable but certainly it originated from the Indian subcontinent and spread to different parts of the world. It is a tropical tree widespread in Asia and Africa (Nigeria) and has long been known to be free from pests and diseases. Its scientific name is derived from ôôazad dirakht-i-hindôô, which in Persian language means the ñfree or noble tree of Indiaò(Anand *et al.,* 2010).

2.5.2 Classification

Kingdom ï Plantae

Order - Rutales

Suborder ï Rutinae

Family ï Meliaceae

Subfamily ï Meliodeae

Tribe ï Melieae

Genus ï *Azardirachta*

Species – *indica*

2.5.3 Phytochemistry

Ethanolic leaf extract of Neem contains saponins, tannins, glycosides, alkaloids, terpenes, flavonoids and reducing sugars (Timothy *et al.,* 2011). Nimbidin demonstrated antifungal activity by inhibiting the growth of *Tinea rubrum* (Murthy *et al.,* 1958). According to Kirtikar *et al.* (1975) and Rao *et al.* (1977), Gedunim isolated from Neem seed oil has been reported to possess both antifungal and antimalaria activities. Sulphur-containing compounds such as cyclic trisulphide (Bhargava *et al*., 1970) and tetrasulphide (Pillai *et al*., 1981) isolated from the steam distillate of fresh, matured Neem leaves have antifungal activity against *Trichophyton mentagrophytes*. Aqueous leaf extract of Neem (concentration 10-60 %) used in a study by Suleiman (2011) reveals mycelial growth inhibition of *Aspergillus viridae, Penicillium digitatum* and *Rhizopus* sp. The effectiveness of vegetative growth decreases in concentration. Zones of

inhibition could also be produced by Neem leaf extract on the growth of dandruff. The higher the concentrations up to 100 % the wider the zone of inhibition. Concentration of 100 % produces a zone of 17.33 mm against 25 % that produces a zone of 6.67 mm (Anand *et al.,* 2010). On the other hand, ethanol extracts of Neem leaves showed MIC and MFC at 250 g/ml concentration for all strains of *T. rubrum, M. nanum*. MIC and MFC observed for strains of *T. mentagrophytes* was 125 g/ml (Natarajan *et al.,* 2003).

2.5.4 Medicinal and traditional uses

Traditionally, dried Neem leaves are mixed with grains for storage. The leaves are spread in 5-7 inches thick layers and grains and Neem fruits are crushed on the inner surfaces of grain containers (Pruthi *et al*., 1944). It is used extensively in Nigeria for the traditional treatment of malaria and other associated conditions in form of decoction, in which unspecified quantities are usually consumed without due regards to toxicological and other adverse effects (Katsaya1 *et al*., 2008). Medicinally, all the parts of the tree are useful. The leaves are used for treating chicken pox; reduce fever caused by malaria and treating various fungal infections and increasing immunity. The oil is used for pest control, cosmetics and medicines. There are personal care products such as skin care- including eczema cream, antiseptic cream, hair care shampoo, hair oil, oral hygiene-toothpaste and so on (Imam *et al.,* 2012). In vitro studies reveal that, neem oil exerted an antibacterial effect and antifungal action against numerous clinical isolates (Subapriya and Nagini, 2005; Rao *et al*., 1986). Neem has been traditionally used as an antimalarial agent (Brahmachari, 2004). The ethanol extracts of neem leaf is said to induce important and dose-dependent hypotensive action in rats, though bradycardia, as well as cardiac arrhythmia, has also been observed (Subapriya and Nagini, 2005). Vaginal neem extract tablets have been studied in sexually active women based on in vitro studies showing efficacy against *Neisserria gonorrhoeae*, *Chlamydia trachomatis* and *Herpes simplex* (Subapriya and Nagini, 2005; Joshi *et al.,* 2005). Vaginal neem oil has been evaluated for safety in women. Endometrial biopsy was normal and no effect on menstrual or ovulatory cycles was shown in a study. Intravaginal neem oil (1 ml) was shown to be spermicidal (Lal *et al.,* 1986; Brahmachari, 2004). The use of Neem oil and chewing of neem twigs have demonstrated varying efficacy against

oral flora and microorganisms responsible for dental caries (Prashant *et al.,* 2007). From animal studies, an estimated safe dose of neem oil about 0.2 ml/kg has been suggested in adults (Boeke *et al*., 2004). Neem oil traditionally has been considered to be a relatively safe product in adults. The oral LD_{50} of neem oil is 14 ml/kg in rats and 24 ml/kg in rabbits. In rats, a dose of up to 80 ml/kg will cause stupor, respiratory distress, depression of activity, diarrhoea, convulsions, and even death (Gandhi *et al.*, 1988). The therapeutic efficacy of many indigenous plants on several disorders has been described by traditional medicine practitioners. Natarajan *et al.* (2003) evaluated the effect *of Azadirachta indica* (Neem) on the growth pattern of Dermatophytes and found out that, the ethanolic extracts of neem leaves showed MIC and MFC at 250 mg/ml concentration for all the strains of *T.rubrum* and *M. nanum* tested. This shows that Neem leaf is efficient in the treatment of fungal infection.

2.6 *Acantus montanus* (Mountain thistle)

2.6.1 General features and distribution

Acantus montanus T. Anderson (Acanthaceae) possesses several medicinal properties. It is used in Cameroon as a folk medicine to treat pain, inflammation and threatened abortion (Asongalem *et al.,* 2008). *Acantus montanus* (Nees) T. Anderson (Acanthaceae) is a shrub widespread in Africa, the Balkans, Romania, Greece and Eastern Mediterranean. It is known as ñBearôs breechesò, ñMountain thistleò or Alligator plantò. It is a striking small shrub with sparse branches and soft stem (Huxley, 1992).

2.6.2 Classification

Kingdom ï Plantae

Unranked ï Angiosperms

Unranked ï Eudicots

Unranked ï Asterids

Order ï Lamiales

Family ï Acanthaceae

Genus – *Acanthus*

Species ï *montanus*

2.6.3 Phytochemistry

The preliminary phytochemical screening revealed the presence of alkaloids, tannins, glycosides, carbohydrates, flavonoids and steroids (Odoh *et al.,* 2010).

2.6.4 Medicinal and traditional uses

Traditionally, where this plant is endemic, the leaves are used for treatment of hypertension and cardiac dysfunction, coughing and gastrointestinal diseases (Lolke *et al.,* 2002).

2.7 Antifungal Activity of Plants Secondary Metabolites

Plants consist of a rich source of thousands of secondary metabolites which are low-molecular weight compounds not necessarily essential for sustaining life, but crucial for the survival of the producing plant (Hadacek, 2002). Plants secondary metabolites are synthetized in specific pathway and sites of production and can vary between kinds of compounds as well as between plant species. Moreover, some molecules can be synthesized by all plant tissues, whereas others are produced in a specific tissue or even cell-specific fashion (Yazdani *et al.,* 2011). The site of synthesis for secondary metabolite is not certainly the site of accumulation.

These secondary metabolites with antifungal activity may be preformed inhibitors that are present constitutively in healthy plants (phytoanticipins) where they represent inbuilt chemical barriers to infection and may protect plants against attack by a wide range of potential pathogens. They may also be synthesized *de novo* in response to pathogen attack or another stress conditions (phytoalexins) (Morrisey and Osbourn, 1999; Osbourn, 1996, 1999; Dixon, 2001) where they are restricted to the tissue colonized by the fungus and the cells surrounding the infection site. They are not present in healthy plants.

Secondary metabolites in plants can be divided into three main groups according to their biosynthetic origin. The terpenes such as mono-, di-, tri-, sesqui- and tetraterpenes, saponins, steroids, cardiac glycosides and sterols.They are frequently highly hydrophobic substances and are stored in resin ducts, oil cells or glandular trichomes. Terpenoids act as phytoalexins (Wink, 2010). The terpenoids, constitute the largest class of secondary products, the diverse substances of this class are generally insoluble in water. The phenolics such as phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins and lignins are chemically heterogeneous group of nearly 10,000 individual compounds. Some are soluble only in organic solvents, some are water-soluble carboxylic acids and glycosides and others are large, insoluble polymers and the nitrogen containing compounds such as alkaloids and glucosinolates. These groups contain compounds with similar biosynthetic properties, and the compounds within the groups do also have some similarities in their structures (Croteau *et al.,* 2000). The most important nitrogen-containing secondary products are the alkaloids, founded in 20 % of higher plants (Dewick, 2002). Some chemical structures of nitrogen- and sulphur-containing plant metabolites act as phytoalexins .These definitions are based on the dynamics of the synthesis of the antifungal molecule, not on its chemical structure, which can be unclear sometimes due to the same compound, can act as phytoalexins in one plant and as phytoanticipin in another. In addition, the same molecule can be a phytoalexin or a phytoanticipin in different organs of the same plant (Grayer and Kokubun, 2001). All microorganisms, insects and biotic stresses such as freezing, salts, heavy metals or ultraviolet radiation can induce the accumulation of phytoalexins in plants (Pelicice *et al.,* 2000). Most phytoalexins are less fungitoxic than synthetic fungicides, but they can accumulate in large quantities within plant tissues, exceeding the concentrations needed to inhibit fungal growth (Jeandet *et al.,* 2002).

2.8 Basis of Antifungal Mechanisms of Action

Fungal cells are eukaryotic hence they have a lot of similarities with mammalian cells, including DNA within the cell nucleus, mitochondria, endoplasmic reticulum and the Golgi apparatus but differ in the cell membrane. Mammalian cells contain mainly cholesterol, while the fungal cells contain mainly ergosterol,

(Walker and White, 2011; McClanahan, 2009). This difference in the sterol content has been a major drug target of interest in the search for antifungal agents. Antifungal activity can be obtained by destroying the pathogenic fungal cell. From looking at the composition of the fungal cell, at least 6 different antifungal mechanisms can be suggested (Walker and White, 2011; Mc Clanahan, 2009).

Inhibition of cell wall formation: The fungal cell wall primarily consists of ß-glucans. If the synthesis of these compounds is inhibited, the cell wall integrity will disrupt (Walker and White 2011 ; McClanahan, 2009).

Cell membrane disruption: The ergosterols are essential for the cell membrane. If these sterols are bound by antifungal drugs, or their syntheses are inhibited by ergosterol biosynthesis inhibitors, the cell membraneôs integrity will disrupt. Thereby the membrane becomes leaky (Walker and White, 2011; McClanahan, 2009).

Dysfunction of the fungal mitochondria: Inhibition of the mitochondrial electron transport will result in reduction in mitochondrial membrane potential. The inhibition can occur via inhibition of the proton pumps in the respiratory chain, leading to reduction in ATP production and subsequent cell death (Kim *et al.,* 2013).

Inhibition of cell division: Inhibition of cell division can happen via inhibition of microtubule polymerization, and thereby inhibiting the formation of the mitotic spindle (Walker and White, 2011; McClanahan, 2009).

Inhibition of RNA/DNA synthesis or protein synthesis: If the antifungal agent enters the cell, for instance via active transport on ATPases, and interferes with the RNA, it can cause faulty RNA synthesis and inhibition of DNA transcription. Inhibition of protein synthesis is also a known antifungal target (McClanahan, 2009).

Inhibition of efflux pumps: Efflux pumps are present in all living cells and their function is to transport toxic substances out of the cell. This transport often

includes transport of accumulated drug out of the fungal cell. Over expression of efflux pumps can lead to drug resistance. By inhibiting the efflux pumps it is believed that drug resistance can be reduced (Kang *et al.,* 2010). An important objective when targeting fungi is to ensure that the mammalian cells are not affected by the antifungal drug, thereby causing side effects. If the antifungal drug is not specific for fungal cells, the drug will inhibit or destroy the mammalian cells as well as the fungal cells. As mentioned above, fungal cells mainly contain ergosterols, while mammalian cells mainly contain cholesterol. So by inhibiting the synthesis of ergosterol or binding of ergosterol to the antifungal drug, mammalian cells may not be affected to the same extent because they have cholesterol instead of ergosterol.

2.9 Dermatophytosis

Cutaneous mycoses are fungal infections of the epidermis and dermis that evoke an inflammatory reaction in the host. Superficial mycoses involve the superficial stratum corneum, thereby not causing a host response. Most cutaneous fungal infections are caused by dermatophytes. Dermatophytic infections are commonly referred to by the region of the body that they inhabit (eg, tinea pedis involving the foot, tinea capitis involving the head, and so forth).

2.9.1 *Trichophyton rubrum*

Trichophyton rubrum is the most common etiological and prominent anthrophilic species of human dermatophytosis known to inhabit moist areas of the human skin (where skin folds), feet or even nails, where keratin is abundant for its growth and survival (White *et al*., 2012). It could contaminate items such as clothing or bedding which could serve as a point of contact. Host invasion depends on the adaptive cellular responses of the pathogen that allow it to penetrate the skin layers, which are mainly composed of proteins and lipids (Maranhão *et al*., 2011). It is not usually a life-threatening infection but is long-lasting, recurring and incredibly difficult to cure. Immunodeficiency, diabetes or treatment with steroids, however, favours widespread disease involving the entire integument. They have the ability to produce and secrete proteolytic enzymes which is a major virulence factor (Chen *et al*., 2010).

Scientific classification:

Kingdom: Fungi

Phylum: Ascomycota

Subphylum: Pezizomycotina

Class: Eurotiomycetes

Order: Onygenales

Family: Arthrodermataceae

Genus: *Trichophyton*

Species: *T. rubrum*

2.9.2 *Trichophyton mentagrophytes*

T. mentagrophytes is a species of communicable fungus found in a variety of environments. *Trichophyton mentagrophytes,* a zoophilic dermatophyte, has at least five different variants: *T. mentagrophytes* var *interdigitale* is anthropophilic, *T.mentagrophytes* var *nodulare* is a rare anthropophilic form occasionally isolated from cases of *tinea pedis*, *T. mentagrophytes* var *mentagrophytes*, *T. mentagrophytes* var *quinckeanum* and *T. mentagrophytes* var *erinacei* are zoophilic dermatophytes. All these variants make up the *T. mentagrophytes* complex, and differentiating these variants is impossible on any one medium (Ajello *et al*., 1967; Houck *et al*., 1996).

The pathogen is keratinophylic and causing a wide range of cutaneous infections of several forms in animals and humans (Weitzman *et al.,* 1995). *T. mentagrophytes* is typically found in moist, carbon-rich environments. They cause a series of infections that affect the feet, face and body. The most well known infection is tinea pedis more commonly known as ‘athlete’s foot’.

2.9.3 *Trichophyton soudanense*

Trichophyton soudanense is also an anthropophilic dermatophyte described in Africa by Joyeux in 1912. Its geographical distribution is restricted to the North-East of tropical Africa, particularly in Nigeria, Ghana, Cameroon, Mauritania, Sudan, Chad and Zaire where most of the isolates from clinical lesions have been reported (Akpata *et al.,* 1992). It is the most frequent etiological agent causing Tinea capitis in children and young adults in this endemic area and in emigrants from Africa residing in Europe (Rubben *et al*., 1996).

2.9.4 *Cladosporium* species

They have a world-wide distribution and are amongst the most common of air-borne fungi.They are of the genus of fungi including some of the most common indoor and outdoor molds, frequently isolated as contaminants. *Cladosporium* sp are pigmented moulds (dematicae) widely distributed in the air as well as decayed organic matter, and very often they are food contaminants. Some species are most widely distributed in the tropics and subtropics (Dixon *et al*., 1999; Hoog *et al*., 2000). Species produce olive-green to brown or black colonies, and have dark-pigmented conidia that are formed in simple or branching chains. They are rarely pathogenic to humans, but have been reported to cause infections of the skin and toenails, as well as sinusitis and pulmonary infections. Exposure to the spores of this fungus is known to cause respiratory allergies. Symptoms include hypersensitivity pneumonitis, asthma, and allergic rhinitis.Severe infections are caused when fungi comes in contact with small cuts or abrasions in the skin (Pritchard *et al*., 1987). Extended exposure to spores may lead to a suppression of the immune system which allows other opportunistic viruses and bacteria to infect the host.

Scientific classification;

Kingdom: Fungi

Division : Ascomycota

Class: Dothideomycetes

Order: Capnodiales

Family: Davidiellaceae

Genus: *Cladosporium*

Type species *Cladosporium herbarum*

2.9.5 *Fusarium* species

Fusarium is a large cosmopolitan genus of pleoanamorphic hyphomycetes whose members are responsible for a wide range of plant diseases (Farr *et al*., 1989).

Collectively the fusaria represent the most important phytopathogen and mycotoxigenic genus of the filamentous fungi (Marasas *et al*., 1984). In humans, *Fusarium* species cause a broad spectrum of infections, including superficial (such as keratitis and onychomycosis), locally invasive, or disseminated infections, with the last occurring almost exclusively in severely immunocompromised patients (Nucci *et al*., 2002). *Fusarium* species may also cause allergic diseases (sinusitis) in immunocompetent individuals (Wickern *et al*., 1993) and mycotoxicosis in humans and animals following ingestion of food contaminated by toxin-producing *Fusarium* sp (Nelson *et al*., 1994). *Fusarium* species are widely distributed in soil, subterranean and aerial plant parts, plant debris, and other organic substrates (Nelson *et al*., 1994) and are present in water worldwide as part of water structure biofilms (Elvers *et al*., 1998).

Classification

Superkingdom: Eukaryota;

Kingdom: Fungi;

Phylum: Ascomycota;

Class: Sordariomycetes;

Order: Hypocreales;

Genus*:* *Fusarium*

CHAPTER THREE

MATERIALS AND METHODS

3.1 Preparation of Plant Materials

Fresh leaves of the plants, *Azadirachta indica* (Neem), *Anacardium occidentale* (cashew). *Euphorbia hirta* (Asthma weed), *Jatropha curcas* (Physic nut), *Acantus montanus* (Mountain thistle) and the *Picralima nitda* (Òsúigwe) Seeds & Rind were collected within the Enugu metropolis and authenticated in the Botany Department of the University of Nigeria, Nsukka.They were washed under running tap water and dried in air under a shade for 5 days (for the leaves) and 12 days (for the seeds and rind).The materials were ground into fine powder and stored in labelled air tight containers.

3.2 Preparation of Crude Extracts

Just 100 g of each of the powdered form of the plant materials were exhaustively extracted by Soxhlet extraction method Tejas *et al*. (2012), using absolute methanol at 30 °C. The 100 g was wrapped in a Whatman No 1 filter paper and stapled. It was then inserted into the extractor chamber. Two hundred (200 ml) of absolute methanol was added into the round bottom flask, and the extractor fitted into it. The condenser was connected to an ice water source and an outlet for the discharge of the water. This set up was then fitted onto the extractor. Heat was then applied using a heating mantle at 30 ^{o}C for five hours (5h). At the end of the procedure the methanol was distilled off and the crude extract was recovered and concentrated by evaporation in Petri dishes placed in a water bath at 30 ºC until solvent was completely evaporated leaving a viscous dark slurry of the extract. The extracts were obtained, weighed and stored separately in labelled universal containers at 4 ºC until used.

3.3 Preparation of fungal isolates

Microscopically identified isolates of *Trichophyton soudanense, T. mentagrophytes, T. rubrum, Cladosporum* sp and *Fusarium* sp, were obtained from the Mycology Laboratory of the University of Nigeria Teaching Hospital, Ituku-Ozalla,Enugu. The isolates were subcultured in Sabouraud Dextrose Agar

(SDA) to get pure cultures. Pure cultures were prepared in slanted cultures, stored in MacCartney bottles and kept at 2-8 ºC for further experimental purposes.

3.4 Determination of Antifungal Activities

3.4.1 Reconstitution of plant extracts

A stock concentration was reconstituted by weighing 2 g of the individual plant residue by adding 1 ml of DMSO to it in a test tube to make a concentration of 2 g/ml (2000 mg/ml).

3.4.2 Preparation of molten SDA plates containing extract

The agar plate method was adopted as described by Onah et *al.* (1994). Volumes of the extracts used to make up 10ml of molten agar with concentrations of 25 mg/ml, 50 mg/ml, 100 mg/ml were calculated using the formula V1C1=V2C2,

Where;

V1	=	Volume of extract needed to prepare the new medium
V2	=	Final volume of medium
C1	=	Concentration of stock
C2	=	Final concentration of medium
V2	=	10 ml, C1= 2000 mg/ml (Stock), C2=25 mg/ml
V1	=	10 ml x 25 mg/ml ÷ 2000 mg/ml
	=	0.125 ml

When

C2	=	50 mg/ml, V1=0.25 ml
C2	=	100 mg/ml, V1=0.5 ml

Table 3.1a A summary of volume of extract added to SDA

Concentration of extract	Volume of extract	Volume of SDA
25 mg/ml	0.125 ml	9.875 ml
50 mg/ml	0.25 ml	9.75 ml
100 mg/ml	0.5 ml	9.5 ml

3.4.3 Preparation of molten SDA containing standard antifungal agent (miconazole).

Standard antifungal culture plates were prepared in like manner. Two grams (2 g) of miconazole nitrate was weighed and dissolved in 10 ml of DMSO to get 2000 µg/ml of stock. Using the formula V1C1 = V2C2 where,

V2 = 10 ml, C1 = 2000 µg/ml

When C2 = 500 µg/ml

V1 = 10 ml x 500 µg/ml ÷ 2000 µg/ml = 2.5 ml

C2 = 250 µg/ml

V1 = 10 ml x 250 µg/ml ÷ 2000 µg/ml = 1.25 ml

C2 = 125 µg/ml

V1 = 10 ml x 125 µg/ml ÷ 2000 µg/ml = 0.625 ml

Table 3.1b A summary of volume of standard antifungal agent added to SDA

Concentration of miconazole	Volume of miconazole solution	Volume of SDA
125 µg/ml	0.625 ml	9.375 ml
250 µg/ml	1.25 ml	8.75 ml
500 µg/ml	2.5 ml	7.5 ml

Miconazole was incorporated into the molten SDA to make up the desired concentrations of 125 ug/ml, 250 ug/ml and 500 ug/ml which served as the positive controls while 0.5 ml of DMSO in molten SDA served as a negative control. The media were allowed to solidify and stored at 2-8 ºC for further use. The antifungal activities were expressed in terms of radius of growth.

3.5 Culturing Process

Culture medium of the different concentrations was inoculated with 2 mm agar disc cut out from the margin of actively growing culture of the dermatophyte. This was carried out in triplicates and incubated at 28 ºC, with controls run concurrently. The radial growth were measured daily for 4 days and the average reading taken for the test, positive and negative controls.

Percentage radial growth inhibition was calculated by employing the following formula:

Percent inhibition = C-T÷ C x 100. Vinit, (2010)

Where,

C = Radial growth of negative control

T = Radial growth of test

3.6 Column Chromatography

This was done using n-haxane, chloroform and ethyl acetate based on increasing polarity (Gambhir, 2008). The separation funnel was plugged with a piece of glass wool to prevent the stationary phase from being washed out of the column. A glass applicator rod was used to tamp it down lightly just enough to prevent the adsorbent from leaking out. The column was filled with dry silica gel adsorbent mesh (stationary phase) by gently adding and tamping it down on the bench top to ensure proper packing. The column was attached to a retort stand and ensured that the column was securely fastened in a vertical position. A pinch clamp was added to the bottom of the column to close the opening. The mobile phase, involved the three solvents (eluents). n-Hexane being the least polar solvent was applied first. It was poured carefully into the column from the top and allowed to drain so that it levels with the top of the packing material. Ten grams of the

extract was emulsified in 5 mls of n-hexane and placed inside the top of the column. Fresh eluting solvent was gradually added to the top to begin the elution process by gravity. The elute was collected in a beaker down the column through the tap. At the point of colour clearing of elute, chloroform was introduced and finally ethyl acetate in the same process. Extracts of the fractions were incorporated into molten SDA to make up concentrations of 100 mg/ml using the formula V1C1 = V2C2 as above. Cultures were carried on the culture media concurrently with negative control culture medium containing 0.5 ml of the respective solvent. Cultures were incubated at 28 ºC for 4 days and the average reading taken.

3.7 Phytochemical Analysis

The phytochemical analysis of fractionated *Picralima nitida* seed extract was carried out using standard methods of Trease and Evans (1996).

3.7.1 Test for alkaloids.

About 2 g of extract and 20 ml of 3 % sulphuric acid in 50 % ethanol was heated in a boiling water bath for 10 mins; it was then cooled and filtered. About 2 ml of the filtrate was tested with few drops of Mayerôs reagent, Dragendoffôs reagent, Wagnerôs reagent and picric acid solution (1 %).

The remaining filtrate was made alkaline with dilute ammonia solution and was placed in a 100 ml separating funnel. The aqueous alkaline solution was separated and extracted with 2.5 ml of dilute sulphuric acid. The extract was tested with few drops of Mayerôs reagent to give a milky precipitate, with Dragendoffôs reagent to give a brick red precipitate and finally with picric acid solution to give a yellowish precipitate.

3.7.2 Test for flavonoids

To 0.2 g of the extract, 10 ml of ethyl acetate was added and heated in a water bath for 3 mins. The mixture was cooled, filtered and the filtrate used for ammonium test:

About 4 ml of filtrate was shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the yellowish color in the ammonia layer indicates the presence of flavonoids.

3.7.3. Test for steroids and terpenoids

About 9 ml of ethanol was added to 1 g of the extract and refluxed for a few minutes and filtered. The filtrate was concentrated to 2.5 ml on a boiling water bath. About 5 ml of hot distilled water was added to the concentrated solution, the mixture was allowed to stand for 1h and the waxy matter was filtered off. The filtrate was extracted with 2.5 ml of chloroform using a separating funnel. About 1 ml of concentrated sulphuric acid was added to 0.5 ml of the chloroform extract in a test tube and a lower layer was formed. A reddish brown interface shows the presence of steroids.

About 0.5 ml of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 mins in a water bath. A grey colour indicated the presence of terpenoids.

3.7.4 Test for saponins

About 0.25 g of the extract and about 20 ml of distilled water was heated in a water bath for 2 minutes. The mixture was filtered while hot and was allowed to cool. The filtrate was used for frothing test.

About 15 ml of distilled water was used to dilute 5 ml of the filtrate and the mixture was mixed vigorously. A stable froth (foam) upon standing indicated the presence of saponins.

3.7.5 Test for tannins

About 1g of the powdered material was boiled with 20 ml of water. It was filtered and used for ferric chloride test.

Few drops of ferric chloride were added to 3 ml of the filtrate. A greenish black precipitate indicated the presence of tannins.

3.7.6 Test for carbohydrate

Molisch Test

About 0.1 g of the extract and 2 ml of distilled water was heated and filtered. Few drops of naphthol solution in ethanol (molisch reagent) were added to the filtrate, concentration sulphuric acid was gently poured down the side of the test tube. A purple interfacial ring indicates the presences of carbohydrate.

3.7.7 Test for glycosides

About 0.1 g of the extract was put into a test tube and about 5 ml of dilute sulphuric acid was added and heated in a water bath for 15 minutes, it was then cooled and neutralised with 20 % potassium hydroxide solution. A mixed of equal parts of Fehlings solutions 1 and 11(10 ml) was added and was allowed to boil for 5 minutes. A dense brick red precipitate indicate the presences of glycosides.

3.7.8 Test for resins (Precipitation test)

About 15 ml of 96 % ethanol was added to 0.2 g of the crude extract. About 20 ml of distilled water and the alcoholic extract was then poured into a beaker. The formation of precipitate indicate the presence of resins.

3.7.9 Test for proteins

About 20 ml of distilled water was added to 0.5 g of the extract and the filtrate was used for Millions test. About two drops of Millions reagent was added to a little portion of the filtrate in a test tube. A white precipitate indicated the presence of proteins. Few drops of picric acid were added to a little portion of the filtrate. A yellow precipitate indicated the presence of proteins.

3.7.10 Test for fats and oil

About 0.1 g of extract was pressed between filter paper and observed. A control was also prepared by placing 2 drops of olive oil on filter paper. Translucency of filter paper indicated the presence of fats and oil.

3.8 Statistical Analysis

All generated data were subjected to statistical analysis using a one-way analysis of variance (ANOVA), followed by Dunnetts test (multiple comparison post test) at $p < 0.05$.

CHAPTER FOUR

RESULTS

Antifungal activities of the methanolic extracts of six medicinal plants were determined against five clinical isolates of fungi species. Miconazole nitrate was used as the standard for comparing the plants extract. The results are represented in Tables 4.1-8b as follows. In Table 4.1, Miconazole nitrate at 500 µm/ml had the highest antifungal effect on the growth of *Cladosporium* sp by inhibiting it completely with a percentage radial growth inhibition (PRGI) of 100 %. *Picralima nitida* seed extract exhibited highest antifungal effect with all the three concentrations Tables 4a and 4b. At 100 mg/ml *the* extract inhibited completely all the investigated isolates with PRGI of 100 %. At 50 mg/ml, there was complete inhibition against all isolates except against *Cladosporium* sp and at 25 mg/ml it inhibited completely *T.soudanense* and *T.rubrum*. The inhibitions exhibited by *T.mentagrophytes*, *Cladosporium* sp and *Fusarium* sp were however significant. *T.soudanense* and *T.rubrum* appeared to be the most sensitive of the fungi under investigation. This was followed by *T.mentagrophytes* and *Fusarium* sp with a PRGI of 90 ï 100 %. *Cladosporium* sp with a PRGI of 91.7 -100 % appeared to be the least sensitive. The inhibition range of *Picralima nitida* seed extract for all the isolates was 90 -100 %. There was no inhibition observed in the negative control. The PRGIs for the antifungal activities of *Picralima nitida* seed extract at 25 mg/ml, 50 mg/ml and 100 mg/ml respectively were significant ($P<0.05$) compared with the standard.

Table 4.1. Percentage radial growth inhibition of positive control antifungal agent (Miconazole) against fungal isolates

Fungal isolates	Concentration of Miconazole nitrate		
	125 µg/ml	250 µg/ml	500 µg/ml
T.soudanense	89.2 %	90 %	93.8 %
T.mentagrophytes	75 %	78 %	85 %
Cladosporium sp	86.7 %	95 %	100 %
T.rubrum	94 %	95 %	98 %
Fusarium sp	82.9 %	75.3 %	95.3 %

Table 4.1a. Radial growth, RG (mm) of *Picralima nitida* seed extract against fungal isolates

Fungal isolates	25 mg/ml $\bar{X}$±SD	50 mg/ml $\bar{X}$±SD	100 mg/ml $\bar{X}$±SD	Pos. Ctrl 125 ug/ml $\bar{X}$±SD	Pos. Ctrl 250 ug/ml $\bar{X}$±SD	Pos. Ctrl 500 ug/ml $\bar{X}$±SD	Negative Ctrl (DMSO) $\bar{X}$±SD
T. soudanense	0±0	0±0	0±0	0.07±0.02	0.065±0.015	0.04±0.01	0.65±0.15
T.mentagrophyte	0.05±0.05	0±0	0±0	0.125±0.025	0.11±0.01	0.075±0.025	0.5±0.1
Cladosporium sp	0.05±0.05	0.03±0.03	0±0	0.08±0.03	0.03±0.03	0±0	0.6±0.2
T.rubrum	0±0	0±0	0±0	0.03±0.03	0.025±0.025	0.01±0.01	0.5±0.15
Fusarium sp	0.05±0.05	0±0	0±0	0.145±0.005	0.21±0.09	0.04±0.01	0.85±0.15

Table 4.1b. Percentage radial growth inhibition of *Picralima nitida* seed extract against fungal isolates

Fungal Isolates	Concentrations of extracts		
	25 mg/ml	**50 mg/ml**	**100 mg/ml**
	Radial Growth inhibition		
T.soudanense	100 %	100 %	100 %
T.mentagrophytes	90 %	100 %	100 %
Cladosporium sp	91.7 %	95 %	100 %
T.rubrum	100 %	100 %	100 %
Fusarium sp	94.1 %	100 %	100 %

Phytochemistry of the chloroform fraction of *Picralima nitida* seed revealed the presence of flavonoids, alkaloids and terpenoids Table 4.9. There was no complete inhibition at 100 mg/ml from the three fractions Tables 4.2a and 4.2b.The highest antifungal effect was exhibited by Chloroform fraction with a PRGI of 88.6 % against *T.rubrum*. All the fungi under investigation appeared sensitive to the chloroform extract. Ethylether fraction, had a lesser antifungal effect on the experimental isolates. There was a significant difference of ($p < 0.05$) in the treatment of chloroform fraction against control.

Table 4.2a. Radial growth RG,(mm) of *Picralima nitida* seed fractions against fungal isolates

Fungal isolates	HEXANE FRACTION		CHLOROFORM FRACTION		ETHYLETHER FRACTION	
	(100mg/ml) $\bar{X}$±SD	Control $\bar{X}$±SD	(100mg/ml) $\bar{X}$±SD	Control $\bar{X}$±SD	(100mg/ml) $\bar{X}$±SD	Control $\bar{X}$±SD
T.soudanense	0.18±0.2	0.4±0.1	0.08±0.03	0.35±0.05	0.22±0.04	0.3±0.05
T.metagrophytes	0.4±0.15	0.3±0.05	0.15±0.03	0.2±0.05	0.5±0.1	0.3±0.05
Cladosporium sp	0.15±0.03	0.2±0.05	0.05±0.05	0.1±0.03	0.3±0.05	0.2±0.05
T.rubrum	0.45±0.1	0.7±0.2	0.08±0.03	0.7±0.2	0.3±0.05	0.7±0.2
Fusarium sp	0.4±0.05	0.35±0.05	0.05±0.03	0.3±0.05	0.3±0.05	0.3±0.05

Table 4.2b. Percentage radial growth inhibition of *Picralima nitida* seed fractions against fungal isolates

Fungal Isolates	Hexane fraction (100 mg/ml)	Chloroform fraction (100 mg/ml)	Ethylether fraction (100 mg/ml)
	Radial growth	**Inhibition**	
T.soudanense	55 %	77.1 %	26.7 %
T.mentagrophytes	-33.3 %	25 %	-66.7 %
Cladosporium sp	25 %	50 %	-50 %
T.rubrum	35.7 %	88.6 %	57.1 %
Fusarium sp	-14.3 %	83.3 %	0%

From Tables 4.3a and 4.3b, *Picralima nitida* rind extract had its highest antifungal effect at 100 mg/ml by inhibiting completely all the investigated isolates with a PRGI of 100 %. RGs of 0.03±0.03 mm to 0.1±0.05 mm at 25 mg/ml and 0.03±0.03 mm to 0.05±0.05 mm at 50 mg/ml were observed for all the isolates as seen in Table 4.3a. At 100 mg/ml a PRGI of 100 % was observed with all isolates. *T.rubrum* with a RG of 0.03±0.03 mm to 0±0 mm appeared to be the most sensitive fungi followed by *T.soudanense* with a RG of 0.05±0.05 mm to 0±0 mm. *T.mentagrophytes* with a RG of 0.1±0.05 mm to 0±0 mm appeared to be the least sensitive. The inhibition range for *P.nitida* rind extract for all the isolates was 0.1±0.05 mm to 0±0 mm. The activities of *Picralima nitida* rind at these concentrations show significant difference ($p < 0.05$) compared to activities of standard antifungal agent.

Table 4.3a. Radial growth RG, (mm) of *Picralima nitida* rind extract

Fungal Isolates $\bar{X}$±SD	25 mg/ml $\bar{X}$±SD	50 mg/ml $\bar{X}$±SD	100 mg/ml $\bar{X}$±SD	Pos. Ctrl 125 ug/ml $\bar{X}$±SD	Pos. Ctrl 250 ug/ml $\bar{X}$±SD	Pos. Ctrl 500 ug/ml $\bar{X}$±SD	Negative Ctrl (DMSO) $\bar{X}$±SD
T. soudanense	0.05±0.05	0.03±0.03	0±0	0.07±0.02	0.065±0.05	0.04±0.01	0.65±0.15
T.mentagrophyte	0.1±0.05	0.03±0.03	0±0	0.125±0.05	0.11±0.01	0.075±0.05	0.5±0.1
Cladosporium sp	0.08±0.02	0.05±0.05	0±0	0.08±0.03	0.03±0.03	0±0	0.6±0.2
T.rubrum	0.03±0.03	0.03±0.03	0±0	0.03±0.03	0.025±0.25	0.01±0.01	0.5±0.15
Fusarium sp	0.08±0.03	0.05±0.05	0±0	0.145±0.05	0.21±0.09	0.04±0.01	0.85±0.15

Table 4.3b. Percentage radial growth inhibition of *Picralima nitida* rind extract against fungal isolates

Fungal isolates	Concentration of Extracts		
	25 mg/ml	50 mg/ml	100 mg/ml
	Radial growth inhibition		
T.soudanense	92.3 %	95.4 %	100 %
T.mentagrophytes	80 %	94 %	100 %
Cladosporium sp	86.7 %	91.7 %	100 %
T.rubrum	94 %	94 %	100 %
Fusarium sp	90.6 %	94.1 %	100 %

Tables 4.4a and 4.4b shows that *Azadirachta indica (Neem)* seed extract did not inhibit any of the fungi completely at its highest concentration of 100 mg/ml. In Table 4.4b the seed had its highest antifungal effect of 95.4 % at 50 mg/ml against *T.soudanense* and 95 % at 100 mg/ml against *T.rubrum*. *T.rubrum* with a PRGI of 94-95 % appeared to be the most sensitive fungi. *Cladosporium* sp appeared to be the least sensitive with a PRGI of 54.2-91.7 %. The inhibition range of Neem seed extract for all the isolates was 54.2-95.4 %. Neem seed was statistically significant ($p < 0.05$) only at 100mg/ml when compared with Miconazole nitrate.

Table 4.4a. Radial growth RG,(mm) of *Azadirachta indica* seed (Neem) extract against fungal isolates

Fungal Isolates $\bar{X}$±SD	25 mg/ml $\bar{X}$±SD	50 mg/ml $\bar{X}$±SD	100 mg/ml $\bar{X}$±SD	Pos. Ctrl 125 ug/ml $\bar{X}$±SD	Pos. Ctrl 250 ug/ml $\bar{X}$±SD	Pos. Ctrl 500 ug/ml $\bar{X}$±SD	Negative Ctrl (DMSO) $\bar{X}$±SD
T. soudanense	0.09±0.04	0.03±0.03	0.07±0.03	0.07±0.02	0.065±0.015	0.04±0.01	0.65±0.15
T.mentagrophyte	0.18±0.07	0.03±0.03	0.03±0.03	0.125±0.025	0.11±0.01	0.075±0.025	0.5±0.1
Cladosporium sp	0.275±0.125	0.2±0.1	0.05±0.05	0.08±0.03	0.03±0.03	0±0	0.6±0.2
T.rubrum	0.03±0.002	0.03±0.03	0.025±0.025	0.03±0.03	0.025±0.025	0.01±0.01	0.5±0.15
Fusarium sp	0.1±0.1	0.175±0.075	0.09±0.06	0.145±0.005	0.21±0.09	0.04±0.01	0.85±0.15

Table 4.4b. Percentage radial growth inhibition of *Azadirachta indica* seed extract against fungal isolates

Fungal isolates	Concentration of Extracts		
	25 mg/ml	50 mg/ml	100 mg/ml
	Radial growth inhibition		
T.soudanense	86.2 %	95.4 %	89.2 %
T.mentagrophytes	64 %	94 %	94 %
Cladosporium sp	54.2 %	66.7 %	91.7 %
T.rubrum	94 %	94 %	95 %
Fusarium sp	88.2 %	79.4 %	89.4 %

Anacardium occidentale (cashew) leaf extract as shown in Tables 4.5a and 4.5b had its highest antifungal effect of 100 % RGI at 100 mg/ml. All investigated isolates were completely inhibited at 100 mg/ml by the extract except for *Cladosporium* sp with a PRGI of 91.7 %. *T.rubrum* had a PRGI of 88-100 % and appeared to be the most sensitive followed by *T.soudanense* with a PRGI of 76.9-100 %. *Fusarium* sp however appeared resistant at 25 mg/ml and 50 mg/ml. The percentage inhibition range of *A.occidentale* extract for all the isolates was between 23.5-100 % giving a significant difference ($p < 0.05$) on all the concentrations with respect to the standard control.

Table 4.5a. Radial growth RG, (mm) of *Anacardium occidentale leaf* (cashew)
extract against fungal isolates

Fungi Isolates	25 mg/ml $\bar{X}$±SD	50 mg/ml $\bar{X}$±SD	100 mg/ml $\bar{X}$±SD	Pos. Ctrl 125 ug/ml $\bar{X}$±SD	Pos. Ctrl 250 ug/ml $\bar{X}$±SD	Pos. Ctrl 500 ug/ml $\bar{X}$±SD	Negative Ctrl (DMSO) $\bar{X}$±SD
T. soudanense	0.15±0.05	0.1±0.05	0±0	0.07±0.02	0.065±0.015	0.04±0.01	0.65±0.15
T.mentagrophytes	0.16±0.07	0.09±0.04	0±0	0.125±0.025	0.11±0.01	0.075±0.025	0.5±0.1
Cladosporium sp	0.34±0.05	0.12±0.03	0.05±0.05	0.08±0.03	0.03±0.03	0±0	0.6±0.2
T.rubrum	0.06±0.01	0.04±0.01	0±0	0.03±0.03	0.025±0.025	0.01±0.01	0.5±0.15
Fusarium sp	0.65±0.1	0.4±0.1	0±0	0.145±0.005	0.21±0.09	0.04±0.01	0.85±0.15

Table 4.5b. Percentage radial growth inhibition of *Anacardium occidentale leaf* (cashew) seed extract against fungal isolates

Fungal isolates	Concentration of Extracts		
	25 mg/ml	50 mg/ml	100 mg/ml
	Radial growth inhibition		
T.soudanense	76.9 %	84.6 %	100 %
T.mentagrophytes	68 %	82 %	100 %
Cladosporium sp	43.3 %	80 %	91.7 %
T.rubrum	88 %	92 %	100 %
Fusarium sp	23.5 %	52.9 %	100 %

From Tables 4.6a and 4.6b, *Euphorbia hirta* exhibited its highest antifungal effect at 100 mg/ml by completely inhibiting all the fungal isolates. No inhibition was observed against most of the isolates under investigation at 25 mg/ml. *T.rubrum* appeared to be the most sensitive fungi with a PRGI of 60 % at 50 mg/ml and 100 % at 100 mg/ml. However, the concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml had a significant difference of ($p < 0.05$) when compared with the Standard.

Table 4.6a. Radial growth RG,(mm) of *Euphorbia hirta* plant extract against fungal isolates

Fungal Isolates	25 mg/ml $\bar{X}$±SD	50 mg/ml $\bar{X}$±SD	100 mg/ml $\bar{X}$±SD	Pos. Ctrl 125 ug/ml $\bar{X}$±SD	Pos. Ctrl 250 ug/ml $\bar{X}$±SD	Pos. Ctrl 500 ug/ml $\bar{X}$±SD	Negative Ctrl (DMSO) $\bar{X}$±SD
T. soudanense	0.45±0.05	0.35±0.05	0±0	0.07±0.02	0.065±0.015	0.04±0.01	0.65±0.15
T.mentagrophyte	0.5±0.1	0.3±0.05	0±0	0.125±0.025	0.11±0.01	0.075±0.025	0.5±0.1
Cladosporium sp	0.6±0.1	0.4±0.1	0±0	0.08±0.03	0.03±0.03	0±0	0.6±0.2
T.rubrum	0.5±0.1	0.2±0.05	0±0	0.03±0.03	0.025±0.025	0.01±0.01	0.5±0.15
Fusarium sp	0.9±0.2	0.55±0.15	0±0	0.145±0.005	0.21±0.09	0.04±0.01	0.85±0.15

Table 4.6b. Percentage radial growth inhibition of *Euphorbia hirta leaf* extract against fungal isolates

Fungal isolates	Concentration of Extracts		
	25 mg/ml	**50 mg/ml**	**100 mg/ml**
	Radial growth inhibition		
T.soudanense	30.8 %	46.2 %	100 %
T.mentagrophytes	0 %	40 %	100 %
Cladosporium sp	0 %	33.3 %	100 %
T.rubrum	0 %	60 %	100 %
Fusarium sp	-5.9 %	35.3 %	100 %

In Tables 4.7a and 4.7b, *Jatropha curcas* exhibited its highest antifungal effect of 100 % at 100 mg/ml by inhibiting all tested isolates except for *Fusarium* sp. Mild inhibition of 58.3 % was observed at 25 mg/ml and 50 mg/ml for *Cladosporium* sp. Others appeared to be resistant at these concentrations. *Cladosporium* sp appeared to be the most sensitive fungi with a PRGI of 58.3-100 %. The extract was significantly higher ($p < 0.05$) at the three concentrations with respect to the standard.

Table 4.7a. Radial growth RG (mm) of *Jatropha curcas* leaves extract against fungal isolates

Fungal Isolates	**25 mg/ml $\bar{X}$±SD**	**50 mg/ml $\bar{X}$±SD**	**100 mg/ml $\bar{X}$±SD**	**Pos. Ctrl 125 ug/ml $\bar{X}$±SD**	**Pos. Ctrl 250 ug/ml $\bar{X}$±SD**	**Pos. Ctrl 500 ug/ml $\bar{X}$±SD**	**Negative Ctrl (DMSO) $\bar{X}$±SD**
T. soudanense	0.4±0.1	0.3±0.1	0±0	0.07±0.02	0.065±0.015	0.04±0.01	0.65±0.15
T.mentagrophyte	0.45±0.2	0.4±0.1	0±0	0.125±0.025	0.11±0.01	0.075±0.025	0.5±0.1
Cladosporium sp	0.25±0.05	0.25±0.1	0±0	0.08±0.03	0.03±0.03	0±0	0.6±0.2
T.rubrum	0.35±0.1	0.3±0.1	0±0	0.03±0.03	0.025±0.025	0.01±0.01	0.5±0.15
Fusarium sp	0.6±0.1	0.45±0.15	0.025±0.025	0.145±0.005	0.21±0.09	0.04±0.01	0.85±0.15

Table 4.7b. Percentage radial growth inhibition of *Jatropha curcas* leaf extract against fungal isolates

Fungal isolates	Concentration of Extracts		
	25 mg/ml	**50 mg/ml**	**100 mg/ml**
	Radial Growth Inhibition		
T.soudanense	38.5 %	53.8 %	100 %
T.mentagrophytes	10 %	20 %	100 %
Cladosporium sp	58.3 %	58.3 %	100 %
T.rubrum	30 %	40 %	100 %
Fusarium sp	29.4 %	47.1 %	97.1 %

In Tables 4.8a and 4.8b shows that, *Acantus montanus* at 100 mg/ml exhibited its highest antifungal effect by completely inhibiting all the tested isolates at a100 % RGI. *T.rubrum* and *Fusarium* sp were not inhibited at 25 mg/ml and 50 mg/ml of extract. *T.soudanense* appeared to be the most sensitive with a PRGI of 84.6-100 % followed by *T.mentagr*ophytes with PRGI of 52-100 %. However, there was a significant difference ($p < 0.05$) in activities of the plant extract to the standard.

Table 4.8a. Radial growth RG, (mm) of *Acantus montanus* leaves extract against fungal Isolates

Fungal Isolates	25 mg/ml $\bar{X}$±SD	50 mg/ml $\bar{X}$±SD	100 mg/ml $\bar{X}$±SD	Pos. Ctrl 125 ug/ml $\bar{X}$±SD	Pos. Ctrl 250 ug/ml $\bar{X}$±SD	Pos. Ctrl 500 ug/ml $\bar{X}$±SD	Negative Ctrl (DMSO) $\bar{X}$±SD
T. soudanense	0.1±0.02	0.05±0.05	0±0	0.07±0.02	0.065±0.05	0.04±0.01	0.65±0.15
T.mentagrophyte	0.24±0.04	0.18±0.03	0±0	0.125±0.025	0.11±0.01	0.075±0.05	0.5±0.1
Cladosporium sp	0.3±0.1	0.2±0.08	0±0	0.08±0.03	0.03±0.03	0±0	0.6±0.2
T.rubrum	0.7±0.2	0.5±0.2	0±0	0.03±0.03	0.025±0.05	0.01±0.01	0.5±0.15
Fusarium sp	0.5±0.1	0.4±0.15	0±0	0.145±0.005	0.21±0.09	0.04±0.01	0.85±0.15

Table 4.8b. Percentage radial growth inhibition of *Acantus montanus* leaves extract against fungal isolates

Fungal Isolates	Concentration of extracts		
	25 mg/ml	**50 mg/ml**	**100 mg/ml**
	Radial Growth inhibition		
T.soudanense	84.6 %	92.3 %	100 %
T.mentagrophytes	52 %	64 %	100 %
Cladosporium sp	50 %	66.7 %	100 %
T.rubrum	-40 %	0 %	100 %
Fusarium sp	41.2 %	52.9 %	100 %

Table 4.9: Phytochemical Constituents of Chloroform extract

S/No	Phytoconstituent	CE
1	Alkaloids	+
2	Carbohydrates	_
3	Flavonoids	+
4	Glycosides	_
5	Fats and oil	_
6	Tannins	_
7	Saponins	_
8	Resins	_
9	Proteins	_
10	Terpenoids	+
11	Steroids	_

KEY:

+ = Present ``
-- = Absent
CE = Chloroform extract

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

Methanolic extracts of six different plant species were assayed for antifungal activities and compared with Miconazole nitrate using the agar plate method. Miconazole belong to the azole group of antifungal agents used clinically against fungal infections. They owe their antifungal activity by inhibiting the enzyme lanosterol 1,4-Ŭ-demethylase; the enzyme necessary to convert lanosterol to ergosterol which is the predominant sterol in fungal cell membranes responsible for maintaining cell integrity, viability, function and normal growth (Ghannoum and Rice, 1999). Results showed that the methanolic extract of *Picralima nitida* (seed and rind), *Azadirachta indica* (Neem), *Anacardium occidentale* (Cashew), *Euphorbia hirta, Acantus montanus* and *Jathropha curcas* exhibit antifungal activity against *T. soudanense, T.mentagrophyte, T. rubrum, Cladosporium* sp and *Fusarium* sp. These plants may be considered to possess biochemically related substances with similar inhibitory properties with miconazole. The antifungal activities vary differently at the same concentrations of each of the extracts against the respective fungal isolate. It generally increases with increase in concentration of the extracts. This finding agrees with the report of Banso *et al*. (1999) that higher concentration of antimicrobial substances will lead to appreciable growth inhibition. Phytochemical studies of these plants reveal the presence of secondary metabolites such as tannins, terpernoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils. The antifungal activities of these plants may probably be due to the presence of these rich secondary metabolites in plants (Cowan, 1999). Baba-Moussa *et al.* (1999) and Reyes-Chilpa *et al*. (2009) have also reported the antifungal properties of tannins and flavonoids respectively. The patterns of radial growth inhibition of the plant extracts were similar to those of miconazole nitrate.This could suggest the presence of similarly active ingredients present in Miconazole nitrate (the control antifungal agent) which is used predominantly as a drug of choice against superficial fungal infection especially *Trichophyton* sp, *Epidermophyton* sp and *Micosporium* sp (Elewski, 1998). The plants extracts equally possess substances capable of binding to the ergosterols or inhibiting its synthesis thereby leading to

the disruption of the cell membraneôs integrity (Walker and White, 2011; McClanahan, 2009). The activities of *Picralima nitida* seed extract appeared to be better than the other plant extracts investigated. This could be based on the concentration of active antifungal ingredients such as the phenolics (Tannins and flavonoids) present in that part of the plant material (Matern *et al*.,1998). The activities of *Picralima nitida* (seed and rind) in this work justifies its use in the treatment of skin conditions including of *Tinea corporis* (ringworm of the skin), *Tinea capitis* (ringworm of the head), (Wosu *et al*.,1989; Ezeamuzieji *et al*.,1994). *Azadirachta indica* (Neem) seed oil has been used in the treatment of various skin infections by alternative system of medicine (Natarajan *et al*., 2003). Its activities in this study are in line with the work done by Khan *et al*. (1986). In their work, neem oil was shown to have different inhibitory effects on different fungi including *T. rubrum, T. mentagrophytes* and *Fusarium* sp. However, though there was no complete inhibition observed in this study with any of the fungi, it could be considered to be in agreement with Ishrat *et al*. (2008) who found out that neem oil from different localities possess different rates of antifungal activities yet with significant effect on all the fungal species tested. This variation could be due to the difference in the quality of the active ingredients in the oil sample. The leaves of *Anacardium occidentale* (cashew) in this work exhibited poor inhibitions at lower concentrations. Rajesh *et al.* (2009) had it that, the nuts have proven antifungal properties of more than 94 % inhibition against *Aspergillus fumigatus, A niger*, *Curvalaria* sp and *Fusarium* sp. This is an indication of uneven distribution of the active ingredients of the plant. *Euphorbia hirta* exhibited resistance at lower concentrations on some of the isolates but complete inhibition at a higher concentration. This finding agrees with the findings of Momoh *et al*. (2011), sensitivity increases with increase in concentration. Though much has not been done on dermatophytes, its activity against *Candida albicans* by Rajesh *et al.* (2007) is a supportive evidence of its antifungal activity. *Jatropha curcas* in this study showed a poor inhibition of 10 % and 30 % at 25 mg/ml for *T. mentagrophytes* and *T.rubrum* respectively. However, Adejumo *et al.* (2009) who worked on *Fusarium* sp observed it to be the most resistant. The difference in their susceptibilities could be attributed to inherent resistant factor of the test organisms among other factors (Ekpo and Etim, 2009). Ayanbimpe *et al.* (2005) in their work, observed that, the leaves of

Jatropha curcas had no inhibitory effect against *T. mentagrophytes* rather it was observed in the seed extract. This discrepancy is likely due to the fact that no two plants of same species may have experienced the same environmental challenges considering the fact that plants phytoanticipins are uniformly distributed within the plants while phytoalexins are restricted to the tissue colonized by the fungus and the cells surrounding the infection site (Morrisey and Osbourn, 1999; Osbourn, 1996, 1999; Dixon, 2001). Some antifungal compounds may be present constitutively in one part of a plant but induced as phytoalexins in other organs. *Acantus montanus* had not really been widely used in folk medicine for the treatment of skin diseases, rather for pain, inflammation and other ailments (Asongalem *et al.,* 2008). Its antifungal activities in this study support its usage in folklore treatment. *T.rubrum* and *T.soudanense* appear to be the most sensitive fungi at lower concentrations of the extracts. This could be due to the difference in the concentrations of the bioactive components in the sample or synergistic reactions of the various phytochemicals in the extract (Govindachari *et al.,* 1998).

Conclusion

Euphorbia hirta (Asthma weed), *Anacardium occidentale L* (Cashew), *Picralima nitida* (Akuamma plant), *Jatropha curcas* (Barbados nut), *Azadirachita indica A* (Neem plant), and *Acantus montanus* (Mountain thistle) have antifungal activities. There antifungal activities increase with increase in consentration. Crude extract of *Picralima nitida* seed possess the highest antifungal activity.The phytochemistry of its chloroform fraction reveal the presence of flavonoids, alkaloids and terpenoids. *Trichophyton rubrum* was the most sensitive fungal under investigation.

Recomendation

Further investigation on the purified components of the seed extracts of *Picralima nitida* in order to determine the metabolites responsible for their activities will make it serve as a good base for consideration in the pharmaceutical industries for the production and packaging of antifungal products. Knowledge of the distribution of the phytoanticipins and phytoalexins of any plant material under investigation will be necessary for higher yield of antifungal products.

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	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E

APPENDIX

STATISTICAL ANALYSIS
1 way ANOVA of Picrilima seed: Tabular results

Table Analyzed	Picrilima seed				
One-way analysis of variance					
P value	0.0150				
P value summary	*				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	6				
F	3.400				
R square	0.3617				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	18.10				
P value	0.0028				
P value summary	**				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.06967	5	0.01393		
Residual (within columns)	0.1229	30	0.004098		
Total	0.1926	35			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
25mg/ml vs 50mg/ml	0.08833	3.380	No	ns	-0.02409 to 0.2008
25mg/ml vs 100mg/ml	0.1017	3.890	No	ns	-0.01076 to 0.2141
25mg/ml vs 125µg/ml	0.001667	0.06378	No	ns	-0.1108 to 0.1141
25mg/ml vs 250µg/ml	-0.006667	0.2551	No	ns	-0.1191 to 0.1058
25mg/ml vs 500µg/ml	0.0550	2.105	No	ns	-0.05743 to 0.1674
50mg/ml vs 100mg/ml	0.01333	0.5102	No	ns	-0.09909 to 0.1258
50mg/ml vs 125µg/ml	-0.08667	3.316	No	ns	-0.1991 to 0.02576
50mg/ml vs 250µg/ml	-0.0950	3.635	No	ns	-0.2074 to 0.01743
50mg/ml vs 500µg/ml	-0.03333	1.275	No	ns	-0.1458 to 0.07909
100mg/ml vs 125µg/ml	-0.1000	3.826	No	ns	-0.2124 to 0.01243
100mg/ml vs 250µg/ml	-0.1083	4.145	No	ns	-0.2208 to 0.004093
100mg/ml vs 500µg/ml	-0.04667	1.786	No	ns	-0.1591 to 0.06576
125µg/ml vs 250µg/ml	-0.008333	0.3189	No	ns	-0.1208 to 0.1041
125µg/ml vs 500µg/ml	0.05333	2.041	No	ns	-0.05909 to 0.1658
250µg/ml vs 500µg/ml	0.06167	2.360	No	ns	-0.05076 to 0.1741

1way ANOVA of +Control 1:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Picrilima				

	seed				
One-way analysis of variance					
P value	0.0150				
P value summary	*				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	6				
F	3.400				
R square	0.3617				
Bartlett's test for equal variances	18.10				
Bartlett's statistic (corrected)	0.0028				
P value	**				
P value summary	Yes				
Do the variances differ signif. (P < 0.05)		df	MS		
	SS	5	0.01393		
	0.06967	30	0.004098		
	0.1229	35			
	0.1926				
ANOVA Table		q	Significant? P < 0.05?	Summary	95% CI of diff
Treatment (between columns)	Mean Diff.	0.0410	No	ns	-0.09986 to 0.09653
Residual (within columns)	-0.001667	2.345	No	ns	-0.01153 to 0.1849
Total	0.08667	5	Yes	*	0.001805 to 0.1982
	0.1000	2.705	No	ns	-0.1065 to 0.08986
Dunnett's Multiple Comparison Test	-0.008333	6	No	ns	-0.04486 to 0.1515
125µg/ml vs 25mg/ml	0.05333	0.2255			
125µg/ml vs 50mg/ml		1.443			
125µg/ml vs 100mg/ml		3			
125µg/ml vs 250µg/ml					
125µg/ml vs 500µg/ml					

1way ANOVA of Picrilima seed:Column statistics

	25mg/ml	50mg/ml	100mg/ml	125µg/ml	250µg/ml	500µg/ml
Number of values	6	6	6	6	6	6
Minimum	0.0300	0.0	0.0	0.0300	0.0300	0.0
25% Percentile	0.0450	0.0	0.0	0.0600	0.0300	0.0075

Median	0.0800	0.0	0.0	0.1050	0.0850	0.0400
75% Percentile	0.1500	0.0425	0.0075	0.1550	0.2175	0.0950
Maximum	0.3000	0.0800	0.0300	0.1700	0.2400	0.1400
Mean	0.1067	0.01833	0.0050	0.1050	0.1133	0.05167
Std. Deviation	0.09791	0.03251	0.01225	0.05357	0.09092	0.05154
Std. Error	0.03997	0.01327	0.0050	0.02187	0.03712	0.02104
Lower 95% CI	0.003915	-0.01578	-0.007853	0.04878	0.01792	-0.002424
Upper 95% CI	0.2094	0.05245	0.01785	0.1612	0.2087	0.1058

1way ANOVA of Treatment:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Picrilima seed				
One-way analysis of variance					
P value					
P value summary	0.0205				
Are means signif. different? (P < 0.05)	*				
	Yes				
Number of groups	3				
F	5.091				
R square	0.4043				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	15.72				
P value	0.0004				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
		df	MS		
	SS	2	0.01832		
ANOVA Table	0.03663	15	0.003598		
Treatment (between columns)	0.05397	17			
Residual (within columns)	0.09060				
Total		q	Significant? P <	Summa	95% CI of diff
	Mean Diff.	3.607	0.05?	ry	-0.001633 to
Tukey's Multiple Comparison	0.08833	4.152	No	ns	0.1783
Test	0.1017	0.544	Yes	*	0.01170 to
25mg/ml vs 50mg/ml	0.01333	5	No	ns	0.1916
25mg/ml vs 100mg/ml					-0.07663 to
50mg/ml vs 100mg/ml					0.1033

1way ANOVA of Treatment:Column statistics

	25mg/ml	50mg/ml	100mg/ml
Number of values	6	6	6
Minimum	0.0300	0.0	0.0

25% Percentile	0.0450	0.0	0.0
Median	0.0800	0.0	0.0
75% Percentile	0.1500	0.0425	0.0075
Maximum	0.3000	0.0800	0.0300
Mean	0.1067	0.01833	0.0050
Std. Deviation	0.09791	0.03251	0.01225
Std. Error	0.03997	0.01327	0.0050
Lower 95% CI	0.003915	-0.01578	-0.007853
Upper 95% CI	0.2094	0.05245	0.01785

1way ANOVA of +Control 2:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Picrilima seed				
One-way analysis of variance					
P value					
P value summary	0.0150				
Are means signif. different? (P < 0.05)	*				
	Yes				
Number of groups	6				
F	3.400				
R square	0.3617				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	18.10				
P value	0.0028				
P value summary	**				
Do the variances differ signif. (P < 0.05)	Yes				
		df	MS		
	SS	5	0.01393		
ANOVA Table	0.06967	30	0.004098		
Treatment (between columns)	0.1229	35			
Residual (within columns)	0.1926				
Total		q	Significant? P <	Summary	95% CI of diff
	Mean Diff.	0.180	0.05?		-0.09153 to
Dunnett's Multiple Comparison	0.006667	4	No	ns	0.1049
Test	0.0950	2.570	No	ns	-0.003195 to
250µg/ml vs 25mg/ml	0.1083	2.931	Yes	*	0.1932
250µg/ml vs 50mg/ml	0.008333	0.225	No	ns	0.01014 to
250µg/ml vs 100mg/ml	0.06167	5	No	ns	0.2065
250µg/ml vs 125µg/ml		1.669			-0.08986 to
250µg/ml vs 500µg/ml					0.1065
					-0.03653 to
					0.1599

1way ANOVA of Picrilima seed:Column statistics

	25mg/ml	50mg/ml	100mg/ml	125µg/ml	250µg/ml	500µg/ml
Number of values	6	6	6	6	6	6

Minimum	0.0300	0.0	0.0	0.0300	0.0300	0.0
25% Percentile	0.0450	0.0	0.0	0.0600	0.0300	0.0075
Media	0.0800	0.0	0.0	0.1050	0.0850	0.0400
	0.1500	0.0425	0.0075	0.1550	0.2175	0.0950
75% Percentile	0.3000	0.0800	0.0300	0.1700	0.2400	0.1400
Maximum						
	0.1067	0.01833	0.0050	0.1050	0.1133	0.05167
Mean	0.09791	0.03251	0.01225	0.05357	0.09092	0.05154
Std. Deviation	0.03997	0.01327	0.0050	0.02187	0.03712	0.02104
Std. Error						
	0.003915	-0.01578	-0.007853	0.04878	0.01792	-0.002424
Lower 95% CI	0.2094	0.05245	0.01785	0.1612	0.2087	0.1058
Upper 95% CI						

1way ANOVA of +Control 3:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Picrilima seed				
One-way analysis of variance					
P value					
P value summary	0.0298				
Are means signif. different? (P < 0.05)	*				
	Yes				
Number of groups	4				
F	3.663				
R square	0.3546				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	15.95				
P value	0.0012				
P value summary	**				
Do the variances differ signif. (P < 0.05)	Yes				
		df	MS		
	SS	3	0.01232		
ANOVA Table	0.03695	20	0.003363		
Treatment (between columns)	0.06725	23			
Residual (within columns)	0.1042				
Total		q	Significant? P <	Summary	95% CI of diff
	Mean Diff.	1.643	0.05?		-0.1400 to
Dunnett's Multiple Comparison	-0.0550	0.995	No	ns	0.03005
Test	0.03333	7	No	ns	-0.05172 to
500µg/ml vs 25mg/ml	0.04667	1.394	No	ns	0.1184
500µg/ml vs 50mg/ml					-0.03838 to
500µg/ml vs 100mg/ml					0.1317

1way ANOVA of Picrilima seed:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E

Table Analyzed	Picrilima seed				
Repeated Measures ANOVA					
P value	0.0004				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	6				
F	6.781				
R square	0.5756				
Was the pairing significantly effective?	0.3716				
R square	6.966				
F	0.0003				
P value	***				
P value summary	Yes				
Is there significant matching? (P < 0.05)		df	MS		
	SS	5	0.01393		
ANOVA Table	0.06967	5	0.01431		
Treatment (between columns)	0.07157	25	0.002055		
Individual (between rows)	0.05137	35			
Residual (random)	0.1926				
Total		q	Significant? P < 0.05?	Summary	95% CI of diff
	Mean Diff.	0.06369	No	ns	-0.07204 to 0.06871
Dunnett's Multiple Comparison Test	-0.001667	3.31	Yes	*	0.01629 to 0.1570
125µg/ml vs 25mg/ml	0.08667	3.82	No	ns	0.02963 to 0.1704
125µg/ml vs 50mg/ml	0.1000	1	No	ns	-0.07871 to 0.06204
125µg/ml vs 100mg/ml	-0.008333	0.31			-0.01704 to 0.1237
125µg/ml vs 250µg/ml	0.05333	2.03			
125µg/ml vs 500µg/ml		8			

1way ANOVA of Picrilima seed:Tabular results

	Data Set-A	Data	Data Set-C	Data Set-D	Data Set-E

		Set-B			
Table Analyzed	Picrilima seed				
Repeated Measures ANOVA					
P value	0.0065				
P value summary	**				
Are means signif. different?	Yes				
(P < 0.05)	4				
Number of groups	6.071				
F	0.5484				
R square					
Was the pairing	0.3534				
significantly effective?	3.630				
R square	0.0237				
F	*				
P value	Yes				
P value summary		df	MS		
Is there significant	SS	3	0.01232		
matching? (P < 0.05)	0.03695	5	0.007364		
	0.03682	15	0.002029		
ANOVA Table	0.03043	23			
Treatment (between	0.1042				
columns)		q	Significant? P	Summ	95% CI of
Individual (between rows)	Mean	2.1	< 0.05?	ary	diff
Residual (random)	Diff.	15	No	ns	-0.1229 to
Total	-0.0550	1.2	No	ns	0.01287
	0.03333	82	No	ns	-0.03454 to
Dunnett's Multiple	0.04667	1.7			0.1012
Comparison Test		95			-0.02121 to
500µg/ml vs 25mg/ml					0.1145
500µg/ml vs 50mg/ml					
500µg/ml vs 100mg/ml					

1way ANOVA of Picri Treatment vs Neg Control:Tabular results

	Data Set-A	Data	Data Set-C	Data Set-D	Data Set-E

		Set-B			
Table Analyzed	Picri Treatment vs Neg Control				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	58.83				
R square	0.8982				
Bartlett's test for equal variances	25.14				
Bartlett's statistic (corrected)	< 0.0001				
P value	***				
P value summary	Yes				
Do the variances differ signif. (P < 0.05)		df	MS		
	SS	3	0.5950		
	1.785	20	0.01012		
ANOVA Table	0.2023	23			
Treatment (between columns)	1.987				
Residual (within columns)		q	Significant?	Summary	95% CI of diff
Total	Mean Diff.	9.644	P < 0.05?		
	0.5600		Yes	***	0.4125 to 0.7075
Dunnett's Multiple Comparison Test	0.6483	11.17	Yes	***	0.5008 to 0.7958
Negative Control vs 25mg/ml	0.6617	11.40	Yes	***	0.5142 to 0.8092
Negative Control vs 50mg/ml					
Negative Control vs 100mg/ml					

1way ANOVA of Picri Treatment vs Neg Control:Column statistics

	25mg/ml	50mg/ml	100mg/ml	Negative Control

Number of values	6	6	6	6
Minimum	0.0300	0.0	0.0	0.5000
25% Percentile	0.0450	0.0	0.0	0.5000
Median	0.0800	0.0	0.0	0.6250
75% Percentile	0.1500	0.0425	0.0075	0.8625
Maximum	0.3000	0.0800	0.0300	0.9000
Mean	0.1067	0.01833	0.0050	0.6667
Std. Deviation	0.09791	0.03251	0.01225	0.1722
Std. Error	0.03997	0.01327	0.0050	0.07032
Lower 95% CI	0.003915	-0.01578	-0.007853	0.4859
Upper 95% CI	0.2094	0.05245	0.01785	0.8474

1way ANOVA of Picri Treatment vs Neg Control:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Picri Treatment vs Neg Control				
Repeated Measures ANOVA P value					
P value summary	< 0.0001				
Are means signif. different? (P < 0.05)	***				
	Yes				
Number of groups	4				
F	81.87				
R square	0.9424				
Was the pairing significantly effective?					
	0.04694				
R square	2.567				
F	0.0719				
P value	ns				
P value summary	No				
Is there significant matching? (P < 0.05)		df	MS		
	SS	3	0.5950		
	1.785	5	0.01866		
ANOVA Table	0.09328	15	0.007268		
Treatment (between columns)	0.1090	23			
Individual (between rows)	1.987				
Residual (random)		q	Significant? P < 0.05?	Summary	95% CI of diff
Total	Mean Diff.	11.38	Yes	***	0.4315 to 0.6885
	0.5600	13.17	Yes	***	0.5199 to 0.7768
Dunnett's Multiple Comparison Test	0.6483	13.44	Yes	***	0.5332 to 0.7901
	0.6617				
Negative Control vs 25mg/ml					
Negative Control vs 50mg/ml					
Negative Control vs 100mg/ml					

1way ANOVA of Picrilima rind:Tabular results

	Data Set-	Data	Data Set-C	Data	Data Set-E

	A	Set-B		Set-D	
Table Analyzed	Picrilimarind				
One-way analysis of variance					
P value	0.3101				
P value summary	ns				
Are means signif. different?	No				
(P < 0.05)	3				
Number of groups	1.267				
F	0.1445				
R square					
Bartlett's test for equal variances	1.460				
	0.4820				
Bartlett's statistic (corrected)	ns				
	No				
P value		df	MS		
P value summary	SS	2	0.01051		
Do the variances differ signif. (P < 0.05)	0.02101	15	0.008291		
	0.1244	17			
	0.1454				
ANOVA Table		q	Significant? P < 0.05?	Summary	95% CI of diff
Treatment (between columns)	Mean Diff.	0.67	No	ns	-0.1116 to 0.1616
Residual (within columns)	0.0250	2.19	No	ns	
Total	0.08167	7	No	ns	-0.05491 to 0.2182
	0.05667	1.52			
Tukey's Multiple Comparison Test		4			-0.07991 to 0.1932
25mg/ml vs 50mg/ml					
25mg/ml vs 100mg/ml					
50mg/ml vs 100mg/ml					

1way ANOVA of Picrilima rind:Column statistics

	25mg/ml	50mg/ml	100mg/ml
Number of values	6	6	6
Minimum	0.0300	0.0300	0.0
25% Percentile	0.0450	0.0300	0.0
Median	0.0800	0.0400	0.0
75% Percentile	0.1500	0.1125	0.0375
Maximum	0.3000	0.3000	0.1500
Mean	0.1067	0.08167	0.0250
Std. Deviation	0.09791	0.1074	0.06124
Std. Error	0.03997	0.04385	0.0250
Lower 95% CI	0.003915	-0.03105	-0.03926
Upper 95% CI	0.2094	0.1944	0.08926

1way ANOVA of + Control 1:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Picrilima rind				
One-way analysis of variance					
P value					
P value summary	0.3475				
Are means signif. different? (P < 0.05)	ns				
Number of groups	No				
F	6				
R square	1.169				
	0.1630				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	4.676				
P value	0.4566				
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				
		df	MS		
	SS	5	0.007531		
ANOVA Table	0.03766	30	0.006444		
Treatment (between columns)	0.1933	35			
Residual (within columns)	0.2310				
Total		q	Significant? P <	Summa	95% CI of diff
	Mean Diff.	0.035	0.05?	ry	-0.1248 to
Dunnett's Multiple Comparison	-0.001667	96	No	ns	0.1215
Test	0.02333	0.503	No	ns	-0.09981 to
125µg/ml vs 25mg/ml	0.0800	4	No	ns	0.1465
125µg/ml vs 50mg/ml	-0.008333	1.726	No	ns	-0.04314 to
125µg/ml vs 100mg/ml	0.05333	0.179	No	ns	0.2031
125µg/ml vs 250µg/ml		8			-0.1315 to
125µg/ml vs 500µg/ml		1.151			0.1148
					-0.06981 to
					0.1765

1way ANOVA of +Control 2:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Picrilima rind				
One-way analysis of variance					
P value	0.3475				
P value summary	ns				
Are means signif. different?	No				
(P < 0.05)	6				
Number of groups	1.169				
F	0.1630				
R square					
Bartlett's test for equal	4.676				
variances	0.4566				
Bartlett's statistic	ns				
(corrected)	No				
P value		df	MS		
P value summary	SS	5	0.007531		
Do the variances differ	0.03766	30	0.006444		
signif. (P < 0.05)	0.1933	35			
	0.2310				
ANOVA Table		q	Significant? P	Summ	95% CI of
Treatment (between	Mean	0.14	< 0.05?	ary	diff
columns)	Diff.	38	No	ns	-0.1165 to
Residual (within columns)	0.006667	0.68	No	ns	0.1298
Total	0.03167	32	No	ns	-0.09148 to
	0.08833	1.90	No	ns	0.1548
Dunnett's Multiple	0.008333	6	No	ns	-0.03481 to
Comparison Test	0.06167	0.17			0.2115
250µg/ml vs 25mg/ml		98			-0.1148 to
250µg/ml vs 50mg/ml		1.33			0.1315
250µg/ml vs 100mg/ml		1			-0.06148 to
250µg/ml vs 125µg/ml					0.1848
250µg/ml vs 500µg/ml					

1way ANOVA of +Control 3:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Picrilimarind				
One-way analysis of variance					
P value	0.3475				
P value summary	ns				
Are means signif. different?	No				
(P < 0.05)	6				
Number of groups	1.169				
F	0.1630				
R square					
Bartlett's test for equal	4.676				
variances	0.4566				
Bartlett's statistic	ns				
(corrected)	No				
P value		df	MS		
P value summary	SS	5	0.007531		
Do the variances differ	0.03766	30	0.006444		
signif. (P < 0.05)	0.1933	35			
	0.2310				
ANOVA Table		q	Significant? P	Summ	95% CI of
Treatment (between	Mean	1.18	< 0.05?	ary	diff
columns)	Diff.	7	No	ns	-0.1781 to
Residual (within columns)	-0.0550	0.64	No	ns	0.06814
Total	-0.0300	73	No	ns	-0.1531 to
	0.02667	0.57	No	ns	0.09314
Dunnett's Multiple	-0.05333	54	No	ns	-0.09648 to
Comparison Test	-0.06167	1.15			0.1498
500µg/ml vs 25mg/ml		1			-0.1765 to
500µg/ml vs 50mg/ml		1.33			0.06981
500µg/ml vs 100mg/ml		1			-0.1848 to
500µg/ml vs 125µg/ml					0.06148
500µg/ml vs 250µg/ml					

1way ANOVA of Picrilima rind 1:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Picrilima rind				
Repeated Measures ANOVA					
P value	0.0050				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	6				
F	4.429				
R square	0.4697				
Was the pairing significantly effective?	0.6529				
R square	17.74				
F	< 0.0001				
P value	***				
P value summary	Yes				
Is there significant matching? (P < 0.05)		df	MS		
	SS	5	0.007531		
ANOVA Table	0.03766	5	0.03016		
Treatment (between columns)	0.1508	25	0.001700		
Individual (between rows)	0.04251	35			
Residual (random)	0.2310				
Total		q	Significant? P < 0.05?	Summary	95% CI of diff
	Mean Diff.	0.07001	No	ns	-0.06569 to 0.06235
Dunnett's Multiple Comparison Test	- 0.001667	0.9801	No	ns	-0.04069 to 0.08735
125µg/ml vs 25mg/ml	0.02333	3.360	Yes	*	0.01598 to 0.1440
125µg/ml vs 50mg/ml	0.0800	0.3500	No	ns	-0.07235 to 0.05569
125µg/ml vs 100mg/ml	- 0.008333	2.240	No	ns	-0.01069 to 0.1174
125µg/ml vs 250µg/ml	0.05333				
125µg/ml vs 500µg/ml					

1way ANOVA of Picrilima rind 2:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Picrilima rind				
Repeated Measures ANOVA					
P value	0.0050				
P value summary	**				
Are means signif. different?	Yes				
(P < 0.05)	6				
Number of groups	4.429				
F	0.4697				
R square					
Was the pairing	0.6529				
significantly effective?	17.74				
R square	< 0.0001				
F	***				
P value	Yes				
P value summary		df	MS		
Is there significant	SS	5	0.007531		
matching? (P < 0.05)	0.03766	5	0.03016		
	0.1508	25	0.001700		
ANOVA Table	0.04251	35			
Treatment (between	0.2310				
columns)		q	Significant? P	Summ	95% CI of
Individual (between rows)	Mean	0.28	< 0.05?	ary	diff
Residual (random)	Diff.	00	No	ns	-0.05735 to
Total	0.006667	1.33	No	ns	0.07069
	0.03167	0	Yes	**	-0.03235 to
Dunnett's Multiple	0.08833	3.71	No	ns	0.09569
Comparison Test	0.008333	0	No	ns	0.02431 to
250µg/ml vs 25mg/ml	0.06167	0.35			0.1524
250µg/ml vs 50mg/ml		00			-0.05569 to
250µg/ml vs 100mg/ml		2.59			0.07235
250µg/ml vs 125µg/ml		0			-0.002352 to
250µg/ml vs 500µg/ml					0.1257

1way ANOVA of Picrilima rind 3:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Picrilima rind				
Repeated Measures ANOVA					
P value	0.0050				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	6				
F	4.429				
R square	0.4697				
Was the pairing significantly effective?					
R square	0.6529				
F	17.74				
P value	< 0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
	SS	df	MS		
ANOVA Table	0.03766	5	0.007531		
Treatment (between columns)	0.1508	25	0.03016		
Individual (between rows)	0.04251	35	0.001700		
Residual (random)	0.2310				
		q	Significant? P < 0.05?	Summary	95% CI of diff
Total	Mean Diff.	2.3	No	ns	-0.1190 to 0.009019
	-0.0550	1.2	No	ns	
Dunnett's Multiple Comparison Test	-0.0300	60	No	ns	-0.09402 to 0.03402
500µg/ml vs 25mg/ml	0.02667	1.1	No	ns	
500µg/ml vs 50mg/ml	-0.05333	20	No	ns	-0.03735 to 0.09069
500µg/ml vs 100mg/ml	-0.06167	2.2			
500µg/ml vs 125µg/ml		40			-0.1174 to 0.01069
500µg/ml vs 250µg/ml		2.5			
		90			-0.1257 to 0.002352

1way ANOVA of Neem seed:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Neem seed				
One-way analysis of variance					
P value	0.1435				
P value summary	ns				
Are means signif. different?	No				
(P < 0.05)	3				
Number of groups	2.216				
F	0.2281				
R square					
Bartlett's test for equal	4.864				
variances	0.0879				
Bartlett's statistic (corrected)	ns				
P value	No				
P value summary		df	MS		
Do the variances differ	SS	2	0.01647		
signif. (P < 0.05)	0.0329	15	0.007431		
	3	17			
ANOVA Table	0.1115				
Treatment (between	0.1444	q	Significant? P <	Summ	95% CI of
columns)		1.8	0.05?	ary	diff
Residual (within columns)	Mean	94	No	ns	-0.06263 to
Total	Diff.	2.9	No	ns	0.1960
	0.0666	36	No	ns	-0.02596 to
Tukey's Multiple	7	1.0			0.2326
Comparison Test	0.1033	42			-0.09263 to
25mg/ml vs 50mg/ml	0.0366				0.1660
25mg/ml vs 100mg/ml	7				
50mg/ml vs 100mg/ml					

1way ANOVA of Neem seed:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Neem seed				
Repeated Measures ANOVA					
P value	0.0561				
P value summary	ns				
Are means signif. different?	No				
(P < 0.05)	3				
Number of groups	3.896				
F	0.4379				
R square					
Was the pairing significantly	0.4792				
effective?	3.274				
R square	0.0521				
F	ns				
P value	No				
P value summary		df	MS		
Is there significant	SS	2	0.01647		
matching? (P < 0.05)	0.0329	5	0.01384		
	3	10	0.004227		
ANOVA Table	0.0692	17			
Treatment (between	0.0422				
columns)	7	q	Significant? P	Summ	95% CI of diff
Individual (between rows)	0.1444	2.5	< 0.05?	ary	-0.03623 to
Residual (random)		12	No	ns	0.1696
Total	Mean	3.8	Yes	*	0.0004324 to
	Diff.	93	No	ns	0.2062
Tukey's Multiple	0.0666	1.3			-0.06623 to
Comparison Test	7	81			0.1396
25mg/ml vs 50mg/ml	0.1033				
25mg/ml vs 100mg/ml	0.0366				
50mg/ml vs 100mg/ml	7				

1way ANOVA of Neem seed:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Neem seed				
One-way analysis of variance					
P value	0.1640				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	6				
F	1.705				
R square	0.2213				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	7.718				
P value	0.1725				
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				
	SS	df	MS		
	0.05127	5	0.01025		
ANOVA Table	0.1804	30	0.006014		
Treatment (between columns)	0.2317	35			
Residual (within columns)					
Total	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
	0.06667	2.106	No	ns	-0.06954 to 0.2029
Tukey's Multiple Comparison Test	0.1033	3.264	No	ns	-0.03287 to 0.2395
25mg/ml vs 50mg/ml	0.06500	2.053	No	ns	-0.07120 to 0.2012
25mg/ml vs 100mg/ml	0.05667	1.790	No	ns	-0.07954 to 0.1929
25mg/ml vs 125µg/ml	0.1183	3.738	No	ns	-0.01787 to 0.2545
25mg/ml vs 250µg/ml	0.03667	1.158	No	ns	-0.09954 to 0.1729
25mg/ml vs 500µg/ml	-0.001667	0.05264	No	ns	-0.1379 to 0.1345
50mg/ml vs 100mg/ml	-0.01000	0.3158	No	ns	-0.1462 to 0.1262
50mg/ml vs 125µg/ml	0.05167	1.632	No	ns	-0.08454 to 0.1879
50mg/ml vs 250µg/ml	-0.03833	1.211	No	ns	-0.1745 to 0.09787
50mg/ml vs 500µg/ml	-0.04667	1.474	No	ns	-0.1829 to 0.08954
100mg/ml vs 125µg/ml	0.0150	0.4738	No	ns	-0.1212 to 0.1512
100mg/ml vs 250µg/ml	-0.008333	0.2632	No	ns	-0.1445 to 0.1279
100mg/ml vs 500µg/ml	0.05333	1.685	No	ns	-0.08287 to 0.1895
125µg/ml vs 250µg/ml	0.06167	1.948	No	ns	-0.07454 to 0.1979
125µg/ml vs 500µg/ml					
250µg/ml vs 500µg/ml					

1way ANOVA of Neem seed:Column statistics

	25mg/ml	50mg/ml	100mg/ml	125µg/ml	250µg/ml	500µg/ml
Number of values	6	6	6	6	6	6
Minimum	0.0300	0.0300	0.0300	0.0300	0.0300	0.0
25% Percentile	0.0750	0.0300	0.0300	0.0600	0.0300	0.0075
Median	0.1400	0.0900	0.0600	0.1050	0.0850	0.0400
75% Percentile	0.3050	0.1850	0.1000	0.1550	0.2175	0.0950
Maximum	0.3200	0.2000	0.1300	0.1700	0.2400	0.1400
Mean	0.1700	0.1033	0.06667	0.1050	0.1133	0.05167
Std. Deviation	0.1187	0.08189	0.03882	0.05357	0.09092	0.05154
Std. Error	0.04844	0.03343	0.01585	0.02187	0.03712	0.02104
Lower 95% CI	0.04547	0.01739	0.02593	0.04878	0.01792	-0.002424
Upper 95% CI	0.2945	0.1893	0.1074	0.1612	0.2087	0.1058

1way ANOVA of + Control 1:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Neem seed				
Repeated Measures ANOVA P value					
P value summary	0.0570				
Are means signif. different? (P < 0.05)	ns				
Number of groups	No				
F	4				
R square	3.132				
	0.3851				
Was the pairing significantly effective?					
R square	0.4573				
F	4.111				
P value	0.0150				
P value summary	*				
Is there significant matching? (P < 0.05)	Yes				
	SS	df	MS		
ANOVA Table	0.03325	3	0.01108		
Treatment (between columns)	0.07274	5	0.01455		
Individual (between rows)	0.05308	15	0.003539		
Residual (random)	0.1591	23			
Total		q	Significant? P < 0.05?	Summary	95% CI of diff
	Mean Diff.	1.893	No	ns	-0.1546 to 0.02464
Dunnett's Multiple Comparison Test	-0.06500	0.0485	No	ns	-0.08798 to 0.09131
125µg/ml vs 25mg/ml	0.001667	3	No	ns	-0.05131 to 0.1280
125µg/ml vs 50mg/ml	0.03833	1.116	No	ns	
125µg/ml vs 100mg/ml					

1way ANOVA of +Control 2:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Neem seed				
Repeated Measures ANOVA					
P value	0.1032				
P value summary	ns				
Are means signif. different?	No				
(P < 0.05)	4				
Number of groups	2.455				
F	0.3293				
R square					
Was the pairing significantly	0.4616				
effective?	3.835				
R square	0.0194				
F	*				
P value	Yes				
P value summary					
Is there significant matching?	SS	df	MS		
(P < 0.05)	0.03293	3	0.01098		
	0.08573	5	0.01715		
ANOVA Table	0.06707	15	0.004471		
Treatment (between columns)	0.1857	23			
Individual (between rows)					
Residual (random)	Mean Diff.	q	Significant? P <	Summary	95% CI of diff
Total	-0.05667	1.468	0.05?	ns	-0.1574 to
	0.01000	0.2590	No	ns	0.04410
Dunnett's Multiple	0.04667	1.209	No	ns	-0.09076 to
Comparison Test			No		0.1108
250µg/ml vs 25mg/ml					-0.05410 to
250µg/ml vs 50mg/ml					0.1474
250µg/ml vs 100mg/ml					

1way ANOVA of +Control 3:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Neem seed				
Repeated Measures ANOVA					
P value					
P value summary	0.0233				
Are means signif. different?	*				
(P < 0.05)	Yes				
Number of groups	4				
F	4.243				
R square	0.4590				
Was the pairing significantly					
effective?	0.3763				
R square	3.345				
F	0.0315				
P value	*				
P value summary	Yes				
Is there significant		df	MS		
matching? (P < 0.05)	SS	3	0.01668		
	0.05005	5	0.01315		
ANOVA Table	0.06577	15	0.003932		
Treatment (between	0.05898	23			
columns)	0.1748				
Individual (between rows)		q	Significant? P <	Summary	95% CI of diff
Residual (random)	Mean	3.269	0.05?	*	-0.2128 to -
Total	Diff.	1.427	Yes	ns	0.02384
	-0.1183	0.4143	No	ns	-0.1462 to
Dunnett's Multiple	-0.05167		No		0.04283
Comparison Test	-0.0150				-0.1095 to
500µg/ml vs 25mg/ml					0.07949
500µg/ml vs 50mg/ml					
500µg/ml vs 100mg/ml					

1way ANOVA of Cashew:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Cashew				
One-way analysis of variance					
P value	0.0232				
P value summary	*				
Are means signif. different?	Yes				
(P < 0.05)	3				
Number of groups	4.888				
F	0.3946				
R square					
Bartlett's test for equal	19.03				
variances	< 0.0001				
Bartlett's statistic (corrected)	***				
P value	Yes				
P value summary					
Do the variances differ signif.	SS	df	MS		
(P < 0.05)	0.3710	2	0.1855		
	0.5692	15	0.03795		
ANOVA Table	0.9402	17			
Treatment (between columns)					
Residual (within columns)	Mean Diff.	q	Significant? P <	Summary	95% CI of
Total	0.1767	2.221	0.05?	ns	diff
	0.3517	4.422	No	*	-0.1155 to
Tukey's Multiple Comparison	0.1750	2.200	Yes	ns	0.4689
Test			No		0.05948 to
25mg/ml vs 50mg/ml					0.6439
25mg/ml vs 100mg/ml					-0.1172 to
50mg/ml vs 100mg/ml					0.4672

1way ANOVA of Cashew:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Cashew				
Repeated Measures ANOVA					
P value	0.0091				
P value summary	**				
Are means signif. different?	Yes				
(P < 0.05)	3				
Number of groups	7.799				
F	0.6093				
R square					
Was the pairing significantly	0.3524				
effective?	2.786				
R square	0.0789				
F	ns				
P value	No				
P value summary					
Is there significant matching?	SS	df	MS		
(P < 0.05)	0.3710	2	0.1855		
	0.3314	5	0.06627		
ANOVA Table	0.2379	10	0.02379		
Treatment (between columns)	0.9402	17			
Individual (between rows)					
Residual (random)	Mean	q	Significant? P <	Summary	95% CI of
Total	Diff.	2.806	0.05?	ns	diff
	0.1767	5.585	No	**	-0.06744 to
Tukey's Multiple Comparison	0.3517	2.779	Yes	ns	0.4208
Test	0.1750		No		0.1076 to
25mg/ml vs 50mg/ml					0.5958
25mg/ml vs 100mg/ml					-0.06911 to
50mg/ml vs 100mg/ml					0.4191

1way ANOVA of Cashew:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Cashew				
One-way analysis of variance					
P value	0.0042				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	6				
F	4.360				
R square	0.4209				
Bartlett's test for equal variances	34.50				
Bartlett's statistic (corrected)	< 0.0001				
P value	***				
P value summary	Yes				
Do the variances differ signif. (P < 0.05)					
	SS	df	MS		
	0.4638	5	0.09276		
ANOVA Table	0.6382	30	0.02127		
Treatment (between columns)	1.102	35			
Residual (within columns)					
Total	Mean Diff.	q	Significant? P <	Summary	95% CI of diff
	0.1767	2.967	0.05?	ns	-0.07949 to 0.4328
Tukey's Multiple Comparison Test	0.3517	5.906	No	**	0.09551 to 0.6078
25mg/ml vs 50mg/ml	0.2550	4.283	Yes	ns	-0.001157 to 0.5112
25mg/ml vs 100mg/ml	0.2467	4.143	No	ns	-0.009491 to 0.5028
25mg/ml vs 125µg/ml	0.3083	5.178	No	*	0.05218 to 0.5645
25mg/ml vs 250µg/ml	0.1750	2.939	Yes	ns	-0.08116 to 0.4312
25mg/ml vs 500µg/ml	0.07833	1.316	No	ns	-0.1778 to 0.3345
50mg/ml vs 100mg/ml	0.07000	1.176	No	ns	-0.1862 to 0.3262
50mg/ml vs 125µg/ml	0.1317	2.211	No	ns	-0.1245 to 0.3878
50mg/ml vs 250µg/ml	-0.09667	1.623	No	ns	-0.3528 to 0.1595
50mg/ml vs 500µg/ml	-0.1050	1.763	No	ns	-0.3612 to 0.1512
100mg/ml vs 125µg/ml	-0.04333	0.7278	No	ns	-0.2995 to 0.2128
100mg/ml vs 250µg/ml	-0.008333	0.1400	No	ns	-0.2645 to 0.2478
100mg/ml vs 500µg/ml	0.05333	0.8957	No	ns	-0.2028 to 0.3095
125µg/ml vs 250µg/ml	0.06167	1.036	No	ns	-0.1945 to 0.3178
125µg/ml vs 500µg/ml			No		
250µg/ml vs 500µg/ml					

1way ANOVA of Cashew:Column statistics

	25mg/ml	50mg/ml	100mg/ml	125µg/ml	250µg/ml	500µg/ml
Number of values	6	6	6	6	6	6
Minimum	0.0600	0.0400	0.0	0.0300	0.0300	0.0
25% Percentile	0.1275	0.0775	0.0	0.0600	0.0300	0.0075
Median	0.2500	0.1100	0.0	0.1050	0.0850	0.0400
75% Percentile	0.6875	0.3625	0.0125	0.1550	0.2175	0.0950
Maximum	0.8000	0.4000	0.0500	0.1700	0.2400	0.1400
Mean	0.3600	0.1833	0.008333	0.1050	0.1133	0.05167
Std. Deviation	0.3007	0.1516	0.02041	0.05357	0.09092	0.05154
Std. Error	0.1228	0.06190	0.008333	0.02187	0.03712	0.02104
Lower 95% CI	0.04440	0.02422	-0.01309	0.04878	0.01792	-0.002424
Upper 95% CI	0.6756	0.3424	0.02975	0.1612	0.2087	0.1058

1way ANOVA of +Control 1:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Cashew				
Repeated Measures ANOVA					
P value	0.0035				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	7.079				
R square	0.5861				
Was the pairing significantly effective?	0.3071				
R square	3.212				
F	0.0361				
P value	*				
P value summary	Yes				
Is there significant matching? (P < 0.05)	SS	df	MS		
	0.3990	3	0.1330		
ANOVA Table	0.3017	5	0.06035		
Treatment (between columns)	0.2818	15	0.01879		
Individual (between rows)	0.9826	23			
Residual (random)					
Total	Mean Diff.	q 3.222	Significant? P < 0.05?	Summary	95% CI of diff -0.4616 to -0.04844
	-0.2550	0.989	Yes	*	
Dunnett's Multiple Comparison Test	-0.07833	8	No	ns	-0.2849 to 0.1282
125µg/ml vs 25mg/ml	0.09667	1.221	No	ns	-0.1099 to 0.3032
125µg/ml vs 50mg/ml					
125µg/ml vs 100mg/ml					

1way ANOVA of +Control 2:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Cashew				
Repeated Measures ANOVA					
P value	0.0026				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	7.548				
R square	0.6015				
Was the pairing significantly effective?	0.3486				
R square	4.028				
F	0.0162				
P value	*				
P value summary	Yes				
Is there significant matching? (P < 0.05)	SS	df	MS		
	0.3934	3	0.1311		
	0.3499	5	0.06999		
ANOVA Table	0.2606	15	0.01737		
Treatment (between columns)	1.004	23			
Individual (between rows)					
Residual (random)	Mean	q	Significant? P <	Summary	95% CI of diff
Total	Diff.	3.241	0.05?	*	-0.4453 to -0.04803
	-0.2467	0.9198	Yes	ns	-0.2686 to 0.1286
Dunnett's Multiple Comparison	-0.07000	1.380	No	ns	-0.09363 to 0.3036
Test	0.1050		No		
250µg/ml vs 25mg/ml					
250µg/ml vs 50mg/ml					
250µg/ml vs 100mg/ml					

1way ANOVA of +Control 3:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Cashew				
Repeated Measures ANOVA					
P value	0.0027				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	7.516				
R square	0.6005				
Was the pairing significantly effective?	0.2745				
	2.841				
R square	0.0533				
F	ns				
P value	No				
P value summary					
Is there significant matching? (P < 0.05)	SS	df	MS		
	0.4497	3	0.1499		
	0.2833	5	0.05667		
ANOVA Table	0.2992	15	0.01994		
Treatment (between columns)	1.032	23			
Individual (between rows)					
Residual (random)	Mean	q	Significant? P <	Summary	95% CI of diff
Total	Diff.	3.782	0.05?	**	-0.5212 to -0.09552
	-0.3083	1.615	Yes	ns	-0.3445 to 0.08115
Dunnett's Multiple Comparison	-0.1317	0.5315	No	ns	-0.1695 to 0.2562
Test	0.04333		No		
500µg/ml vs 25mg/ml					
500µg/ml vs 50mg/ml					
500µg/ml vs 100mg/ml					

1way ANOVA of Euphobia hirta:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Euphobia hirta				
One-way analysis of variance					
P value					
P value summary	< 0.0001				
Are means signif. different?	***				
(P < 0.05)	Yes				
Number of groups	3				
F	31.96				
R square	0.8310				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)					
P value	ns				
P value summary	No				
Do the variances differ		df	MS		
signif. (P < 0.05)	SS	2	0.4892		
	0.9783	13	0.01531		
ANOVA Table	0.1990	15			
Treatment (between	1.177				
columns)		q	Significant? P <	Summary	95% CI of diff
Residual (within columns)	Mean Diff.	4.157	0.05?	*	0.02334 to
Total	0.2300	11.14	Yes	***	0.4367
	0.5900	6.796	Yes	***	0.3921 to
Tukey's Multiple	0.3600		Yes		0.7879
Comparison Test					0.1621 to
25mg/ml vs 50mg/ml					0.5579
25mg/ml vs 100mg/ml					
50mg/ml vs 100mg/ml					

1way ANOVA of Euphobia hirta:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 6				
Repeated Measures ANOVA					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	44.78				
R square	0.9180				
Was the pairing significantly effective?	0.1108				
R square	3.038				
F	0.0847				
P value	ns				
P value summary	No				
Is there significant matching? (P < 0.05)	SS	df	MS		
	0.8843	2	0.4422		
	0.1200	4	0.0300		
ANOVA Table	0.07900	8	0.009875		
Treatment (between columns)	1.083	14			
Individual (between rows)					
Residual (random)	Mean Diff.	q	Significant? P <	Summary	95% CI of diff
Total	0.2300	5.175	0.05?	*	0.05041 to 0.4096
	0.5900	13.28	Yes	***	0.4104 to 0.7696
Tukey's Multiple Comparison Test	0.3600	8.101	Yes	**	0.1804 to 0.5396
25mg/ml vs 50mg/ml			Yes		
25mg/ml vs 100mg/ml					
50mg/ml vs 100mg/ml					

1way ANOVA of +Control 1:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 6				
Repeated Measures ANOVA					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	45.11				
R square	0.9185				
Was the pairing significantly effective?	0.08821				
R square	3.563				
F	0.0388				
P value	*				
P value summary	Yes				
Is there significant matching? (P < 0.05)					
	SS	df	MS		
	1.074	3	0.3579		
ANOVA Table	0.1131	4	0.02827		
Treatment (between columns)	0.09521	12	0.007934		
Individual (between rows)	1.282	19			
Residual (random)	Mean Diff.	q	Significant? P <	Summary	95% CI of diff
Total	-0.4980	8.840	0.05?	***	-0.6491 to -0.3469
	-0.2680	4.757	Yes	**	-0.4191 to -0.1169
Dunnett's Multiple Comparison Test	0.0920	1.633	Yes	ns	-0.05914 to 0.2431
			No		
125µg/ml vs 25mg/ml					
125µg/ml vs 50mg/ml					
125µg/ml vs 100mg/ml					

1way ANOVA of +Control 2:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 6				
Repeated Measures ANOVA					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	48.66				
R square	0.9240				
Was the pairing significantly effective?	0.1016				
R square	4.468				
F	0.0193				
P value	*				
P value summary	Yes				
Is there significant matching? (P < 0.05)	SS	df	MS		
	1.080	3	0.3601		
ANOVA Table	0.1323	4	0.03307		
Treatment (between columns)	0.08881	12	0.007401		
Individual (between rows)	1.301	19			
Residual (random)					
Total	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
	-0.5020	9.226	Yes	***	-0.6480 to -0.3560
Dunnett's Multiple Comparison Test	-0.2720	4.999	Yes	***	-0.4180 to -0.1260
250µg/ml vs 25mg/ml	0.0880	1.617	No	ns	-0.05797 to 0.2340
250µg/ml vs 50mg/ml					
250µg/ml vs 100mg/ml					

1way ANOVA of +Control 3:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 6				
Repeated Measures ANOVA					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	42.21				
R square	0.9134				
Was the pairing significantly effective?	0.06542				
R square	2.426				
F	0.1052				
P value	ns				
P value summary	No				
Is there significant matching? (P < 0.05)	SS	df	MS		
	1.184	3	0.3947		
ANOVA Table	0.09073	4	0.02268		
Treatment (between columns)	0.1122	12	0.009349		
Individual (between rows)	1.387	19			
Residual (random)	Mean	q	Significant? P <	Summary	95% CI of diff
Total	Diff.	9.092	0.05?	***	-0.7201 to -0.3919
	-0.5560	5.331	Yes	***	-0.4901 to -0.1619
Dunnett's Multiple Comparison Test	-0.3260	0.5560	Yes	ns	-0.1301 to 0.1981
	0.0340		No		
500µg/ml vs 25mg/ml					
500µg/ml vs 50mg/ml					
500µg/ml vs 100mg/ml					

1way ANOVA of Euphobia hirta:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Euphobia hirta				
One-way analysis of variance					
P value					
P value summary	< 0.0001				
Are means signif. different? (P < 0.05)	***				
	Yes				
Number of groups	6				
F	28.30				
R square	0.8348				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)					
P value					
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				
		df	MS		
	SS	5	0.2709		
ANOVA Table	1.354	28	0.009570		
Treatment (between columns)	0.2680	33			
Residual (within columns)	1.622				
Total		q	Significant? P <	Summary	95% CI of diff
	Mean Diff.	5.257	0.05?	*	0.04075 to 0.4192
Tukey's Multiple Comparison	0.2300	14.09	Yes	***	0.4088 to 0.7712
Test	0.5900	11.58	Yes	***	0.3038 to 0.6662
25mg/ml vs 50mg/ml	0.4850	11.38	Yes	***	0.2955 to 0.6579
25mg/ml vs 100mg/ml	0.4767	12.85	Yes	***	0.3571 to 0.7195
25mg/ml vs 125µg/ml	0.5383	8.594	Yes	***	0.1788 to 0.5412
25mg/ml vs 250µg/ml	0.3600	6.088	Yes	**	0.07381 to 0.4362
25mg/ml vs 500µg/ml	0.2550	5.889	Yes	**	0.06548 to 0.4279
50mg/ml vs 100mg/ml	0.2467	7.361	Yes	***	0.1271 to 0.4895
50mg/ml vs 125µg/ml	0.3083	2.629	Yes	ns	-0.2778 to 0.06776
50mg/ml vs 250µg/ml	-0.1050	2.838	No	ns	-0.2861 to 0.05942
50mg/ml vs 500µg/ml	-0.1133	1.294	No	ns	-0.2244 to 0.1211
100mg/ml vs 125µg/ml	-0.05167	0.2087	No	ns	-0.1811 to 0.1644
100mg/ml vs 250µg/ml	-0.008333	1.335	No	ns	-0.1194 to 0.2261
100mg/ml vs 500µg/ml	0.05333	1.544	No	ns	-0.1111 to 0.2344
125µg/ml vs 250µg/ml	0.06167		No		
125µg/ml vs 500µg/ml					
250µg/ml vs 500µg/ml					

1way ANOVA of Euphobia hirta:Column statistics

	25mg/ml	50mg/ml	100mg/ml	125µg/ml	250µg/ml	500µg/ml
Number of values	6	6	6	6	6	6
Minimum	0.4500	0.2000	0.0	0.0300	0.0300	0.0
25% Percentile	0.4750	0.2500	0.0	0.0600	0.0300	0.0075
Median	0.5000	0.3500	0.0	0.1050	0.0850	0.0400
75% Percentile	0.7500	0.4750	0.0	0.1550	0.2175	0.0950
Maximum	0.9000	0.5500	0.0	0.1700	0.2400	0.1400
Mean	0.5900	0.3600	0.0	0.1050	0.1133	0.05167
Std. Deviation	0.1817	0.1294	0.0	0.05357	0.09092	0.05154
Std. Error	0.08124	0.05788	0.0	0.02187	0.03712	0.02104
Lower 95% CI	0.3644	0.1993	0.0	0.04878	0.01792	-0.002424
Upper 95% CI	0.8156	0.5207	0.0	0.1612	0.2087	0.1058

1way ANOVA of Euphobia hirta 1:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Euphobia hirta				
One-way analysis of variance					
P value					
P value summary	< 0.0001				
Are means signif. different? (P < 0.05)	***				
Number of groups	Yes				
F	4				
R square	32.03				
	0.8422				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)					
P value					
P value summary					
Do the variances differ signif. (P < 0.05)	ns				
	No				
		df	MS		
	SS	3	0.3797		
ANOVA Table	1.139	18	0.01185		
Treatment (between columns)	0.2134	21			
Residual (within columns)	1.352				
Total		q	Significant? P <	Summary	95% CI of diff
	Mean Diff.	7.357	0.05?	***	-0.6540 to -0.3160
Dunnett's Multiple Comparison	-0.4850	3.868	Yes	**	-0.4240 to -0.08602
Test	-0.2550	1.670	Yes	ns	-0.05612 to 0.2661
125µg/ml vs 25mg/ml	0.1050		No		
125µg/ml vs 50mg/ml					
125µg/ml vs 100mg/ml					

1way ANOVA of Euphobia hirta2:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Euphobia hirta				
One-way analysis of variance					
P value					
P value summary	< 0.0001				
Are means signif. different? (P < 0.05)	***				
	Yes				
Number of groups	4				
F	28.09				
R square	0.8240				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)					
P value					
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				
		df	MS		
	SS	3	0.3751		
ANOVA Table	1.125	18	0.01335		
Treatment (between columns)	0.2403	21			
Residual (within columns)	1.366				
Total		q	Significant? P <	Summary	95% CI of diff
	Mean Diff.	6.813	0.05?	***	-0.6560 to -0.2973
Dunnett's Multiple Comparison Test	-0.4767	3.525	Yes	**	-0.4260 to -0.06731
250µg/ml vs 25mg/ml	-0.2467	1.699	Yes	ns	-0.05767 to 0.2843
250µg/ml vs 50mg/ml	0.1133		No		
250µg/ml vs 100mg/ml					

1way ANOVA of Euphobia hirta 3:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Euphobia hirta				
One-way analysis of variance					
P value					
P value summary	< 0.0001				
Are means signif. different? (P < 0.05)	***				
	Yes				
Number of groups	4				
F	35.07				
R square	0.8539				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)					
P value					
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				
		df	MS		
	SS	3	0.4136		
ANOVA Table	1.241	18	0.01179		
Treatment (between columns)	0.2123	21			
Residual (within columns)	1.453				
Total		q	Significant? P <	Summary	95% CI of diff
	Mean Diff.	8.186	0.05?	***	-0.7069 to -0.3698
Dunnett's Multiple Comparison	-0.5383	4.689	Yes	***	-0.4769 to -0.1398
Test	-0.3083	0.8240	Yes	ns	-0.1090 to 0.2124
500µg/ml vs 25mg/ml	0.05167		No		
500µg/ml vs 50mg/ml					
500µg/ml vs 100mg/ml					

1way ANOVA of Acantus montanus :Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Acantus montanus				
One-way analysis of variance					
P value	0.0003				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	6				
F	6.646				
R square	0.5427				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	27.36				
P value	< 0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.5016	5	0.1003		
Residual (within columns)	0.4227	28	0.01509		
Total	0.9242	33			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
25mg/ml vs 50mg/ml	0.1020	1.856	No	ns	-0.1357 to 0.3397
25mg/ml vs 100mg/ml	0.3597	6.837	Yes	***	0.1321 to 0.5872
25mg/ml vs 125µg/ml	0.2630	4.999	Yes	*	0.03545 to 0.4906
25mg/ml vs 250µg/ml	0.2547	4.841	Yes	*	0.02711 to 0.4822
25mg/ml vs 500µg/ml	0.3163	6.013	Yes	**	0.08878 to 0.5439
50mg/ml vs 100mg/ml	0.2577	4.898	Yes	*	0.03011 to 0.4852
50mg/ml vs 125µg/ml	0.1610	3.061	No	ns	-0.06655 to 0.3886
50mg/ml vs 250µg/ml	0.1527	2.902	No	ns	-0.07489 to 0.3802
50mg/ml vs 500µg/ml	0.2143	4.074	No	ns	-0.01322 to 0.4419
100mg/ml vs 125µg/ml	-0.09667	1.927	No	ns	-0.3136 to 0.1203
100mg/ml vs 250µg/ml	-0.1050	2.093	No	ns	-0.3220 to 0.1120
100mg/ml vs 500µg/ml	-0.04333	0.8639	No	ns	-0.2603 to 0.1736
125µg/ml vs 250µg/ml	-0.008333	0.1661	No	ns	-0.2253 to 0.2086
125µg/ml vs 500µg/ml	0.05333	1.063	No	ns	-0.1636 to 0.2703
250µg/ml vs 500µg/ml	0.06167	1.229	No	ns	-0.1553 to 0.2786

1way ANOVA of Acantus montanus:Column statistics

	25mg/ml	50mg/ml	100mg/ml	125µg/ml	250µg/ml	500µg/ml
Number of values	6	6	6	6	6	6
Minimum	0.1000	0.0500	0.0	0.0300	0.0300	0.0
25% Percentile	0.1700	0.1150	0.0	0.0600	0.0300	0.0075
Median	0.3000	0.2000	0.0	0.1050	0.0850	0.0400
75% Percentile	0.6000	0.4500	0.0125	0.1550	0.2175	0.0950
Maximum	0.7000	0.5000	0.0500	0.1700	0.2400	0.1400
Mean	0.3680	0.2660	0.008333	0.1050	0.1133	0.05167
Std. Deviation	0.2348	0.1811	0.02041	0.05357	0.09092	0.05154
Std. Error	0.1050	0.08097	0.008333	0.02187	0.03712	0.02104
Lower 95% CI	0.07649	0.04119	-0.01309	0.04878	0.01792	-0.002424
Upper 95% CI	0.6595	0.4908	0.02975	0.1612	0.2087	0.1058

1way ANOVA of Acantus montanus:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Acantus montanus				
One-way analysis of variance P value					
P value summary	0.0085				
Are means signif. different? (P < 0.05)	**				
Number of groups	Yes				
F	3				
R square	7.044				
	0.5201				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	15.70				
P value	0.0004				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
		df	MS		
	SS	2	0.1916		
ANOVA Table	0.3833	13	0.02721		
Treatment (between columns)	0.3537	15			
Residual (within columns)	0.7370				
Total		q	Significant? P <	Summary	95% CI of diff
	Mean Diff.	1.383	0.05?	ns	-0.1735 to 0.3775
Tukey's Multiple Comparison	0.1020	5.093	No	**	0.09588 to 0.6234
Test	0.3597	3.648	Yes	ns	-0.006116 to
25mg/ml vs 50mg/ml	0.2577		No		0.5214
25mg/ml vs 100mg/ml					
50mg/ml vs 100mg/ml					

1way ANOVA of Acantus montanus:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Acantus montanus				
Repeated Measures ANOVA P value					
P value summary	0.0041				
Are means signif. different? (P < 0.05)	**				
Number of groups	Yes				
F	3				
R square	11.85				
	0.7476				
Was the pairing significantly effective?					
R square	0.3224				
F	3.769				
P value	0.0522				
P value summary	ns				
Is there significant matching? (P < 0.05)	No				
		df	MS		
ANOVA Table	SS	2	0.1805		
Treatment (between columns)	0.3610	4	0.05743		
Individual (between rows)	0.2297	8	0.01524		
Residual (random)	0.1219	14			
Total	0.7126				
		q	Significant? P < 0.05?	Summary	95% CI of diff
Tukey's Multiple Comparison Test	Mean Diff.	1.848	No	ns	-0.1211 to 0.3251
25mg/ml vs 50mg/ml	0.1020	6.666	Yes	**	0.1449 to 0.5911
25mg/ml vs 100mg/ml	0.3680	4.819	Yes	*	0.04293 to 0.4891
50mg/ml vs 100mg/ml	0.2660		Yes		

1way ANOVA of +Control 1:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Acantus montanus				
Repeated Measures ANOVA					
P value					
P value summary	0.0003				
Are means signif. different? (P < 0.05)	***				
Number of groups	Yes				
F	6				
R square	7.777				
	0.6604				
Was the pairing significantly effective?					
R square	0.1328				
F	2.254				
P value	0.0994				
P value summary	ns				
Is there significant matching? (P < 0.05)	No				
	SS	df	MS		
	0.5185	5	0.1037		
ANOVA Table	0.1202	4	0.03005		
Treatment (between columns)	0.2667	20	0.01333		
Individual (between rows)	0.9053	29			
Residual (random)		q	Significant? P < 0.05?	Summary	95% CI of diff
Total	Mean Diff.	3.779	Yes	**	-0.4757 to -0.07628
	-0.2760	2.383	No	ns	-0.3737 to 0.02572
Dunnett's Multiple Comparison Test	-0.1740	1.260	No	ns	-0.1077 to 0.2917
125µg/ml vs 25mg/ml	0.0920	0.05477	No	ns	-0.1957 to 0.2037
125µg/ml vs 50mg/ml	0.004000	0.7942	No	ns	-0.1417 to 0.2577
125µg/ml vs 100mg/ml	0.0580		No		
125µg/ml vs 250µg/ml					
125µg/ml vs 500µg/ml					

1way ANOVA of +Control 2:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Acantus montanus				
Repeated Measures ANOVA					
P value					
P value summary	0.0003				
Are means signif. different? (P < 0.05)	***				
Number of groups	Yes				
F	6				
R square	7.777				
	0.6604				
Was the pairing significantly effective?					
R square	0.1328				
F	2.254				
P value	0.0994				
P value summary	ns				
Is there significant matching? (P < 0.05)	No				
	SS	df	MS		
	0.5185	5	0.1037		
ANOVA Table	0.1202	4	0.03005		
Treatment (between columns)	0.2667	20	0.01333		
Individual (between rows)	0.9053	29			
Residual (random)		q	Significant? P < 0.05?	Summary	95% CI of diff
Total	Mean Diff.	3.834	Yes	**	-0.4797 to -0.08028
	-0.2800	2.437	No	ns	-0.3777 to 0.02172
Dunnett's Multiple Comparison Test	-0.1780	1.205	No	ns	-0.1117 to 0.2877
250µg/ml vs 25mg/ml	0.0880	0.05477	No	ns	-0.2037 to 0.1957
250µg/ml vs 50mg/ml	-0.004000	0.7394	No	ns	-0.1457 to 0.2537
250µg/ml vs 100mg/ml	0.0540		No		
250µg/ml vs 125µg/ml					
250µg/ml vs 500µg/ml					

1way ANOVA of +Control 3:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Acantus montanus				
Repeated Measures ANOVA					
P value					
P value summary	0.0003				
Are means signif. different? (P < 0.05)	***				
	Yes				
Number of groups	6				
F	7.777				
R square	0.6604				
Was the pairing significantly effective?					
	0.1328				
R square	2.254				
F	0.0994				
P value	ns				
P value summary	No				
Is there significant matching? (P < 0.05)		df	MS		
	SS	5	0.1037		
	0.5185	4	0.03005		
ANOVA Table	0.1202	20	0.01333		
Treatment (between columns)	0.2667	29			
Individual (between rows)	0.9053				
Residual (random)		q	Significant? P <	Summary	95% CI of diff
Total	Mean Diff.	4.573	0.05?	***	-0.5337 to -0.1343
	-0.3340	3.177	Yes	*	-0.4317 to -
Dunnett's Multiple Comparison	-0.2320	0.4656	Yes	ns	0.03228
Test	0.0340	0.7942	No	ns	-0.1657 to 0.2337
500µg/ml vs 25mg/ml	-0.0580	0.7394	No	ns	-0.2577 to 0.1417
500µg/ml vs 50mg/ml	-0.0540		No		-0.2537 to 0.1457
500µg/ml vs 100mg/ml					
500µg/ml vs 125µg/ml					
500µg/ml vs 250µg/ml					

1way ANOVA of Jatropha curcas leaves:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Jatropha curcas leaves				
One-way analysis of variance					
P value					
P value summary	< 0.0001				
Are means signif. different? (P < 0.05)	***				
Number of groups	Yes				
F	3				
R square	28.73				
	0.8272				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	11.82				
P value	0.0027				
P value summary	**				
Do the variances differ signif. (P < 0.05)	Yes				
	SS	df	MS		
ANOVA Table	0.4823	2	0.2411		
Treatment (between columns)	0.1007	12	0.008393		
Residual (within columns)	0.5830	14			
Total					
	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Tukey's Multiple Comparison Test	0.08000	1.953	No	ns	-0.07459 to 0.2346
25mg/ml vs 50mg/ml	0.4140	10.10	Yes	***	0.2594 to 0.5686
25mg/ml vs 100mg/ml	0.3340	8.152	Yes	***	0.1794 to 0.4886
50mg/ml vs 100mg/ml					

1way ANOVA of Jatropha curcas leaves:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Jatropha curcas leaves				
Repeated Measures ANOVA					
P value					
P value summary	< 0.0001				
Are means signif. different? (P < 0.05)	***				
Number of groups	Yes				
F	3				
R square	59.64				
	0.9371				
Was the pairing significantly effective?					
R square	0.1173				
F	4.228				
P value	0.0395				
P value summary	*				
Is there significant matching? (P < 0.05)	Yes				
	SS	df	MS		
ANOVA Table	0.4823	2	0.2411		
Treatment (between columns)	0.06837	4	0.01709		
Individual (between rows)	0.03235	8	0.004043		
Residual (random)	0.5830	14			
Total					
	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Tukey's Multiple Comparison Test	0.08000	2.813	No	ns	-0.03491 to 0.1949
25mg/ml vs 50mg/ml	0.4140	14.56	Yes	***	0.2991 to 0.5289
25mg/ml vs 100mg/ml	0.3340	11.75	Yes	***	0.2191 to 0.4489
50mg/ml vs 100mg/ml					

1way ANOVA of +Control 1:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Jatropha curcas leaves				
Repeated Measures ANOVA					
P value					
P value summary	< 0.0001				
Are means signif. different? (P < 0.05)	***				
	Yes				
Number of groups	6				
F	60.01				
R square	0.9375				
Was the pairing significantly effective?					
R square	0.09770				
F	8.664				
P value	0.0003				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
	SS	df	MS		
	0.7467	5	0.1493		
ANOVA Table	0.08623	4	0.02156		
Treatment (between columns)	0.04977	20	0.002488		
Individual (between rows)	0.8827	29			
Residual (random)					
		q	Significant? P < 0.05?	Summary	95% CI of diff
Total	Mean Diff.	10.40		***	-0.4143 to -0.2417
	-0.3280	7.861	Yes	***	-0.3343 to -0.1617
Dunnett's Multiple Comparison Test	-0.2480	2.726	Yes	ns	-0.0002799 to 0.1723
	0.0860	0.1268	No	ns	
125µg/ml vs 25mg/ml	0.004000	1.838	No	ns	-0.08228 to 0.09028
125µg/ml vs 50mg/ml	0.0580		No		
125µg/ml vs 100mg/ml					-0.02828 to 0.1443
125µg/ml vs 250µg/ml					
125µg/ml vs 500µg/ml					

1way ANOVA of +Control 2:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Jatropha curcas leaves				
Repeated Measures ANOVA					
P value					
P value summary	< 0.0001				
Are means signif. different? (P < 0.05)	***				
Number of groups	Yes				
F	6				
R square	60.01				
	0.9375				
Was the pairing significantly effective?					
R square	0.09770				
F	8.664				
P value	0.0003				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
	SS	df	MS		
ANOVA Table	0.7467	5	0.1493		
Treatment (between columns)	0.08623	4	0.02156		
Individual (between rows)	0.04977	20	0.002488		
Residual (random)	0.8827	29			
Total					
	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Dunnett's Multiple Comparison Test	-0.3320	10.52	Yes	***	-0.4183 to -0.2457
250µg/ml vs 25mg/ml	-0.2520	7.988	Yes	***	-0.3383 to -0.1657
250µg/ml vs 50mg/ml	0.0820	2.599	Yes	ns	-0.004280 to 0.1683
250µg/ml vs 100mg/ml	-0.004000	0.1268	No	ns	-0.09028 to 0.08228
250µg/ml vs 125µg/ml	0.0540	1.712	No	ns	-0.03228 to 0.1403
250µg/ml vs 500µg/ml			No		

1way ANOVA of +Control 3:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Jatropha curcas leaves				
Repeated Measures ANOVA					
P value					
P value summary	< 0.0001				
Are means signif. different? (P < 0.05)	***				
	Yes				
Number of groups	6				
F	60.01				
R square	0.9375				
Was the pairing significantly effective?					
	0.09770				
R square	8.664				
F	0.0003				
P value	***				
P value summary	Yes				
Is there significant matching? (P < 0.05)		df	MS		
	SS	5	0.1493		
	0.7467	4	0.02156		
ANOVA Table	0.08623	20	0.002488		
Treatment (between columns)	0.04977	29			
Individual (between rows)	0.8827				
Residual (random)		q	Significant? P < 0.05?	Summary	95% CI of diff
Total	Mean Diff.	12.23	Yes	***	-0.4723 to -0.2997
	-0.3860	9.699	Yes	***	-0.3923 to -0.2197
Dunnett's Multiple Comparison Test	-0.3060	0.8875	Yes	ns	-0.05828 to 0.1143
	0.0280	1.838	No	ns	-0.1443 to 0.02828
500µg/ml vs 25mg/ml	-0.0580	1.712	No	ns	-0.1403 to 0.03228
500µg/ml vs 50mg/ml	-0.0540		No		
500µg/ml vs 100mg/ml					
500µg/ml vs 125µg/ml					
500µg/ml vs 250µg/ml					

1way ANOVA of Jatropha curcas leaves:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Jatropha curcas leaves				
One-way analysis of variance					
P value					
P value summary	< 0.0001				
Are means signif. different? (P < 0.05)	***				
Number of groups	Yes				
F	6				
R square	22.89				
	0.8091				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)					
P value	15.19				
P value summary	0.0096				
Do the variances differ signif. (P < 0.05)	**				
	Yes				
		df	MS		
ANOVA Table	SS	5	0.1439		
Treatment (between columns)	0.7193	27	0.006285		
Residual (within columns)	0.1697	32			
Total	0.8890				
		q	Significant? P < 0.05?	Summary	95% CI of diff
Tukey's Multiple Comparison Test	Mean Diff.	2.256	No	ns	-0.07378 to 0.2338
25mg/ml vs 50mg/ml	0.08000	11.68	Yes	***	0.2602 to 0.5678
25mg/ml vs 100mg/ml	0.4140	9.280	Yes	***	0.1678 to 0.4622
25mg/ml vs 125µg/ml	0.3150	9.034	Yes	***	0.1594 to 0.4539
25mg/ml vs 250µg/ml	0.3067	10.85	Yes	***	0.2211 to 0.5156
25mg/ml vs 500µg/ml	0.3683	9.421	Yes	***	0.1802 to 0.4878
50mg/ml vs 100mg/ml	0.3340	6.923	Yes	***	0.08777 to 0.3822
50mg/ml vs 125µg/ml	0.2350	6.678	Yes	***	0.07943 to 0.3739
50mg/ml vs 250µg/ml	0.2267	8.494	Yes	***	0.1411 to 0.4356
50mg/ml vs 500µg/ml	0.2883	2.917	No	ns	-0.2462 to 0.04823
100mg/ml vs 125µg/ml	-0.0990	3.162	No	ns	-0.2546 to 0.03990
100mg/ml vs 250µg/ml	-0.1073	1.345	No	ns	-0.1929 to 0.1016
100mg/ml vs 500µg/ml	-0.04567	0.2575	No	ns	-0.1487 to 0.1320
125µg/ml vs 250µg/ml	-0.008333	1.648	No	ns	-0.08705 to 0.1937
125µg/ml vs 500µg/ml	0.05333	1.905	No	ns	-0.07871 to 0.2020
250µg/ml vs 500µg/ml	0.06167				

1way ANOVA of Jatropha curcas leaves:Column statistics

	25mg/ml	50mg/ml	100mg/ml	125µg/ml	250µg/ml	500µg/ml
Number of values	6	6	6	6	6	6
Minimum	0.2500	0.2500	0.0	0.0300	0.0300	0.0
25% Percentile	0.3000	0.2750	0.0	0.0600	0.0300	0.0075
Median	0.4000	0.3000	0.0	0.1050	0.0850	0.0400
75% Percentile	0.5500	0.4250	0.0150	0.1550	0.2175	0.0950
Maximum	0.6000	0.4500	0.0300	0.1700	0.2400	0.1400
Mean	0.4200	0.3400	0.0060	0.1050	0.1133	0.05167
Std. Deviation	0.1351	0.08216	0.01342	0.05357	0.09092	0.05154
Std. Error	0.06042	0.03674	0.0060	0.02187	0.03712	0.02104
Lower 95% CI	0.2523	0.2380	-0.01066	0.04878	0.01792	-0.002424
Upper 95% CI	0.5877	0.4420	0.02266	0.1612	0.2087	0.1058

2way ANOVA of Data 10:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Data 10			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Interaction	6.37	0.2325		
Column Factor	12.30	0.0671		
Row Factor	19.01	0.0051		
Source of Variation	P value summary	Significant?		
Interaction	ns	No		
Column Factor	ns	No		
Row Factor	**	Yes		
Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	2	0.08149	0.04074	1.532
Column Factor	2	0.1575	0.07874	2.961
Row Factor	1	0.2434	0.2434	9.151
Residual	30	0.7979	0.02660	
Number of missing values	0			
Bonferroni posttests				
Hexane vs Chloroform				
Row Factor	Hexane	Chloroform	Difference	95% CI of diff.
Extract	0.3217	0.07833	-0.2433	-0.4935 to 0.006872
Control	0.4417	0.3750	-0.06667	-0.3169 to 0.1835
Row Factor	Difference	t	P value	Summary
Extract	-0.2433	2.584	P < 0.05	*
Control	-0.06667	0.7081	P > 0.05	ns
Hexane vs Ethyl Ether				
Row Factor	Hexane	Ethyl Ether	Difference	95% CI of diff.
Extract	0.3217	0.3067	-0.01500	-0.2652 to 0.2352
Control	0.4417	0.3833	-0.05833	-0.3085 to 0.1919
Row Factor	Difference	t	P value	Summary
Extract	-0.01500	0.1593	P > 0.05	ns
Control	-0.05833	0.6195	P > 0.05	ns

1way ANOVA of Data 10:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 10				
One-way analysis of variance					
P value	0.5527				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	0.7272				
R square	0.3265				
	SS	df	MS		
ANOVA Table	0.02625	2	0.01312		
Treatment (between columns)	0.05414	3	0.01805		
Residual (within columns)	0.08039	5			
Total					
	Mean	q	Significant? P <	Summary	95% CI of diff
Tukey's Multiple Comparison Test	Diff.	1.632	0.05?	ns	-0.4064 to 0.7164
Hexane vs Chloroform	0.1550	0.3860	No	ns	-0.5248 to 0.5981
Hexane vs Ethyl Ether	0.03667	1.246	No	ns	-0.6798 to 0.4431
Chloroform vs Ethyl Ether	-0.1183		No		

1way ANOVA of Data 10:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 10				
Repeated Measures ANOVA					
P value	0.3410				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	1.933				
R square	0.6590				
Was the pairing significantly effective?					
R square	0.5046				
F	5.973				
P value	0.1345				
P value summary	ns				
Is there significant matching? (P < 0.05)	No				
	SS	df	MS		
ANOVA Table	0.02625	2	0.01312		
Treatment (between columns)	0.04056	1	0.04056		
Individual (between rows)	0.01358	2	0.006791		
Residual (random)	0.08039	5			
Total					
	Mean Diff.	q	Significant? P <	Summary	95% CI of diff
Tukey's Multiple Comparison Test	0.1550	2.660	0.05?	ns	-0.3304 to 0.6404
Hexane vs Chloroform	0.03667	0.6293	No	ns	-0.4488 to 0.5221
Hexane vs Ethyl Ether	-0.1183	2.031	No	ns	-0.6038 to 0.3671
Chloroform vs Ethyl Ether			No		

1way ANOVA of Data 10:Tabular results

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 10		
Error:See Commentary			
P value	0.3410		
P value summary	ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	3		
F	1.933		
R square	0.6590		
Was the pairing significantly effective?			
R square	0.5046		
F	5.973		
P value	0.1345		
P value summary	ns		
Is there significant matching? (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	0.02625	2	0.01312
Individual (between rows)	0.04056	1	0.04056
Residual (random)	0.01358	2	0.006791
Total	0.08039	5	

t test of Hexane dissolved:Tabular results

	Data Set-A
Table Analyzed	Hexane dissolved
Column A	Extract
vs	vs
Column B	Control
Paired t test	
P value	0.1634
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.633 df=5
Number of pairs	6
How big is the difference?	
Mean of differences	-0.1200
95% confidence interval	-0.3089 to 0.06893
R square	0.3478
How effective was the pairing?	
Correlation coefficient (r)	0.5251
P Value (one tailed)	0.1424
P value summary	ns
Was the pairing significantly effective?	No

t test of Hexane dissolved:Column statistics

	Extract	Control
Number of values	6	6
Minimum	0.1500	0.2000
25% Percentile	0.1725	0.2750
Median	0.3750	0.3750
75% Percentile	0.4125	0.7000
Maximum	0.4500	0.7000
Mean	0.3217	0.4417
Std. Deviation	0.1258	0.2108
Std. Error	0.05134	0.08604
Lower 95% CI	0.1897	0.2205
Upper 95% CI	0.4536	0.6628

t test of Chloroform dissolved:Tabular results

	Data Set-A
Table Analyzed	Chloroform dissolved
Column A	Extract
vs	vs
Column B	Control
Paired t test	
P value	0.0292
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=3.026 df=5
Number of pairs	6
How big is the difference?	
Mean of differences	-0.2967
95% confidence interval	-0.5487 to -0.04462
R square	0.6468
How effective was the pairing?	
Correlation coefficient (r)	-0.1432
P Value (one tailed)	0.3933
P value summary	ns
Was the pairing significantly effective?	No

t test of Chloroform dissolved:Column statistics

	Extract	Control
Number of values	6	6
Minimum	0.0500	0.1000
25% Percentile	0.0500	0.1750
Median	0.0700	0.3250
75% Percentile	0.0975	0.6250
Maximum	0.1500	0.7000
Mean	0.07833	0.3750
Std. Deviation	0.03764	0.2318
Std. Error	0.01537	0.09465
Lower 95% CI	0.03883	0.1317
Upper 95% CI	0.1178	0.6183

t test of Ethyl-ether dissolved:Tabular results

	Data Set-A
Table Analyzed	Ethyl-ether dissolved
Column A	Extract
vs	vs
Column B	Control
Paired t test	
P value	0.4473
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.8243 df=5
Number of pairs	6
How big is the difference?	
Mean of differences	-0.07667
95% confidence interval	-0.3158 to 0.1625
R square	0.1196
How effective was the pairing?	
Correlation coefficient (r)	-0.2056
P Value (one tailed)	0.3480
P value summary	ns
Was the pairing significantly effective?	No

t test of Ethyl-ether dissolved:Column statistics

	Extract	Control
Number of values	6	6
Minimum	0.2200	0.2000
25% Percentile	0.2200	0.2750
Median	0.3000	0.3000
75% Percentile	0.3500	0.5500
Maximum	0.5000	0.7000
Mean	0.3067	0.3833
Std. Deviation	0.1025	0.1835
Std. Error	0.04185	0.07491
Lower 95% CI	0.1991	0.1908
Upper 95% CI	0.4142	0.5759

1way ANOVA of Solvent efficacy:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Solvent efficacy				
One-way analysis of variance					
P value					
P value summary	0.0049				
Are means signif. different? (P < 0.05)	**				
	Yes				
Number of groups	7				
F	3.830				
R square	0.3963				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	14.77				
P value	0.0221				
P value summary	*				
Do the variances differ signif. (P < 0.05)	Yes				
		df	MS		
	SS	6	0.09355		
ANOVA Table	0.5613	35	0.02443		
Treatment (between columns)	0.8550	41			
Residual (within columns)	1.416				
Total		q	Significant?	Summary	95% CI of diff
	Mean Diff.	1.881	P < 0.05?	ns	-0.4024 to 0.1624
Tukey's Multiple Comparison	-0.1200	3.814	No	ns	-0.03910 to 0.5258
Test	0.2433	0.8359	No	ns	-0.3358 to 0.2291
H.Extract vs H.Control	-0.05333	0.2351	No	ns	-0.2674 to 0.2974
H.Extract vs C.Extract	0.01500	0.9665	No	ns	-0.3441 to 0.2208
H.Extract vs C.Control	-0.06167	1.881	No	ns	-0.4024 to 0.1624
H.Extract vs E.Extract	-0.1200	5.694	No	**	0.08090 to 0.6458
H.Extract vs E.Control	0.3633	1.045	Yes	ns	-0.2158 to 0.3491
H.Extract vs No solvent	0.06667	2.116	No	ns	-0.1474 to 0.4174
H.Control vs C.Extract	0.1350	0.9142	No	ns	-0.2241 to 0.3408
H.Control vs C.Control	0.05833	0.0	No	ns	-0.2824 to 0.2824
H.Control vs E.Extract	0.0	4.650	No	*	-0.5791 to -0.01423
H.Control vs E.Control	-0.2967	3.579	Yes	ns	-0.5108 to 0.05410
H.Control vs No solvent	-0.2283	4.780	No	*	-0.5874 to -0.02256
C.Extract vs C.Control	-0.3050	5.694	Yes	**	-0.6458 to -0.08090
C.Extract vs E.Extract	-0.3633	1.071	Yes	ns	-0.2141 to 0.3508
C.Extract vs E.Control	0.06833	0.1306	No	ns	-0.2908 to 0.2741
C.Extract vs No solvent	-0.008333	1.045	No	ns	-0.3491 to 0.2158
C.Control vs E.Extract	-0.06667	1.202	No	ns	-0.3591 to 0.2058
C.Control vs E.Control	-0.07667	2.116	No	ns	-0.4174 to 0.1474
C.Control vs No solvent	-0.1350	0.9142	No	ns	-0.3408 to 0.2241
E.Extract vs E.Control	-0.05833		No		
E.Extract vs No solvent					
E.Control vs No solvent					

1way ANOVA of Solvent efficacy:Column statistics

	H.Extract	H.Control	C.Extract	C.Control	E.Extract	E.Control	No solvent
Number of values	6	6	6	6	6	6	6
	0.1500	0.2000	0.0500	0.1000	0.2200	0.2000	0.3500
Minimum	0.1725	0.2750	0.0500	0.1750	0.2200	0.2750	0.3500
25% Percentile	0.3750	0.3750	0.0700	0.3250	0.3000	0.3000	0.4250
Median	0.4125	0.7000	0.0975	0.6250	0.3500	0.5500	0.5250
75% Percentile	0.4500	0.7000	0.1500	0.7000	0.5000	0.7000	0.6000
Maximum							
	0.3217	0.4417	0.07833	0.3750	0.3067	0.3833	0.4417
Mean	0.1258	0.2108	0.03764	0.2318	0.1025	0.1835	0.1068
Std. Deviation	0.05134	0.08604	0.01537	0.09465	0.04185	0.07491	0.04362
Std. Error							
	0.1897	0.2205	0.03883	0.1317	0.1991	0.1908	0.3295
Lower 95% CI	0.4536	0.6628	0.1178	0.6183	0.4142	0.5759	0.5538
Upper 95% CI							

1way ANOVA of Solvent efficacy:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 14				
One-way analysis of variance					
P value	0.0110				
P value summary	*				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	6				
F	3.627				
R square	0.3768				
Bartlett's test for equal variances	13.59				
Bartlett's statistic (corrected)	0.0185				
P value	*				
P value summary	Yes				
Do the variances differ signif. (P < 0.05)					
	SS	df	MS		
	0.4824	5	0.09647		
ANOVA Table	0.7979	30	0.02660		
Treatment (between columns)	1.280	35			
Residual (within columns)					
Total	Mean	q	Significant? P <	Summary	95% CI of diff
	Diff.	1.802	0.05?	ns	-0.4064 to 0.1664
Tukey's Multiple Comparison Test	-0.1200	3.655	No	ns	-0.04308 to 0.5298
H.Extract vs H.Control	0.2433	0.8011	No	ns	-0.3398 to 0.2331
H.Extract vs C.Extract	-0.05333	0.2253	No	ns	-0.2714 to 0.3014
H.Extract vs C.Control	0.01500	0.9262	No	ns	-0.3481 to 0.2248
H.Extract vs E.Extract	-0.06167	5.457	No	**	0.07692 to 0.6498
H.Extract vs E.Control	0.3633	1.001	Yes	ns	-0.2198 to 0.3531
H.Control vs C.Extract	0.06667	2.028	No	ns	-0.1514 to 0.4214
H.Control vs C.Control	0.1350	0.8762	No	ns	-0.2281 to 0.3448
H.Control vs E.Extract	0.05833	4.456	No	*	-0.5831 to -0.01025
H.Control vs E.Control	-0.2967	3.430	Yes	ns	-0.5148 to 0.05808
C.Extract vs C.Control	-0.2283	4.581	No	*	-0.5914 to -0.01858
C.Extract vs E.Extract	-0.3050	1.026	Yes	ns	-0.2181 to 0.3548
C.Extract vs E.Control	0.06833	0.1252	No	ns	-0.2948 to 0.2781
C.Control vs E.Extract	-0.008333	1.152	No	ns	-0.3631 to 0.2098
C.Control vs E.Control	-0.07667		No		
E.Extract vs E.Control					

1way ANOVA of Solvent efficacy:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 14				
One-way analysis of variance					
P value	0.8813				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	0.2201				
R square	0.03196				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	2.694				
P value	0.4413				
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.02365	3	0.007882		
Residual (within columns)	0.7162	20	0.03581		
Total	0.7399	23			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P <	Summary	95% CI of diff
No solvent vs H.Control	0.0	0.0	0.05?	ns	-0.2776 to 0.2776
No solvent vs C.Control	0.06667	0.6102	No	ns	-0.2109 to 0.3442
No solvent vs E.Control	0.05833	0.5339	No	ns	-0.2192 to 0.3359
			No		

1way ANOVA of Solvent efficacy: abular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 14				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	14.11				
R square	0.6791				
Bartlett's test for equal variances	5.671				
Bartlett's statistic (corrected)	0.1288				
P value	ns				
P value summary	No				
Do the variances differ signif. (P < 0.05)					
	SS	df	MS		
	0.4143	3	0.1381		
ANOVA Table	0.1958	20	0.009789		
Treatment (between columns)	0.6101	23			
Residual (within columns)					
Total	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
		2.101		ns	-0.02512 to 0.2651
Dunnett's Multiple Comparison	0.1200	6.361	No	***	0.2182 to 0.5084
Test	0.3633	2.363	Yes	ns	-0.01012 to 0.2801
No solvent vs H.Extract	0.1350		No		
No solvent vs C.Extract					
No solvent vs E.Extract					

1way ANOVA of Solvent efficacy:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 14				
Repeated Measures ANOVA	0.3423				
P value	ns				
P value summary	No				
Are means signif. different? (P < 0.05)	4				
Number of groups	1.204				
F	0.1940				
R square					
	0.8353				
Was the pairing significantly effective?	18.87				
R square	< 0.0001				
F	***				
P value	Yes				
P value summary					
Is there significant matching? (P < 0.05)	SS	df	MS		
	0.02365	3	0.007882		
	0.6180	5	0.1236		
	0.09823	15	0.006549		
ANOVA Table	0.7399	23			
Treatment (between columns)	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Individual (between rows)	0.0	0.0	No	ns	-0.1219 to 0.1219
Residual (random)	0.06667	1.427	No	ns	-0.05528 to 0.1886
Total	0.05833	1.249	No	ns	-0.06361 to 0.1803
			No		
Dunnett's Multiple Comparison Test					
No solvent vs H.Control					
No solvent vs C.Control					
No solvent vs E.Control					

STATISTICAL ANALYSIS SUMMARY

DESCRIPTION	*N(groups)*	*P value*	*Summary*
Picralima nitida seed.			
General	6	0.015	*
25mg/ml extract vs std antifungal agent	6	0.015	*
50mg/ml extract vs std antifungal agent	6	0.015	*
100mg/ml extract vs std antifungal agent	6	0.0298	*
Picralima nitida rind.			
25mg/ml extract vs std antifungal agent	6	0.005	**
50mg/ml extract vs std antifungal agent	6	0.005	**
100mg/ml extract vs std antifungal agent	6	0.005	**
Azadirachta indica (Neem) seed.			
General	6	0.1640	NS
25mg/ml extract vs std antifungal agent	6	0.0570	NS
50mg/ml extract vs std antifungal agent	6	0.1032	NS
100mg/ml extract vs std antifungal agent	6	0.0233	*
Anacardium occidentale (Cashew).			
General	6	0.0042	**
25mg/ml extract vs std antifungal agent	6	0.0035	**
50mg/ml extract vs std antifungal agent	6	0.0026	**
100mg/ml extract vs std antifungal agent	6	0.0027	**
Euphobia hirta.			
General	6	< 0.0001	***
25mg/ml extract vs std antifungal agent	6	< 0.0001	***
50mg/ml extract vs std antifungal agent	6	<0.0001	***
100mg/ml extract vs std antifungal agent	6	< 0.0001	***
Acantus montanus.			
General	6	0.0003	***
25mg/ml extract vs std antifungal agent	6	0.0003	***
50mg/ml extract vs std antifungal agent	6	0.0003	***
100mg/ml extract vs std antifungal agent	6	0.0003	***
Jatropha curcas.			
General	6	<0.0001	***
25mg/ml extract vs std antifungal agent	6	< 0.0001	***
50mg/ml extract vs std antifungal agent	6	< 0.0001	***
100mg/ml extract vs std antifungal agent	6	< 0.0001	***
Picralima nitida seed fractions.			
General	6	<0.3410	NS
Hexane	6	< 0.1634	NS
Chloroform	6	< 0.0292	*
Ethyl ether	6	< 0.4474	NS

NS: Not Significant.

*** :Significant.**

PLANTS USED

Figure 1a: Picture of *Euphorbia hirta* plant

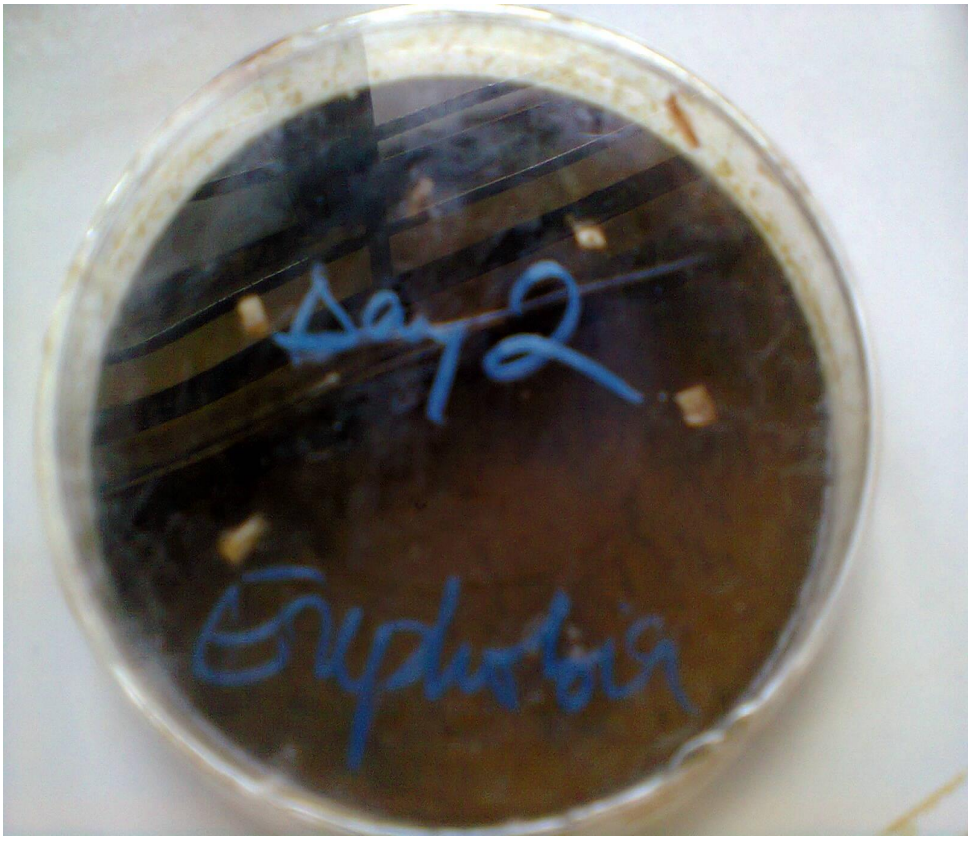

Figure 1b. **Effect of 100 mg/ml (100 %) Methanolic extract of *Euphorbia hirta* plant on *Fusarium* sp ,*T. soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

Figure 2a. Picture of *Anacardium occidentale* (Cashew) plant.

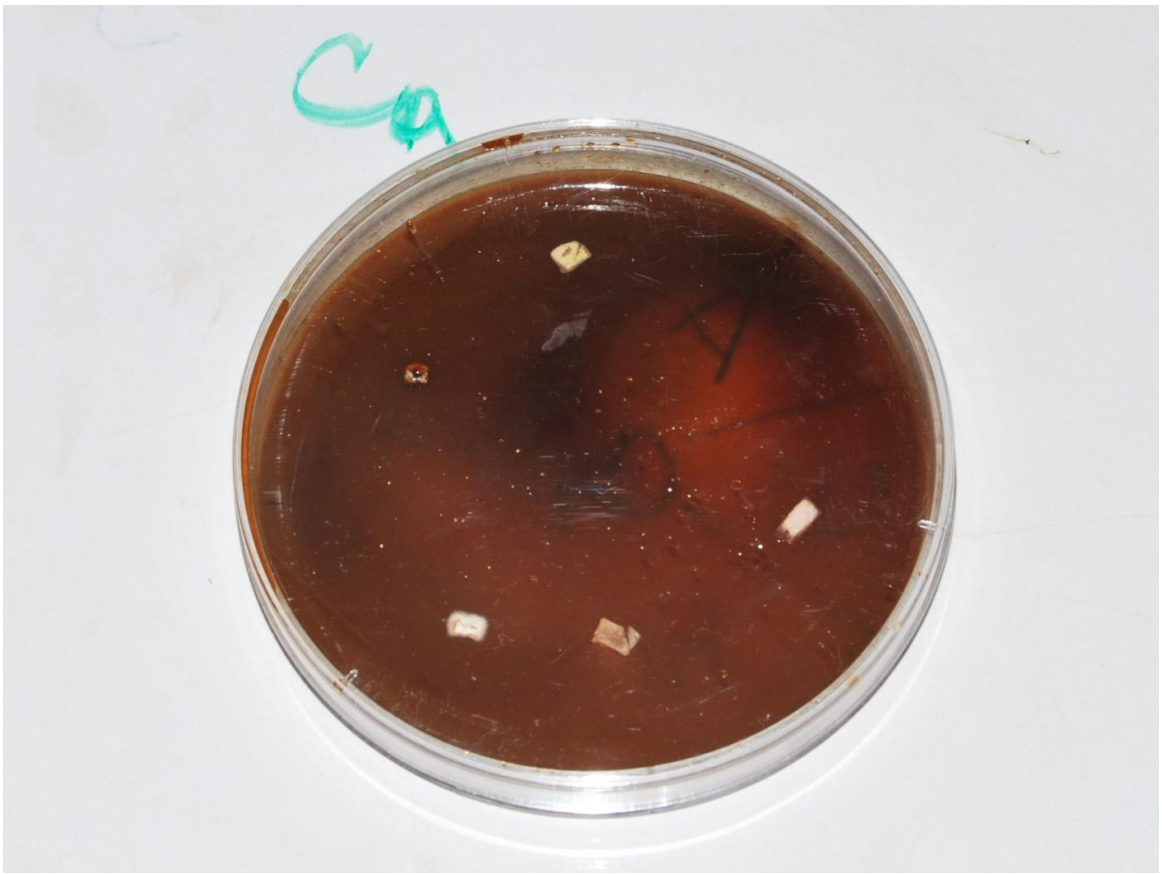

Figure 2b. **Effect of 100 mg/ml (100 %), Methanolic extract of *Anacardium occidentale* (Cashew) leaf on *Fusarium* sp, *T. soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

KEY:
R- *T.rubrum*
S- T. soudanense
M- *T.mentagrophyte*
F-*Fusrium* sp
C- *Cladosporium* sp

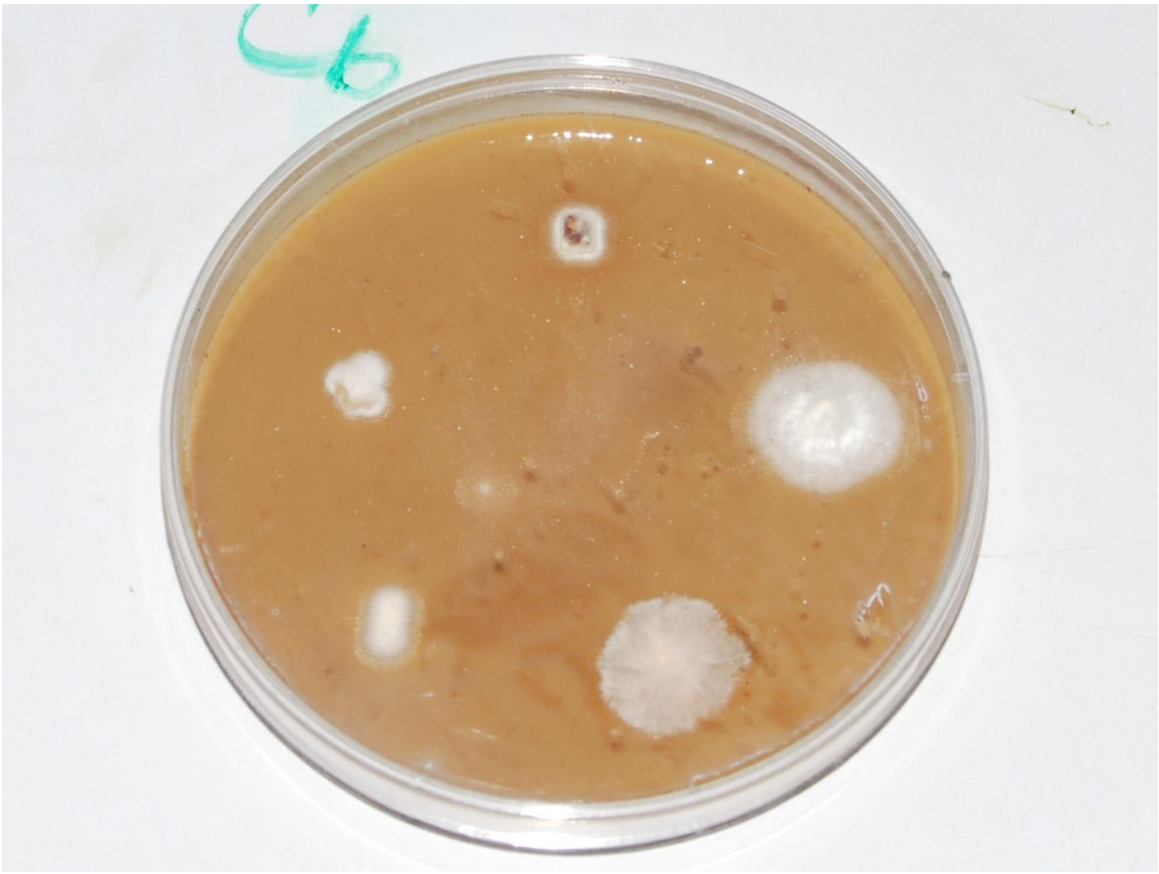

Figure 2c. **Effects of 50 mg/ml (50 %), Methanolic extract of *Anacardium occidentale* (Cashew) leaf on *Fusarium* sp, *T. soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

KEY:
R- *T.rubrum*
S- T. soudanense
M- *T.mentagrophyte*
F-*Fusarium* sp
C-*Cladosporium* sp

Figure 3a.`Picture of *Picralima nitida* plant

Figure 3b. **Effect of 100 mg/ml(100 %) Methanolic extract of *Picralima nitida* seed on *Fusarium* sp, *T. soudanense*,*T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

KEY:
R- ***T.rubrum***
S- T. soudanense
M- ***T.mentagrophyte***
F-*Fusarium* sp
C-*Cladosporium* sp

Figure 3c. **Effect of 50mg/ml (50%) Methanolic extract of *Picralima nitida seed* on *Fusarium* sp, *T. soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

KEY:
R- ***T.rubrum***
S- T. soudanense
M- ***T.mentagrophyte***
F-*Fusarium* sp
C-*Cladosporium* sp

Figure 3d. **Effect of 25 mg/ml (25 %) Methanolic extract of *Picralima nitida seed* on *Fusarium* sp, *T. soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

KEY:
R- ***T.rubrum***
S- T. soudanense
M- ***T.mentagrophyte***
F-*Fusarium* sp
C-*Cladosporium* sp

Figure 3e. **Effect of 100 mg/ml (100 %) Methanolic extract of *Picralima nitida* rind on *Fusarium* sp, *T. soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

KEY:
R- ***T.rubrum***
S- T. soudanense
M- ***T.mentagrophyte***
F-*Fusarium* sp
C-*Cladosporium* sp

Figure 3f. **Effect of 50 mg/ml(50 %) Methanolic extract of *Picralima nitida* rind on *Fusarium* sp, *T. soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

KEY:
R- ***T.rubrum***
S- T. soudanense
M- ***T.mentagrophyte***
F-*Fusarium sp*
C-*Cladosporium* sp

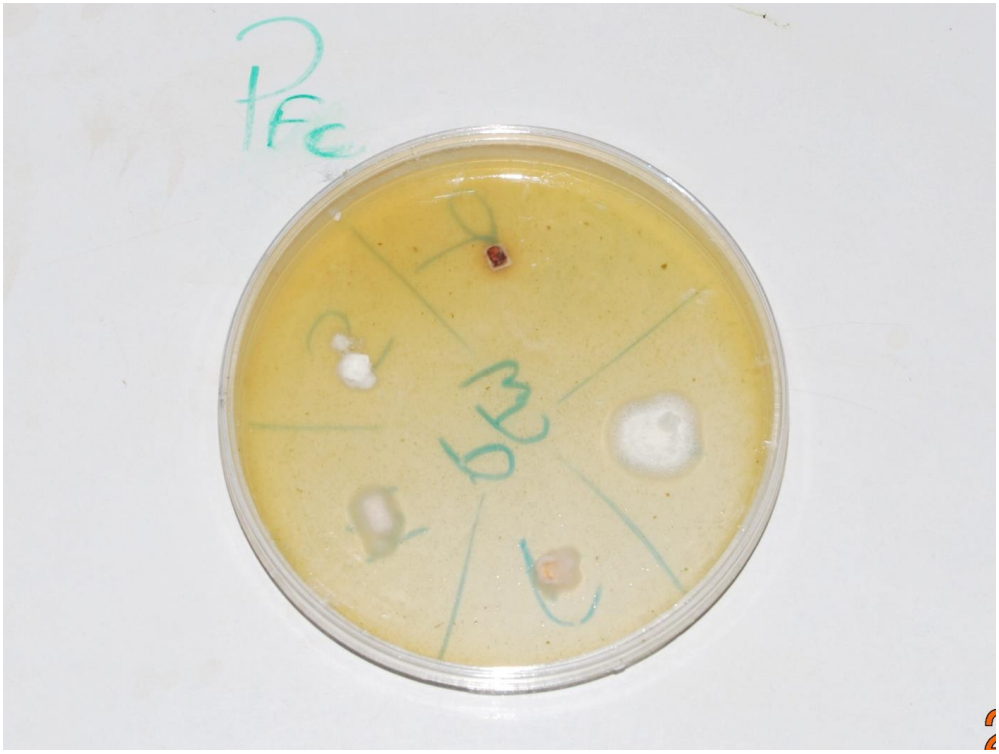

Figure 3g. **Effect of 25 mg/ml (25 %) Methanolic extract of *Picralima nitida* rind on Fusarium spp, *T. soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

KEY:
R- ***T.rubrum***
S- T. soudanense
M- ***T.mentagrophyte***
F-*Fusarium* sp
C-*Cladosporium* sp

Figure 4a. Picture of *Jatropha curcas* plant.

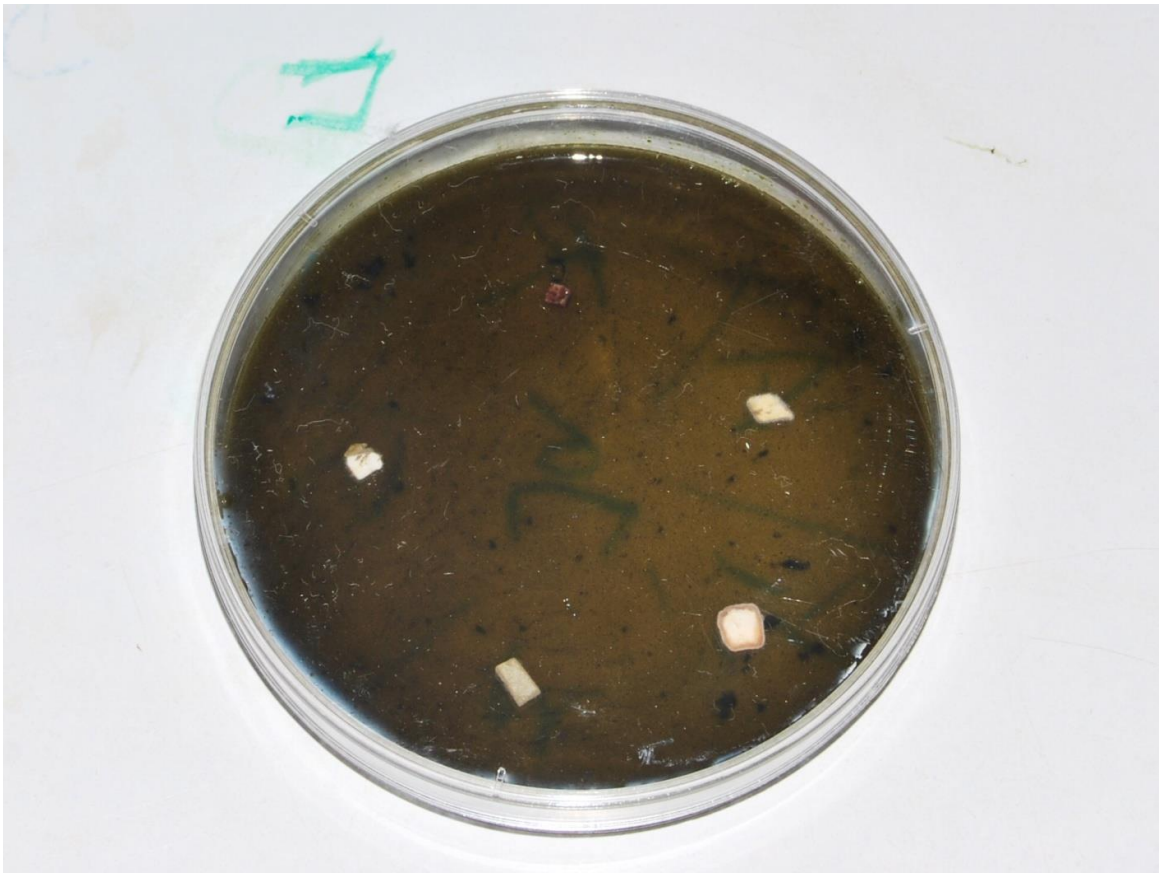

Figure 4b. **Effect of 100 mg/ml (100 %) Methanolic extract of *Jatropha curcas* on *Fusarium* sp, *T. soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

KEY:
R- ***T.rubrum***
S- T. soudanense
M- ***T.mentagrophyte***
F-*Fusarium* sp
C-*Cladosporium* sp

Figure 4c. **Effect of 50 mg/ml (50 %) Methanolic extract of *Jatropha curcas* on *Fusarium* sp, *T. soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

Figure 5a.Picture of *Azadirachta indica (Neem)* Plant.

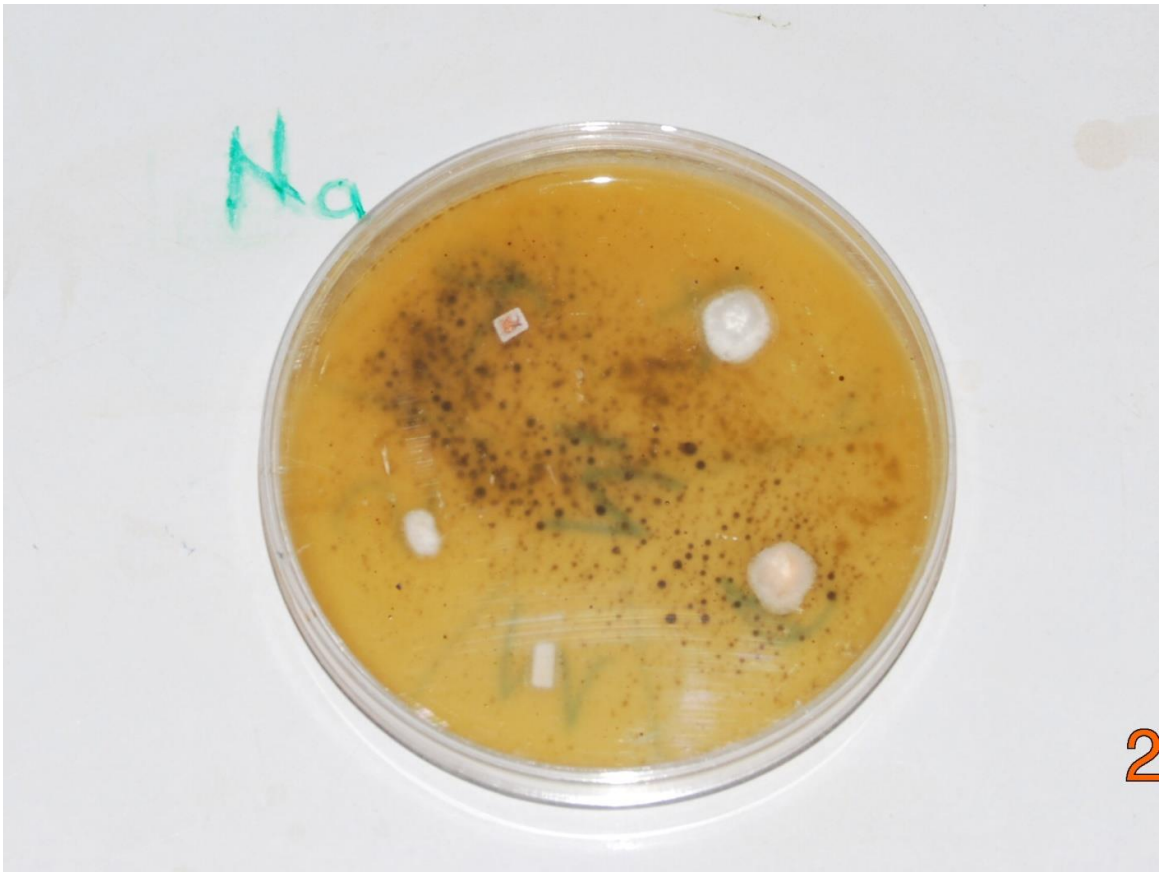

Figure 5b. **Effect of 100 mg/ml (100 %) Methanolic extract of *Azadirachta indica (Neem) seed* on *Fusarium* sp, *T. soudanense, T.mentagrophyte, Cladosporium* sp and *T.rubrum.***

KEY:
R- ***T.rubrum***
S- T. soudanense
M- ***T.mentagrophyte***
F-*Fusarium* sp
C-*Cladosporium* sp

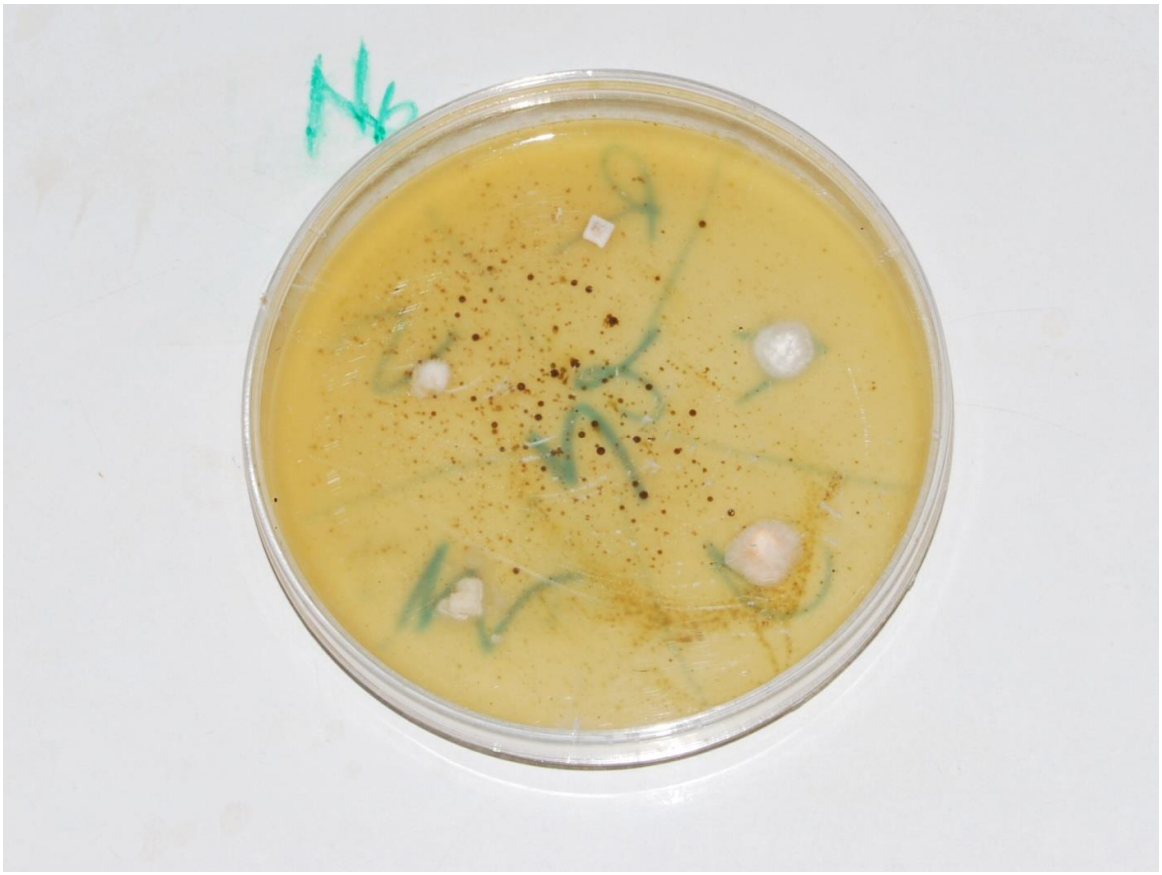

Figure 5c. **Effect of 50 mg/ml (50 %) Methanolic extract of *Azadirachta indica (Neem) seed* on *Fusarium* sp, *T. Soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

KEY:
R- ***T.rubrum***
S- T. soudanense
M- ***T.mentagrophyte***
F-*Fusarium* sp
C-*Cladosporium* sp

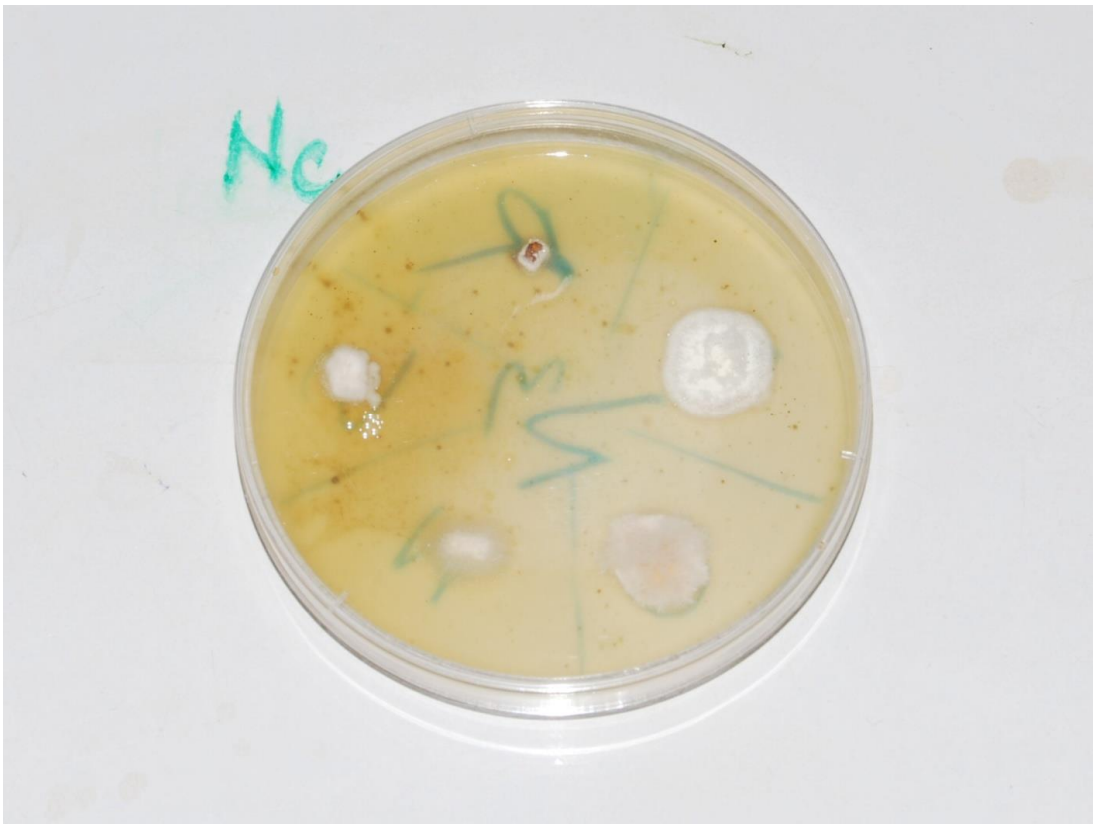

Figure 5d. **Effect of 25 mg/ml (25 %) Methanolic extract of *Azadirachta indica (Neem) seed* on *Fusarium* sp, *T. soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

KEY:
R- ***T.rubrum***
S- T. soudanense
M- ***T.mentagrophyte***
F-*Fusarium* sp
C-*Cladosporium* sp

***Figure 6*a. Picture of *Acantus montanus* plant.**

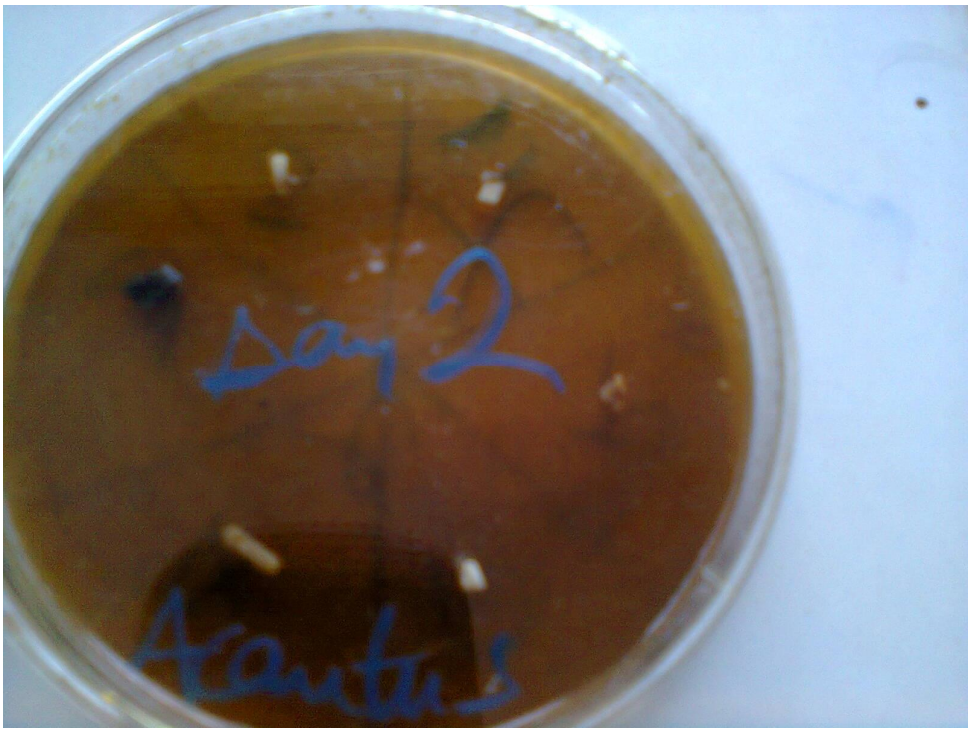

Figure 6b. **Effect of 100 mg/ml (100 %) Methanolic extract of *Acantus montanus* plant extract on *Fusarium* sp, *T. soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

KEY:
R- ***T.rubrum***
S- T. soudanense
M- ***T.mentagrophyte***
F-*Fusarium* sp
C-*Cladosporium* sp

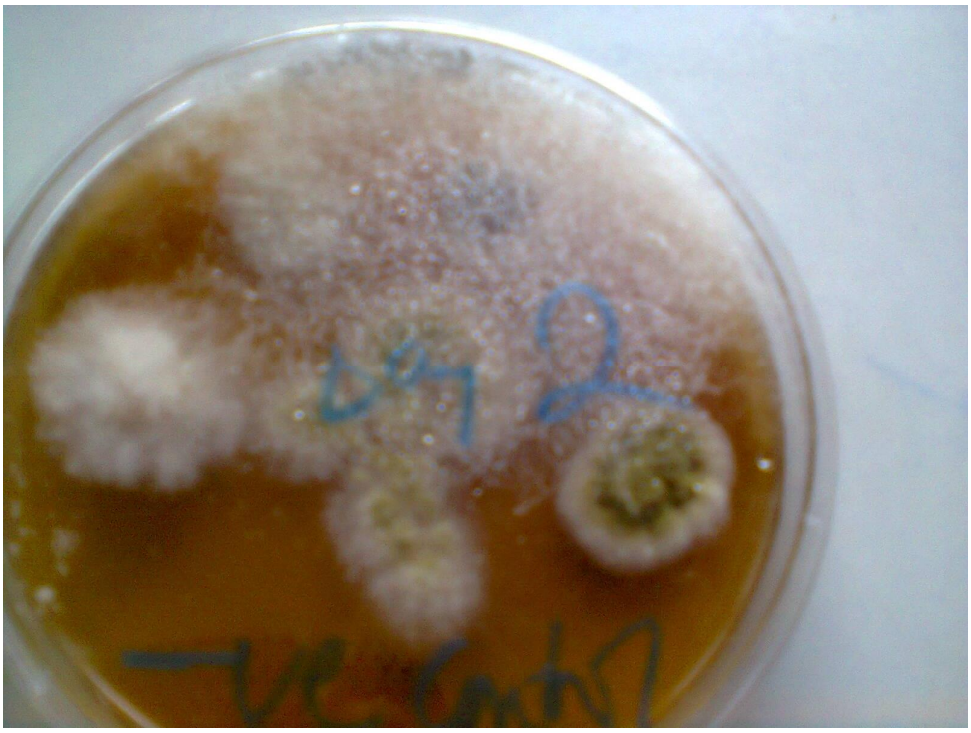

Figure 7. Negative control. Culture plate without extract.

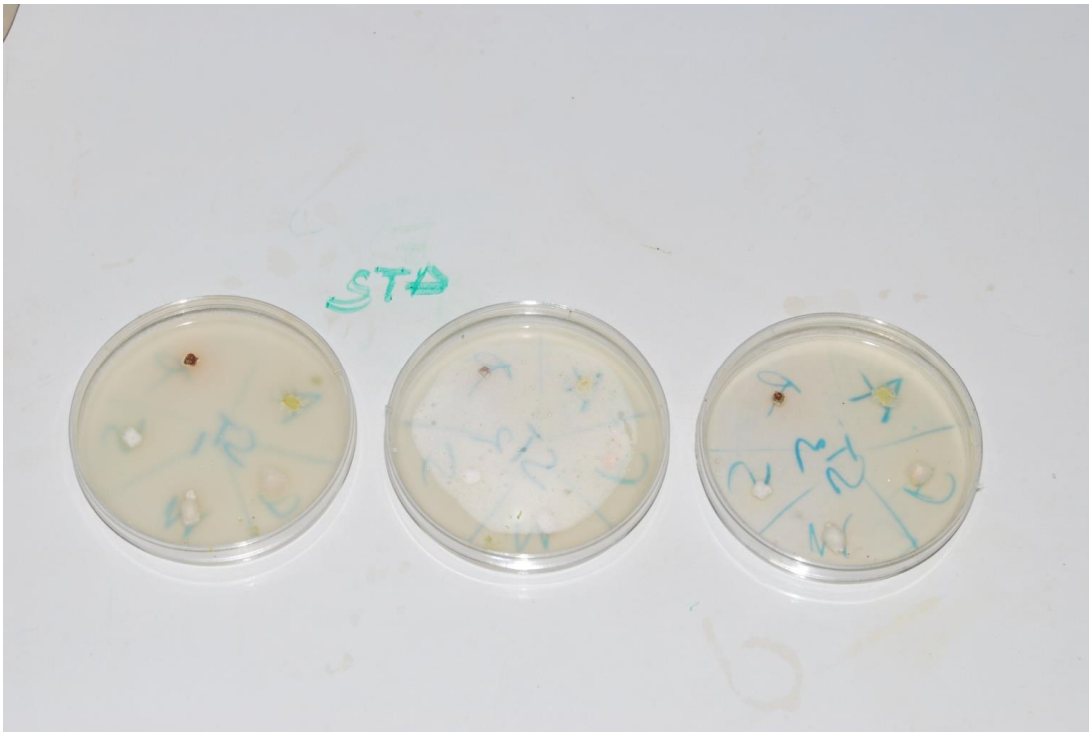

Figure 8. **Effects of 500 ug/ml, 250 ug/ml, 125 ug/ml of Miconazole nitrate in SDA respectively on *Fusarium* sp, *T.soudanense, T.mentagrophyte*, *Cladosporium* sp and *T.rubrum.***

KEY:
R- *T.rubrum*
S- T. soudanense
M- *T.mentagrophyte*
F- *Fusarium* sp
C-*Cladosporium* sp

Figure 9. Set up of Soxhlet extraction procedure.