EVALUATION OF ANTIDEPRESSANT ACTIVITY OF THE PHYTEXPONENT PREPARATION

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DECLARATION

This research project is exclusively my own work and has not been submitted for examination or award of any degree in any university for any other award.

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Signature …….. Date…….

SUPERVISOR APPROVAL

I can confirm that this project has been submitted for examination with my approval as university supervisor.

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Signature…… date…….

DEDICATION

This research project is dedicated to my parents Mr. and Mrs. Warui, and my sister Charity Warui for their words of encouragement to finish this study.

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ABSTRACT

Background: Depression is a common ailment that is spread globally and it affects both younger and older generations. The spreading rate of this disease has been on the rise in the recent years. The reports indicates about 4.4 % of the entire global population was affected with depression by the year 2015. About 5 % of the adult population was those with 60 years of age and above. Suicidal thoughts are the major experienced thoughts among many people under depression in the recent days. The conventional antidepressant have shown to be limited in managing depression. This is has been due to the poor understanding of the onset mechanism of depression. Additionally the conventional drugs are only designed for specific mechanism of action and the mechanism of occurrence of depression is complex hence choice of a suitable conventional drug is a challenge. Alternative therapies that involve use of herbal remedies are currently employed in treatment of depression. Plants are potential agents based on their ability to act through different mechanisms as a result of the different bioactive constituents. For this reason the plant based remedies are able to treat various diseases that occur through various biological pathways.

Objective: This study aimed at evaluating the antidepressant activity of the phytexponent preparation, a natural plant based product.

Methods: The forced swim test and tail suspension test were used as the models for evaluating the antidepressant activity. Fluoxetine at dose level of 20 mg/kg bw was used as the standard antidepressant drug for both models. Phyt^{exponent} preparation was evaluated at three dose levels 100%, 50 % and 25% respectively. The positive control drug and phyt^{exponent} were prepared in normal saline and orally administered 60 minutes prior to the study. The immobility time in both the test was used as an indicator of depression. The depressive like conditions were induced by suspending the animal at a given height and allowing the mice swim in a controlled container respectively.

Results: The results showed that fluoxetine was able to increase the mobility of the mice in the tail suspension test as compared to the phyt^{exponent}. The immobility time of fluoxetine was 2.643±0.0633 and of phyt^{exponent} both models hence reducing depression. 3.547±0.4334, 3.327±0.5519 and for 100 %, 50 % and 25% respectively in the tail suspension test. In the forced swim test, fluoxetine recorded immobility time of 2.448±0.0.2951 and phyt^{exponent} 2.375±0.2.980, 1.975±0.02179 and 2.733±0.09196 minutes for 100 %, 50 % and 25% respectively. The phyt^{exponent} at 50 % dose level recorded the best antidepressant activity.

Conclusion: In conclusion, the study has shown phyt^{exponent} has antidepressant properties and it can be used as a potential herbal remedy for depression like conditions.

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LIST OF ABBREVIATIONS

Bw	Body weight
Cm	Centimeter
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
MAOIS	Monoamine oxidase inhibitors
SEM	Standard error of mean
SNRLs	Serotonin/norepinephrine reuptake inhibitors
SSRLs	Selective serotonin reuptake inhibitors
TCA	Tricyclic antidepressants

CHAPTER ONE: INTRODUCTION

1.1 Background information

Depression is a mental disorder that interferes with the social life and work performance. It's widely spread and the most common psychiatric complaint among many people. About 20 % of the population has been affected with this disorder that does not affect the patients alone but the family and friends too (Rabiei & Rabiei, 2017). The world health organization data indicated, in the year 2020 depression was estimated to be the second most cause of mortality and morbidity after cardiovascular disorder and in addition to other neurodegenerative diseases such as Alzheimer's diseases and Parkinson's disease (Sultana et al., 2018). This mental disorder may vary from mild to severe which is accompanied by hallucinations and delusions. Depression is characterized by various symptoms that include reduced concentration, altered sleep patterns (hypersomnia), eating pattern and weight loss, feeling of hopelessness or worthlessness, fatigue, increased agitation and recurring suicidal thoughts (Fekadu et al., 2016). This conditions that alters the mood is caused by various etiological factors including both personal and environmental conditions, genetic and biochemical parameters. Depression is as well linked to cause the disturbance in the physical health. Stress which mainly leads to depression in its prolonged state, has been involved in the pathogenesis of various diseases such as immunosuppression, endocrine disorders such as diabetics peptic ulcers, hypertension, cognitive dysfunction and ulcerative colitis (Aleza et al., 2015).

Medication currently in use for managing depression and depression related conditions involves use of the synthetic antidepressant agents. These agents are grouped in three major categories: the monoamine oxidase (MAO) inhibitors, the tricyclic antidepressants, and the second-generation antidepressants (Lee & Bae, 2017). The monoamine oxidase inhibitors constitute the antidepressant agents that elicit their role by inhibiting the monoamine oxidase enzyme family. These agents are considered as the first line of depression therapy and include drugs such as tranylcypromine, isocarboxazid, phenelzine, and moclobemide. The MAO inhibitors block both norepinephrine and serotonin transporter, and this rises their levels in the synaptic C hence increase neurotransmission. The use of the tricyclic antidepressant is on the decline in the recent days and their

use is being replaced by new antidepressant agents associated with fewer side effects. The second-generation antidepressant includes agents such as the norepinephrine reuptake inhibitors, the selective serotonin reuptake inhibitors, and the serotonin–norepinephrine reuptake inhibitors. However, even with major advances and development in the modern agents that treat depression, many gaps are still being noted. These antidepressant agents have shown to be less effective with less than 50% of the depression cases being treated completely. Additionally, they have as well been reported to result into side effects such as anxiety, diaphoresis, tachycardia, tremor, sedation, insomnia, serotonin syndrome, Parkinsonism, postural hypotension, blurred vision, and so forth. These outcomes have limited the use of the synthetic antidepressants hence creating an avenue for search of an alternative depression treating agents from medicinal plants (Md Rashidur Rahman, Mohammad Ali, Mostakim Sharif, 2017).

Plants of medicinal values contain many biologically active compounds. These compounds are responsible for various therapeutic effects observed upon use of plants remedies. A single plant species is able to cure various ailments whose pathogenesis follows different mechanisms. This is as a result of the different mechanism of action of the various phytochemical compounds present in these plants. The genesis and progress of depression follows many mechanisms which are not well understand, therefore use of plant based remedies is appropriate as plants owing to the complexity of depression occurrence mechanism many researchers have dedicated much time in evaluating antidepressant pharmacological property of the medicinal plants through various studies. Through these studies many plants have been identified as potential antidepressant alternatives. These plants spread globally including in both in Kenya and globally and this include poppy (opium), deadly nightshade (*Atropa belladonna*), Indian hemp (hashish), henbane (hyoscyamine), thorn apple (scopolamine), and St. John's wort (Hypericum oil) (Rabiei & Rabiei, 2017). Many more plants thought to have the antidepressant properties are available however, the study on them has not been conducted. Many of the plants in the resent days are prepared and combined to make poly-herbal preparations which are combination of many medicinal plants. One of this polyherbal preparation is the phytexponent. Its poly herbal preparation obtained

from a combination of five ethanolic extracts of medicinally known plants; *Matricaria chamomilla, Echinacea purpura, Triticum repens, Allium sativum* and *Viola tricolor* (Moriasi et al., 2021). Its marked as an immune-modulatory agent in its country of origin; Belgium. The individual plants have been studied and shown to confer various pharmacological properties such as antioxidant and anti-inflammatory. The well-studied and validated pharmacological properties of the phytexponent that have been studied

1.2 Problem statement and justification

The depression cases significantly increases by 18.4 % between the 2002 and 2015 %. However, the total population living with the depression cases varied depending on the region. The western pacific region for instance recorded as low as 2.6 % depression cases in male while African region had 5.9% cases in the female (Vos et al., 2016). This condition is the commonly identified mental disorder which in many cases culminates into suicide thoughts. It has been characterized as the second leading cause of mortality among all ages of people with more preference in females (WHO, 2017). Drugs currently used in treatment of depression have adverse effects that has been seen to affect the quality of life of patients. This has worsened the situation as non-compliance to medication by depression patients is on the rise and this further complicates the problem (Pratap et al., 2012).These synthetic antidepressants are characterized with about characterized with about 60 % success. Additionally, other drugs have been reported to take along time of about two weeks after treatment to relive the signs and symptoms of depression observed. These drugs have as well been observed to have many side effects including dry mouth, fatigue, gastrointestinal or respiratory problems, anxiety, agitation, drowsiness, and cardiac arrhythmias (Akhilesh Kumar, 2020). The cost of obtaining the depression drugs is high hence high cost of managing depression and its related disorders. Based on the social, economic and health problems of depression alternative agents from natural sources with ability to lower depression and at the same elicit no toxic effect is urgently need (Ashok Kumar et al., 2013).

Plants of medicinal importance have been part and parcel of man for ages. They were the only source of remedy for the ailments before the discovery of the allopathic medicines. Their use in the current years has gained popularity due

to the few or no side effects upon use, very cheap and affordable and as well high efficacy. Medicinal plants synthesize a wide range of phytochemicals which are the active components that are responsible for the biological activities witnessed in plant remedies. These phyto-compounds include alkaloids, phenols and flavonoids which elicit pharmacological activities including antioxidant, anti-inflammatory, anticancer and antidepressant. The potential of medicinal plants as remedies for the many diseases with no enough data to validate these claims has ignited commencement of researches on plants. This is to explore the many plants which have only been claimed to act against various conditions such as depression. Additionally, with the entry of poly-herbal preparations in the market, their therapeutic property should as well be validated. Hence this study aims evaluate the antidepressant activity of the phytexponent preparation.

1.3 Objectives

1.3.1 General objective

To evaluate the antidepressant activity of the phytexponent preparation

1.3.2 Specific objectives

I. To investigate the antidepresaent activity of phytexponent buy forced swim test
II. To evaluate the antidepressant potential of phytexponent by tail suspension test

1.4 Research questions

I. Does the phytexponent have antidepressant potential against depressive state induced through forced swim test?
II. Does the phytexponent have antidepressant potential against depressive state induced through tail suspension test?

CHAPTER TWO: LITERATURE REVIEW

2.1 Depression

Depression is a common psychiatric disorder that is characterized by changes in the mood, reduced interest in the surroundings and impairment in both psychosocial and physical status (Adongo, 2015). The symptoms of depression are categorized in various groups; cognitive symptoms, physiological symptoms and behavioral symptoms. The cognitive symptoms includes the increased sense of being hopeless, helpless and worthless. The physiological symptoms include loss of appetite towards food, change in the sleep patterns, fatigue and concerns towards aches and pain (Farahani et al., 2015). Similarly, the depressed individuals are as well reported to hav4 diminished sexual interests (Farahani et al., 2015). Lastly, behavioral symptoms include calmness in previously active individuals which results in loss of interest and pleasure towards activities that previously were interesting.

Depression is a vital public health problem all over the world. This is as result of its high lifetime prevalence that ranges between 2 % to 15% and due to its high rate of causing disability. The reports of WHO indicated that depression by the year 2020 would be the second largest cause of diseases only one spot behind heart diseases (Bakhshaei Si, 2017). Based on its transmission processes, the higher percentage is through inheritance even though the specific genes are not known. The genetic cause of depression carries about 40-50 % while the remaining percentage is for the other factors.

2.2 Biological Mechanism of depression

For a very a long time science has focused on monoamine theory of depression. However, more recently there has been many evidence focusing on oxidation and inflammation associated with depression.

Inflammation is very vital process in the protection of the body against foreign substance including infections. However, the inflammation process that proceeds unchecked results into acute inflammation and later chronic inflammation which has negative effects on the body organs and tissues. The inflammatory mediators identified as cytokines have a beneficial role of which including maintaining redox balance in the body. However, during the chronic inflammation, these cytokines are produced in high levels as well as free radicals which result into damage to tissues. The excessive increase in the

cytokines has been reported to cause shock as well as death. On the other hand mild levels of cytokines has been shown to result into depression. The inflammatory cytokines are key in activation of the indoleamine 2, 3-dioxygenase an enzyme responsible for decomposition of the tryptophan to kynurenine hence inhibition of biosynthesis of tryptophan to serotonin (Monji, 2021). The activation of this pathway results into production of the neurotoxic substances that have the excitatory amino acid receptors stimulating actions that include the quinolinic acids. The increased levels of quinolinic acids have been linked to increased impulsivity and suicide- related behavior. The high levels of quinolinic acid are reported in cerebrospinal fluid of any individual with thoughts of suicide (Monji, 2021).

2.3 Epidemiology of depression

According to the world health organization survey, depression is the major cause of disability and has become the leading cause of disease (Faquih et al., 2019). The alteration in mood of any individual occurs daily in the entire life of a human being. With persistence in the changes in mood of an individual is an indication of depression like conditions. When compared across the gender, depression is more susceptible in women than in men. Depending on the age, depression occurs at any age and the mid-20s being the average age for the onset of depression. Depression is at its highest in women at the ages of 35-45 years (Pratt & Brody, 2014). The major depressive disorder is less common as compared to the minor depressive disorder. For instance in India, population estimation of 3-4% have complained of major depressive disorders while 7-10% suffer from minor depressive disorders.

2.4 Etiology of depression

As it is with other psychiatric disorders, main cause of depression is not known to date. However, various factors that contribute to its genesis are known. These factors include biochemical, endocrine, genetic, environmental and hormonal factors (Searle, 2011).

Environmental factors have greatly played major role in causing depression responsible for depression. These factors such as water, air, synthetic chemicals, food additives and food pollution, hormones, pesticides, drugs and industrial byproducts are bombarding our bodies at an extreme rate. Other sources include

stress electrical pollution, natural disasters, noise pollution and other catastrophic environmental events. Other events such as lose of the loved one, divorce, job loss, financial problems and disabling illness or injury sometimes called as social and relational causes of depression (Searle, 2011).

2.5 Treatment of depression

2.5.1 Conventional treatment of depression

Many antidepressants are available in the market. Majority of these antidepressants have been established through many controlled trials to be able to modulate monoaminergic neurotransmission primarily the serotonin and norepinephrine systems. These drugs elicit their action by inhibiting reuptake of norepinephrine and/or serotonin from synapse, inhibiting monoamine oxidase or acting on the receptors that modulate monoaminergic transmission. The drugs that acts as the monoaminergic modulators includes, the monoamine oxidase inhibitors (MAOIs), Tricyclic antidepressants (TCA), serotonin/norepinephrine reuptake inhibitors (SNRIs) and selective serotonin reuptake inhibitors (SSRIs) (Farahani et al., 2015).

The tricyclic antidepressants include drugs such as imipramine, trimipramine, doxepin, clomipramine, desipramine and amoxapine. These antidepressants inhibit the norepinephrine and/or serotonin reuptake. Similarly, these agents tend to block muscarinic cholinergic receptors and due to this they have been linked to anticholinergic side effects such as dry mouth, dry eyes, constipation, blurred vision, urinary hesitancy, mild cognitive disturbance and increased heart beat rate. Similarly, these agents have antihistamine effects hence they cause sedation and weight gain. These side effects in addition to cardiac toxicity observed upon overdose has seen there replacement as the first line agents for depression treatment (Adongo, 2015).

The monoamine oxidase inhibitors are responsible for the maintenance of all monoamines in the presynaptic terminal by preventing their degradation. These antidepressants are potent against depression, however, they can interact with other agents that have effect on the monoaminergic system producing accompanied and deleterious effect. The monoamine oxidase inhibitors include drugs such as isocarboxazid, phenelzine, selegiline and tranylcypromine.

The selective serotonin reuptake inhibitors are largely preferred as compared to the TCAs and MAOIs as the first line agent in treatment of depression. This has been drawn from their less deleterious side effects profile and reduced drug-drug interactions. These antidepressants strongly and selectively inhibit the reuptake of serotonin into the presynaptic terminal. Additionally, they have very little effects on norepinephrine and cholinergic or histaminergfic functions. However, the vigorous proserotonergic activity has aided various and relatively unique side effects such as gastrointestinal distress, insomnia, nervousness and agitation and sexual dysfunction. The selective serotonin reuptake inhibitors include drugs such as citalopram, escitalopram, fluoxetine, paroxetine and fluvoxamine.

Other antidepressants that are not in the same group as the tricyclic antidepressants, monoamine oxidase inhibitors and selective serotonin reuptake inhibitors are available and used for treating depression. These drugs include bupropion, mirtazapine, nefazodone, and trazodone .The mechanism of action of these agents are not well known and elaborate but are thought to cause modulation of monoaminergic systems.

2.5.2 Complementary management of depression and depression related disorders

The complementary management of depression involves use of medicinal plants and other natural products as the alternatives to the synthetic medicines. Medicinal plants are characterized by least side effects and low cost as compared to the synthetic medicines and this makes then suitable for managing chronic conditions such as depression (Farahani et al., 2015). The merits of medicinal plants remedies that are high efficacy, low adverse effects and low cost play a huge part in use of herbal remedies (Sultana et al., 2018). The rich source of wealth of the plant kingdom inform of phytochemicals can be novel source of newer compounds that show significant therapeutic properties such as antianxiety and antidepressant.

Many medicinal plants with potential antidepressant activity against mental disorders that include depression and anxiety have been identified. These plants through trials have been found to be effective against depression like conditions

and their use in the psychiatry has gone a step higher (Bakhshaei Si, 2017). For instance in the Iranian and other traditional medicines plants such as lemon balm (*Melissa officinalis*), lavender (*Lavandulaangustifolia*), Cinnamon (*Cinnamomum zeylanicum*), Banafsha (*Viola Odorata*), Echium (*Echium amoneum*), valerian (*Valeriana officinalis*),Aloysia (*Aloysia triphylla*), Citrus (*Citrus aurantium*) and Salix (*Salixa egyptica*) have been reported to have potential antidepressant effects (table 2.1) (Dobetsberger & Buchbauer, 2011).

The knowledge on combination of many medicinal products from natural sources that have similar therapeutic properties has increased. This has been done with an aim of getting a product with increased synergetic effect (Bakhshaei Si, 2017). In France for example a herbal preparation made up of three plants; *Passiflora incarnata, Valeriana officinalis* and *Crataegus oxyacantha* has been developed and is used as an antianxiety remedy (Bakhshaei Si, 2017).

Table 2. 1 List of medicinal plants used as antidepressants

Plant	**Plant Part used**	**Bioactive Compounds**	**Screened Activity**
Aloysia triphylla	Roots	Flavonoids (Artemitin and Hesperidin)	Antidepressant, anti-inflammatory
Citrus aurantium	Flowers	Phenolic compounds like flavanone glycosides, hydroxycinnamic acids	Antidepressant, anticonvulsant, antianxiety, antioxidant
Echium amoneum	Leaves and flowers	Phenolic compounds like rosmarinic acid, cyanidin, and delphinidin	Antidepressant, ant-hyperlipidemia, anti-cholesterol, antibacterial, anti-diabetic and antioxidant
Lavandula angustifolia	Flowers	Phenolic compounds like hydroxycinnamic acids and flavone glycosides	Antidepressant, anticonvulsive, anxiolytic, antioxidant
Melissa officinalis	Leaves and stems	Citronellal, citral (citronellol, linalool), geranial, threeterpinene, phenol carbon-acid (rosmarinic	Antidepressant, antimicrobial, antispasmodic, antioxidant

		acid), and flavonglychoside acids	
Salix aegyptiaca	Leaves and stem bark	Phenolic compounds like gallic acid, caffeic acid, vanillin and p-coumaric acid, myricetin, catechin, epigallocatechin gallate, rutin, quercetin and Salicin	Antidepressant, antioxidant, antivertigo, anti-anemia
Viola odorata	Leaves and flowers	Alkaloid, glycoside, saponins, methyl slicylate, mucilage and vitamin C, Cycloviolacin O2 (CyO 2)	Antidepressant, antihyperlipidemia, anti-cholesterol, anti-blood pressure, anti-cancer and anti-tumor
Valeriana officinalis	Leaves	Alkaloid, glycoside, saponins, methyl salicylate and mucilage	Antidepressant, antioxidant, antiinflammatory, laxative, anti-septic, anti-hyperlipidemia
Cinnamomum zeylanicum	Stem bark and leaves	Eugenol, Cinnamaldehyde, camphor, procyanidins and catechins	Antidepressant, antimicrobial, antioxidant, anti-diabetic and anti-inflammatory

2.6 Depression evaluation models

Many models that utilize animals for evaluating the antidepressant potential of novel compounds such as natural products are available (Ayuob, 2017). Presently, animals models widely used for evaluating depression studies are those that incorporate stress exposure and depressive-like conditions. Models that employ acute or sub-chronic stress exposure such as learned helpless, forced swim test and tail suspension test are widely used. These models generally involves exposing the study animals to short term inescapable or uncontrollable stress (Ayuob, 2017).

In the forced swim test the animal is allowed to swim in a small controlled container for a period of six minutes in order to develop stress. The stressed

animals remain immobile and only show the movements that are only suitable for survival. The novel compounds with potential as antidepressants overturns this and allows the animal to increase the mobility period (Gorman-Sandler & Hollis, 2021).

The tail suspension test involves suspending the animal for a height of 50 cm by pinning it to the edge of the bench using masking tape for 6 minutes. The animal develops depressive like conditions to the difference in height and remains immobile. The mobility period is monitored in the last four minutes (Faquih et al., 2019).

Models that involves long term exposure to stress chronic mild stress model, repeated open-space model, early-life stress models and social conflict models. These models have the ability to accurately stimulate processes that results to depression. The chronic depression evaluation model are considered to be more practical in inducing depressive like conditions and are more better related to human situation. Models are usually evaluated to correctly predict outcome in humans (predictive validity), their ability to reproduce in animals aspects of the illness in humans (face validity) and the extent to which the model the true disease process or its etiology in humans (construct or etiologic validity).

2.7 Phyt^{exponent}

Phyt^{exponent} is a natural product that is classified under the class of poly- herbal preparations. It's prepared from herbals that are naturally available and whose medicinal value has been evaluated from different previous studies. This poly-herbal preparation originated from Belgium and comprises of five different medicinal plants/herbs (Moriasi et al., 2021). The phyt^{exponent} is marketed all over the world including Kenya where it's supplied by Pharmapath Belgium under the licence of fizzer pharmaceutical company. The plants and herbs that constitute the phytexponent preparation are *Echinacea purpura, Allium sativum, Triticum repens, Matricaria chamomilla* and *Viola tricolor* (Moriasi et al., 2021).

2.7.1 Preparation of the phyt^{exponent}

The phytexponent is an ethanolic extract of five natural plants/herbs of medicinal importance. The respective plants are parked in individual extraction

vessels and ethanol solution added in all the vessels. The materials of the respective plants are macerated in the solvent for a period of 48 hours. The material of the individual plants are then filtered and the filtrate mixed in the respective composition to make the final product. The mixing of the plant extracts is as follows *Viola Tricolor* - 3.77% *Echinacea purpurea*- 26.42% Allium sativum- 11.32% *Triticum repens*- 26.42% *Matricaria chamomilla*- 32.08%. The final product is composed of 62.1 % ethanol and the rest of the percentage being the five different plant extract to make 100 % product.

2.7.2 Components of phyt[exponent]

Viola tricolor

Viola tricolor is a small plant that is taxonomically classified under family *Violaceae*. This plant is of the creeping and ramping habit and it grows to about 150 mm tall. It's characterized with flowers that are 15mm in diameter. Viola tricolor grows in short grasslands on farms and wasteland. Viola tricolor is identified by common names such as Heartsease, Johnny Jump up, Call-me-to-you, or Bird's Eye (Hellinger et al., 2015). This plant contains cyclotides compounds which have been proven have cytotoxic properties hence have the ability to treat cancer (Moriasi et al., 2021). Similarly, the viola tricolor extracts have antimicrobial, antioxidant and anti-inflammatory properties (Koike et al., 2015). The plant extract has shown to be a potential anti-inflammatory against acute inflammation induced in male Wister rats. The antioxidant property that is found in the flowers can be attributed to the flavonoids such as quercetin, luteolin and rutin. Other compounds such as colorless crystalline compounds that have been found to potential remedy for management of cardiovascular disorders, diabetes, inflammation, immune disorders and liver disorders among many other complications. (Hellinger et al., 2015).

Echinacea purpurea

Echinacea purpurea is an herbaceous perennial herb that grows to about 47 inches and about 25 cm wide at maturity. This herb is characterized by cone-shaped flower heads that are usually purple in color while in the wild. The individual flowers in the flower head are hermaphroditic; having both male and female organs in one flower. This herb is native to North America. The

Echinacea purpurea contains various bioactive compounds such as alkamides, caffeic acid derivatives, polysaccharides and glycoproteins (Manayi et al., 2015). These compounds are thought to have stimulating effects on the immune system. The flavonoid compounds are as well present in the herb with nicotiflorin dominating followed by rutin.

Traditionally, *Echinacea purpurea* has been used by Native Americans to treat various conditions such as wounds, insect bites, reducing toothache pain, throat infections, pain, cough, stomach cramps and snake bites. Through various researches, it has been proven that *Echinacea purpurea* is an immune modulator that has seen an increased use of this plant to treat immunodeficiency disorders(Manayi et al., 2015), lower, glycemia and act as an antianxiety, antidepressant and anti-inflammatory agent.

As an anti-inflammatory agent *Echinacea purpurea* is employed in pharmaceutics due to its ability in mobilizing the leukocytes, activating phagocytosis and well stimulate fibroblast formation (Manayi et al., 2015).

Allium sativum

Allium sativam is plant species of the bulbous flowering plants in the larger onion genus allium family alliaceae (Moutia et al., 2018). Its perenial in nature and grows from the bulb with erected flowering srem that is about 3 feerts tall. The leaf blades are usually flat, linear, solid and approximately 1.25-2.5 cm wide with an ac ute apex. Commonly identified as garlic is widely used as a spice in most of the countries and the most sold herbal product globally (Majewski, 2014). Garlic has a close relation to the onion, shallot, leek, chive, welsh onion and the chines onion. This species native to central Asia and northeastern iran. Alluim sativum contain the main compounds containing sufer that are responsible for the flavor and these compounds are responsible for the many known medicinal properties such as antioxidant, anticancer, antidiabetic, antimicrobial and immunomodulatiry activity (Martins et al., 2016).

The aqueous garlic extracts have been reported to have antioxidant activity by inhibiting reactive oxygen species (ROS) and enhancing enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (Arreola et al., 2015).

Matricaria chamomilla

is amedicinal plant found in the family *Asteraceae* . the componds isolated from this plant have been used in about 26 drugs. It has as well been used as antiinflammtory and antispasmodic drug. It has as well been used in traeting pain that results from the disturbace from the stomach. Its has as well been used extensivel as a tea or tonic and treating hysteria, anxiety, insomnia, and nightmares(Satyal et al., 2015).

Tricum vulgare

Tricum vulgare is commonly identified as cough grass and is an invasive weed whose roots and leaves are used as medicine. Its is used in managing constipation,swollen bladder, cough, fever, hypertension, kidney stones and inflammation (Sanguigno et al., 2018). It has as well been incoparated in some pharmaceuticl formulations for treatment of burns, skin lesions and decubitus ulcers. The antiinflammatory properties of this weed has been an area of corncern.

2.7.3 Photochemistry of phytexponent

Phytochemicals are secondary metabolites found in natural products are responsible for the various observed therapeutic properties of herbal medicine. Phytexponent is characterized by presence of many phytochemicals that include; flavonoids, alkaloids, phenols, glycosides, steroids, terpenoids, tannins, saponins and anthraquinones.

2.7.4 Bioactivities of phytexpont

2.7.4.1Antioxidant activity

The study conducted by revealed the antioxidant activity of the phytexponent preparation. In this study the dpph free radical scavenging activity, hydroxyl radical scavenging activity and catalase enzyme activity were evaluated. The phytexponent was able to scavenge both dpph free radical and the hydroxyl radical that was depicted by the lower IC 50 recorded in both tests; 0.00733 and 0.716 for dpph and hydroxyl respectively. Similarly, the phytexponent increased the activity of catalase enzyme (Moriasi et al., 2021).

2.7.4.2 Anti-inflammatory activity

The anti-inflammatory activity of the phytexponemt was studied in the in vitro assays. The phytexponent was able to prevent the denaturation of the protein a similar property showed by etenercept. However this activity was not significantly different at all concentrations100%, 50, 25 and 12.5 % and etenercept (25 mg/ml). Similarly, the phytexponent showed anti-inflammatory activity through the membrane stabilization assay. Its ability to stabilize the red blood cell membrane induced to hypotonic solution and heat stress was in a dose depended manner. In both the heat induced and hypo-tonicity induced, the phytexponent stabilized more the red blood membrane more at 100 5 and 50 % concentration as compared to the standard anti-inflammatory drug etenercept (25 mg/ml) (Moriasi et al., 2021).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Source of the phytexponent

The phytoexponent preparation used in this study was purchased from Maendeleo pharmacy in Nairobi. This product was then be transported to Mount Kenya University and kept according to the manufacturer's instruction until the experimental day.

3.2 Experimental animals

The Swiss albino mice of both gender and aged four to five weeks were used in this study. They were bought from the animal breeding laboratories in Jomo Kenyatta University of agriculture and technology kiambu County. The study animals were then transported to Mount Kenya university research laboratories in well covered cages with rice harks as beddings. In the MKU- research laboratories, they were allowed four days of acclimatization prior to commencing the study. In the laboratory the animals were allowed to have 12-hour day and night cycle with free access to clean water and standard rodent pellets. The wood shavings were changed on the daily basis to avoid dampness that would affect the physiological function of the animals. All the ethical consideration regarding the animal handling were properly followed to the latter.

3.3.1 Animal sorting and groping

This study consisted of five groups with each grouping consisting of five mice (n=5). The groups were allotted as follows (Table 3.1), negative control group (group I) administered normal saline, positive control group (group II) administered standard antidepressant drug (Fluoxetine at 20 mg/kg bw) while three experimental groups (groups III, IV and V and VI) that were administered the three selected dose 100 %, 50 % and 25% of Phyt^{exponent}.

Table 3. 1 Experimental treatment groups

Treatment groups	Dose administered
Negative control I	Normal saline
Positive control II	Normal saline + Fluoxetine(20 mg/kg bw)

Experimental group III	Phyt^{exponent} 100%
Experimental group IV	Phyt^{exponent} 50%
Experimental group V	Phyt^{exponent} 25%

3.4 Antidepressant activity of the phytexponent poly herbal preparation

The ability of the phytexponent to act as an antidepressant agent was evaluated via two methods: forced swim test (FST) and tail suspension test (TST). The phyt^{exponent} polyherbal preparation was diluted in normal saline to make the three dose levels whose calculations followed the OCED guidelines (Oghenesuvwe et al., 2014).

3.4.1 Forced swim test

The antidepressant activity via the forced swim test as described by Kaur et al.(2015) was adopted with minor modifications. The forced swim test apparatus consisted of an open cylindrical container with measurements of 20 cm diameter and 25 cm height. This container was filled with clean tap water to the 15 cm mark and the water temperature maintained at 25±1° C. The study mice were trained to swim in the pretest for period of 6 min per day for two days prior to the test session. On the test day the respective mice in the groups were administered with the respective drug at calculated dosage 60 minutes to the test time. All the mice were subjected to forced swim and the duration of in which the mice did not moving any more (immobility) was noted and recorded over the 4 minutes of the 6 min swim time. The immobility of the mice was judged as the ability of the mice to stop moving with only motion being noted was that which helped the mice to keep the head above the water. The decrease in the duration of immobility was taken as an indicator of antidepressant like effects.

3.4.2 Tail suspension test

The antidepressant activity of the phytexponent via the tail suspension test was conducted by following the protocols described by Kaur et al. (2015) with minor modifications. This involved suspending all the mice at time interval 60 mins after the treatment with the respective drugs on the edge of a table with a height of 60 cm above the floor for 6 min. Mice were suspended with the help of masking tape which was placed approximately 1 cm from tip of the tail. The total time of immobility as a result to tail suspension was recorded within the

last 6 minutes of 10 minutes study period. The mice were considered to be immobile if there was no body movements noted while still under suspension. The decrease in time of immobility was regarded as the indicator of antidepressant effects.

3.5 Data management and statistical analysis

All the set of data that consisted of immobility time in minutes were tabulated in an excel spread sheet and then exported to graph pad prism data analysis software for descriptive statistics. The data was then presented as Mean ± SEM and further analysis by one way a nova followed by post hoc tukeys test which was be conducted to determine the level of significance between the means. The mean immobility time in both forced swim-test and tail suspension test were compared to each other at confidence interval of 90% $p<0.01$. The results were then presented in form of tables and graphs.

CHAPTER FOUR: RESULTS AND DISCUSSION

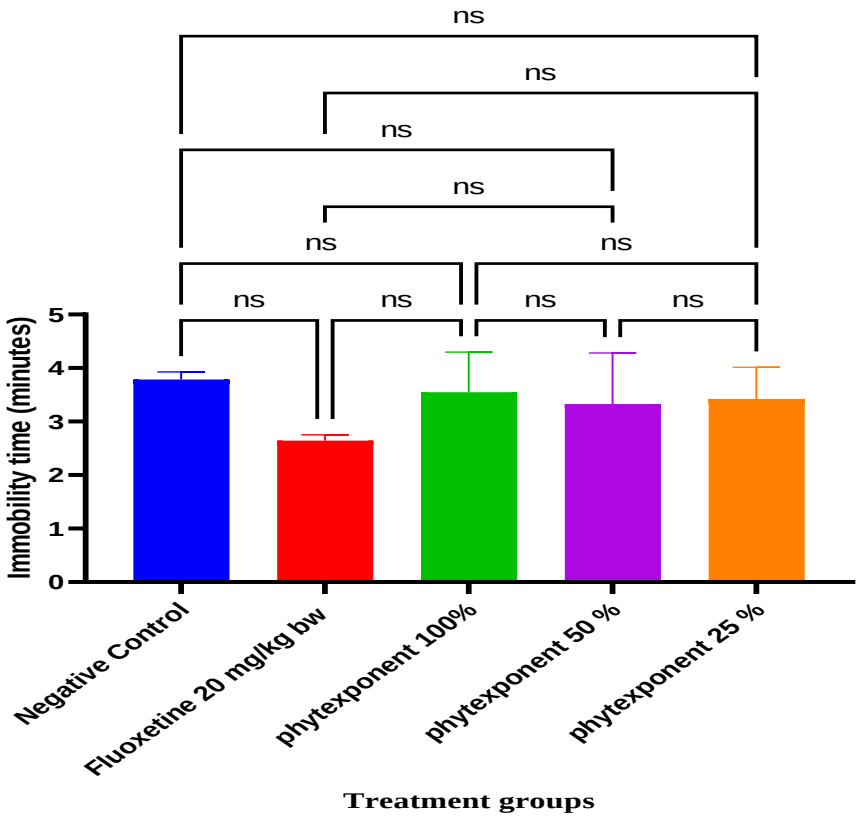
4.1 Effects of phytexponent on the immobility time in the tail suspension test.

The results for antidepressant activity of phytexponent conducted via the tail suspension test are presented in table 4.1 and figure 4.1. The oral administration of the fluoxetine at dose level of 20 mg/kg body weight reduced the immobility time of the mouse while suspended on the bench. The reduction in time was more as compared to phytexponent and the negative control groups. The administration of normal saline as the negative control did not reduce the ability of the mouse suspended on the bench to remain immobile. However, the administration of the phytexpionent at the three different doses showed a reduction in the immobility time in all the three groups. The 50% phytoexponent reduced more the immobility time as compared to 100 % and 25 %. While the 25 % phytexponent reduced the immobility time more as compared to the 100% phytexponet (table 4.1 and figure 4.1).

Table 4. 1 Effect of phytexponent on the immobility time in tail suspension test

Treatment group	Immobility time (Mean±SEM)
Negative control	3.783±0.0833
Fluoxetine 20 mg/kg bw	2.643±0.0633
phytexponent 100%	3.547±0.4334
phytexponent 50%	3.327±0.5519
phytexponent 25%	3.423±0.3421

Figure 4. 1 Tail suspension test



4.2 Effect of the phytoxponent on the immobility time in forced swim test

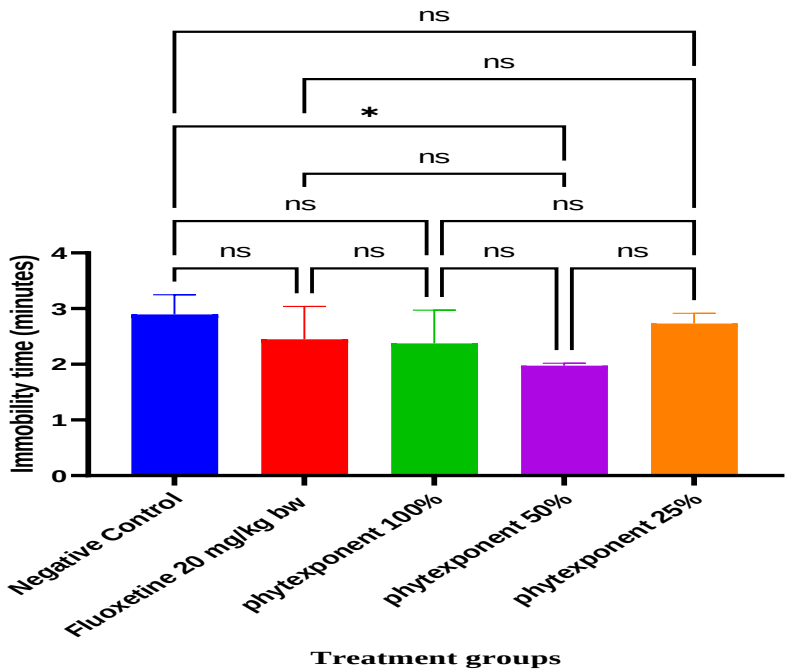
The immobility time recorded by different groups in the tail suspension test is summarized in table 4.2 and figure 4.2. The results showed that suspending mouse at a particular height induces depression like conditions increasing the immobility time as showed in negative control group. Upon administration of the standard antidepressant drug fluoxetine at dose level of 20 mg/kg bw

reduced the immobility time as compared to the negative control (table 4.2 and fig 4.2). Similarly, oral administration of the phytexponent at the three doses reduced the immobility time recorded in the experimental groups as compared to the negative control. The phytexpobnent at 50 % dose reduced more the immobility time as compared to phytexponent at 100 and 25 % and fluoxetine at 20 mg/kg bw. The immobility time recorded by the phytexponent at dose level of 50% was significantly lower as compared to the immobility time recorded by the negative control group ($p<0.05$;fig 4.2). However, in all the other groups no significant difference was noted in the respective immobility time recorded ($p>0.05$; fig 4.2).

Table 4. 2Effect of phytexponent on immobility time in forced swim test

Treatment group	Immobility time (Mean±SEM)
Negative control	2.895±0.1760
Fluoxetine 20 mg/kg bw	2.448±0.0.2951
phytexponent 100%	2.375±0.2.980
phytexponent 50%	1.975±0.02179
phytexponent 25%	2.733±0.09196

Figure 4. 2 Forced swim test



4.2 Discussion

The present study investigated the antidepressant potential of the phytexponent in animal models. Two widely used and most common models were adopted; forced swim test and tail suspension test (Cryan et al., 2002). The forced swimming and tail suspension tests are behavioral despair models that are used to show the mechanism of depression and as well as test for evaluating the potential antidepressant agents (Hsu et al., 2012). Both models are sensitive in evaluating different types of antidepressant with different action mechanisms such as serotonin reuptake inhibitors and monoamine oxidase inhibitors. The phytexponent was administered in three dose levels of 100%, 50% and 25% while fluoxetine at dose level of 10 mg/kg bw was used as the standard antidepressant.

The forced swim test model is a two-day model that basically involves placing the study animal mainly a rodent in an inescapable pool of water with 20 cm diameter and 25 cm height. The rodent is trained to swim for a period of 15 minutes a day prior to the experiment and six minutes during the test day. The floating and exhibition of the immobility behavior shown by the animal is interpreted as behavioral despair, learned helplessness, passive coping and anxiety. The ability of the compound to reduce the immobility behavior is regarded as a potential antidepressant. In this study, the oral administration of the fluoxetine at 20 mg/kg bw significantly reduced the behavioral despair by reducing the time taken by the mice to remain immobile on water. Similarly, in the tail suspension test administration of fluoxetine at 20 mg/kg bw reduced the immobility time. The phyt^{exponent} at 50 % dose in the tail suspension test and forced swim test recorded the best antidepressant activity as compared to 100 % and 25 % respectively. In the oral administration of phyt^{exponent} elicited more similar antidepressant activity asw compared to the standard antidepressant drug; fluoxetine at 20 mg/kg bw. The mechanism of action of phyt^{expont} as antidepressant can therefore be through inhibition the reuptake of serotonin the mechanism shown by fluoxetine.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study illustrated that phyt^{exponent} has antidepressant activity that is comparable to fluoxetine the standard antidepressant drug. This antidepressant activity may be attributed to the varied phytochemicals that are present in the phyt^{exponent}. The antidepressant activity of the phyt^{exponent} may be through the inhibition the reuptake of serotonin mode of action.

5.2 Recommendation

From this study the following recommendations were made:

I. Antidepressant activity at lower concentration of 12.5 % be done

II. Toxicity of phyrexponent to be done

III. Antianxiety potential of phytexponent to be evaluated

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