ANTIDEPRESSANT AND ANXIOLYTIC PROPERTIES OF NATURE GIFT® SUPPLEMENT

BY

Divine Osarugue OSAGIE (Miss)

LSC2007346

PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY FACULTY OF LIFE SCIENCES UNIVERSITY OF BENIN, BENIN CITY

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCE, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA. IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE BACHELOR DEGREE (BSc.) IN SCIENCE LABORATORY TECHNOLOGY DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY (PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES) FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY

NOVEMBER, 2025

CERTIFICATION

This is to certify that this project work, titled "**ANTIDEPRESSANT AND ANXIOLYTIC PROPERTIES OF NATURE GIFT® SUPPLEMENT"** was carried out by Divine Osarugue OSAGIE (Miss) with Matriculation Number LSC2007346 of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City, Edo state under the supervision of Dr. D.O. Uwaya.

_____ _____

Dr. D. O. Uwaya **Date**
(Project Supervisor)

_____ _____

Dr. P. O. Alonge **Date**
(Project Coordinator)

_____ _____

Prof. J. O. Osarumwense **Date**
(Head of Department)

_____ _____

External Supervisor **Date**

DEDICATION

This work is dedicated to God Almighty for His guidance, grace and mercies towards the successful completion of this work

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ABSTRACT

Medicinal plants have been essential to medicine for millennia, both modern and traditional. Nature's Gift® (*Allium sativum*, turmeric, ginseng, and bilberry) was tested for antidepressant and anxiolytic properties. The forced swim test, tail suspension test, and elevated plus maze were used to study antidepressants and anxiolytics. Twenty-five 20–30 gram mice were split into five groups of five. Group 1 received 10 ml/kg distilled water, Groups 2, 3, and 4 received 50, 100, and 200 mg/kg Nature Gift® extract, and Group 5 received 20 mg/kg oral fluoxetine. The mice were placed in an inescapable transparent cylinder filled with water at 25°C for one hour after receiving Nature's Gift® and fluoxetine. Animal immobility was recorded after 5 minutes of swimming. Twenty 20–30 gram mice were randomly assigned to five four-animal groups. Group 1 received distilled water (10 ml/kg), groups 2–4 Nature Gift extract (50, 100, and 200 mg/kg), and group 5 diazepam (10 mg/kg). An hour after receiving Nature's Gift® extracts and diazepam, the animals explored the central maze for five minutes. The number of entries and open arms time were recorded. Compared to the control, Nature's Gift® extract at 200 mg/kg and fluoxetine (20 mg/kg) reduced immobility time in the forced swimming test ($p<0.05$). Compared to the control, Nature's Gift® extract (50, 100, and 200 mg/kg) and fluoxetine (20 mg/kg) reduced tail suspension immobility time ($p<0.05$). Both Nature's Gift® extract (100 and 200 mg/kg) and diazepam (10 mg/kg) increased time spent in the open arm compared to the control ($p<0.05$). Nature's Gift® Supplement is an antidepressant and anxiolytic.

CHAPTER ONE

INTRODUCTION

1.1. BACKGROUND OF THE STUDY

For millennia, medicinal plants have been a mainstay of medicine; they remain absolutely vital in both modern and traditional medicine. Ekor (2014) asserts that the use of these substances stems from their bioactive components, which provide medicinal benefits and serve as the basis for numerous essential medications. According to WHO estimates from 2013, nearly 80% of the world's population relies on herbal remedies for primary health care needs. These plants are valued not only for their medicinal properties but also for their cultural and economic importance. Apart from their medicinal benefits, these plants are prized for their cultural and financial significance.

Phytochemicals found in abundance in medicinal plants include alkaloids, flavonoids, tannins, terpenoids, and glycosides; they have pharmacological actions including anti-inflammatory, antimicrobial, antidiabetic, and anticancer as well as mustard (Fabricant and Farnsworth, 2001). Plants have provided several contemporary medications. For instance, morphine comes from Papaversomniferum (opium poppy), quinine from Cinchona bark, and artemisinin, an efficient malaria therapy, from Artemisia annua (Rates, 2001; Tu, 2011). These instances show that understanding of conventional medicine has offered an excellent basis for contemporary drug development and still inspires pharmaceutical research.

About a quarter of prescribed medicines today, roughly 25%, come from plants (Newman and Cragg, 2016). To create safe, efficient, and economical medications, contemporary scientists increasingly look at therapeutic plants. Additionally highly culturally important, herbal medicines are used by traditional healers in many societies in physical and spiritual healing

processes (Balick and Cox, 2021). Driven by consumer choice for natural products and complementary treatments, the herbal medicine sector has been expanding swiftly both domestically and internationally (Rafieian-Kopaei, 2012).

Despite these advantages, medicinal plants encounter several difficulties. Overharvesting, habitat destruction, and the absence of stringent laws for quality control in herbal products can all influence and restrict their availability (Sharma *et al.*, 2019). Furthermore, underlining the necessity of standardisation, scientific validation of plant systems Ekor (2014) emphasises the necessity of standardisation, scientific validation, and monitoring systems to ensure the effectiveness and safety of medicinal plant systems.

In essence, for human health, cultural customs, and economic growth, medicinal plants continue to be a priceless resource. They provide both preventative and therapeutic advantages, and they help connect modern and traditional healthcare systems. However, for their potential to be fully realised, there must be a balance between utilisation and conservation, as well as scientific efforts to validate their effectiveness. With good control and more investigation, medicinal plants will continue to be a major source of contribution to world health.

1.2. AIM OF THE STUDY

The aim was to evaluate the antidepressant and anxiolytic properties of the Nature's Gift® supplement (*Allium sativum*, turmeric, ginseng, and bilberry).

1.3. OBJECTIVES OF THE STUDY

· To evaluate the antidepressant properties of Nature Gift® supplement (*Allium sativum*, turmeric, ginseng, bilberry) using the forced swimming and tail suspension tests

· To help determine the anxiolytic property of Nature Gift® supplement using the elevated plus maze test.

CHAPTER TWO

LITERATURE REVIEW

2.1. NATURE GIFT SUPPLEMENT

2.1.1. Description of Nature Gift® Supplement

Nature Gift® Supplement is a polyherbal mix that has become somewhat well-known in certain regions of West Africa, especially Nigeria, where it is sold as a natural health supplement for enhancing general well-being, neurological, and heart. Based on distributor product information, the supplement usually combines recognized therapeutic herbs, especially garlic (*Allium sativum*), turmeric (*Curcuma longa*), ginseng (*Panax spp.*), blueberry (*Vaccinium myrtillus*). Long ethnomedicinal histories abound for each of these plants, along with a rising body of scientific data pointing roles in controlling oxidative stress, inflammatory reactions, and Factors linked to sadness and anxiety disorders include neuronal signaling pathways (Kennedy *et al.*, 2001; Hewlingsand; Kalman, 2017; Ghosh, 2024). Usually in capsule or powdered form, the formulation fits with the nutraceutical industry's tendency of transforming conventional botanicals into standardized, readily available items.

Nature Gift® is a commercial polyherbal solution, where several plants are mixed with the expectation of synergistic effects - unlike single-plant treatments typical in traditional medicine. Advocates claim that, according to Williamson 2001, combining phytochemicals like allicin (from garlic), curcumin (from turmeric), ginsenosides (from ginseng), and anthocyanins (from bilberry) may provide more widespread therapeutic coverage than single-compound formulas. Particularly in the absence of strict clinical studies (Posadzki *et al.,* 2013), such combinations also seeks questions about dose standardizing, pharmacokinetic interactions, and safety.

Manufacturers of Nature Gift® Supplement (MyHealthMySkin, 2022) usually promote it for conditions including stroke, epilepsy, high and low blood pressure, memory loss, anxiety, and depression. Though they correspond with local health-seeking patterns and the cultural dependence on plant-based medicine, such assertions are mostly driven by marketing rather than supported by evidence. As of 2025, no peer-reviewed random controlled trial (RCT) has been released particularly assessing the effectiveness of Nature Gift Supplement. This gap highlights the importance of methodical preclinical and clinical studies on its pharmacological characteristics and safety profile. Nature Gift Supplement, therefore, inhabits a liminal space between contemporary commercial nutraceuticals and traditional ethnomedicine: it derives legitimacy from historic plant use while as a regular product for modern consumers. This dual identity makes it more attractive but also begs issues of scientific validation and governmental control.

2.1.2. Distribution of Nature Gift® Supplement

The Nature Gift® Supplement's distribution depicts the expanding herbal medicine industry in Africa. Herbal Resources Limited (Benin City, Nigeria) mostly produces it; sales are made via Nature’s Renaissance International Ltd. (NRI), with item listings discovered on local Distribution takes several channels:

- **Community-based sales:** Local distributors and herbal practitioners are important in the value chain by marketing of the supplement to people (especially consumers) who are less privilege to have access to the conventional health care.
- **Pharmacies and Herbal Shops:**The product is typically found alongside both prescription medications and over-the-counter herbal remedies, thereby erasing the boundary between nutraceuticals and medicines.

- **Online platforms:** E-commerce has spread access beyond Nigeria into nearby nations such Ghana and Cameroon, and diaspora communities in Europe and North America. purchasing the supplement online (Konga, 2025)

Regional branded formulation Nature Gift® is made and sold in West Africa using sourced raw botanical materials from imports or local sources. Its distribution thus reflects market-driven routes of nutraceutical products rather than natural geographic separation.

Still an issue is regulatory supervision. Under a category separate from prescription, NAFDAC regulates herbal supplements in Nigeria, which requires less exacting proof of effectiveness (Ekor, 2014). Although this helps companies to enter the market, it also lets products like Nature Gift® spread broadly without concrete effectiveness and safety data. By contrast, in Europe and North America herbal products are often marketed under dietary supplement categories governed by government like the U. S. Food and which need precise labeling but not proof of therapeutic benefit prior to marketing (U. S. FDA, 2023).

Nature Gift® Supplement summarizes the hybrid commercial model of several African herbal formulations: locally branded, somewhat distributed, and culturally reliable but not as sold.

2.2. *Allium sativum* (Garlic)

2.2.1. Description of *Allium sativum* (Garlic)

Commonly referred to as garlic, *Allium sativum* is a member of the Amaryllidaceae family, genus Allium, which also comprises onions, leeks, and shallots. Usually 30–60 cm tall, garlic is a perennial bulbous plant with a compound bulb made up of 10–20 cloves encapsulated in papery sheaths (Brewster, 2008). Although leaves and scapes are eaten in certain societies, the primary medical and culinary component of the plant is the bulb. A variety of organosulfur

chemicals (OSCs) found in garlic are thought to be responsible for its therapeutic promise. The most remarkable is allicin (diallylthiosulfinate), which results from the enzymatic from crushed garlic cloves' conversion of the precursor alliin via the enzyme alliinase (AnkriandMirelman, 1999). Allicin is unstable and quickly breaks down into molecules with various pharmacological activities including ajoene, diallyl disulfide (DADS), diallyltrisulfide (DATS), and S-allylcysteine (SAC).

Moreover synergistically contributing to garlic's biological activities are flavonoids (quercetin), saponins, vitamins (C and B6), and trace elements (selenium) Garlic has been used medicinally for over 3, 000 years. It was prescribed in ancient Egyptian papyri (Ebers Papyrus, c. 1550 BCE) for circulatory and parasitic disorders (Rivlin, In Greek and Roman medicine garlic was given for infections and to increase strength; in Traditional Chinese Medicine it was used for respiratory and digestive problems. Such historical applications set the groundwork for its ongoing popularity as both a medical treatment and culinary spice.

2.2.2. Distribution *Allium sativum* (Garlic)

Though it has spread widely, *Allium sativum*, often known as garlic is thought to have originated in Central Asia, particularly areas around Iran and Turkmenistan. (Block, 2010) has been developed over millennia throughout many different societies. From its centre of origin, garlic migrated to ancient Egypt, India, and China, where it grew as both a regular meal and therapeutic plant (Rivlin, 2001).

Garlic is grown and spread worldwide nowadays. China, India, South Korea, Egypt, Russia, and the United States are among the top manufacturers; according to FAO 2020, China alone accounts for almost 70–80% of world output. According to Brewster (2008), the crop grows best in areas with chilly winters and hot summers, a range of temperate and subtropical environments.

Among the most widely distributed vegetable plants, garlic is cultivated on almost every continent. Its flexibility in temperature and soils has helped it to be used in several farming practices and culinary customs. Besides extensive commercial farming in Asia and Europe, smallholder farmers in Africa and South America grow garlic for local consumption and trade (Gebreyohannes and Gebreyohannes, 2013).

Allum sativum has gone from its Central Asian roots to become a worldwide crop, with China as the top producer. Its great flexibility guarantees its presence in developed and developing nations alike, where it is still a crucial food and medical plant.

2.2.3. Ethno-Medicinal Uses of *Allium sativum* (Garlic)

Garlic has been extensively used in traditional medicine among many cultures for: Infections (antibacterial, antifungal, antiparasitic action), cardiac diseases General tonic for strength and vitality (GebreyohannesandGebreyohannes, 2013). Many of these applications have been confirmed by studies. Examples are

- Cardiovascular health: Meta-analyses indicate garlic supplementation may modestly lower systolic blood pressure by around 58 mmHg and total cholesterol by 1015 mg/dL(Ried *et al*., 2013; Reinhart *et al*., 2009)
- Broad-spectrum antimicrobial activity of OSCs like allicin and ajoene against Staphylococcus aureus, Candida albicans, and protozoa like Giardialamblia (AnkriandMirelman, 1999)
- Although human data are unclear (Iciek *et al*., 2009), garlic-derived chemicals scavenge free radicals and modulate phase II detoxification enzymes, therefore suggesting antioxidant and anticancer ability.

Therefore, garlic is a well-characterized therapeutic herb with robust antimicrobial and cardiovascular evidence; yet, its neuropsychiatric potential is underexplored and under-validated clinically, hence representing anPolyherbal treatments like Nature Gift seek to close an important gap.

2.3. *Curcuma longa* (Tumeric)

2.3.1. Description of *Curcuma longa* (Tumeric)

Curcuma longa usually growing between 0.5 and 1.0 m, the plant boasts short stems and big oblong leaves. The subterranean organ of economic and medicinal value is harvested, boiled, dried and milled rhizome yields the well-known yellow-orange powder employed as medicine and spice (FAO, 2018). Thanks to volatile essential oils (turmerones, ar-turmerone) and curcuminoid pigments in the rhizome, it has a distinctive earthy scent and brilliant orange-yellow cortex. Turmeric's pharmacology comes from two major chemical families:

(1) curcuminoids, polyphenolicdiarylheptanoids, and

(2) Volatile oils. Curcumin (diferuloyl methane), demethoxycurcumin (DMC), and bis-demethoxycurcumin (BDMC); curcumin is the most researched and is often used as the marker compound in analytical standardizing (Lin *et al*., 2022).

With conjugated double bonds and phenolic groups, curcumin is a lipophilic chemical with antioxidant capability and the capacity to modulate several intracellular signalling pathways. Some experimental systems show anti-inflammatory and neuroactive benefits from -turmerone, -turmerone, ar-turmerone, zingiberene, and others in the volatile fraction (Fu *et al*. , 2021). For extract standardization, curcuminoids and volatile oil components are routinely measured using analytical techniques (HPLC, LC-MS).

As an anti-inflammatory, digestive, wound-healing, and blood-circulation agent.Ayurveda, Unani, and Traditional Chinese Medicine have been paralleled in South Asia for ritual and culinary uses, from jaundice to joint ailments, medicinal uses for diseases ranging from these long-standing ethnomedical assertions stirred current pharmacological attention in curcumin and turmeric extracts.

2.3.2. Distribution of *Curcuma longa* (Tumeric)

Indigenous to South and Southeast Asia, particularly the Indian subcontinent, turmeric (*Curcuma longa*) has been grown for more than 4, 000 years India, which supplies almost 75–80% of the world's supply and meets most of international demand (Ravindran *et al*. 2007). According to Prasad and Aggarwal, 2011, the plant thrives in tropics and subtropics, thrives in warm temperatures (20-30 C), high humidity, and well-drained loamy soils. Although India is the main producer, other Asian countries including Bangladesh, Pakistan, Sri Lanka, China, Myanmar, Thailand, and Indonesia (Sasikumar, 2005) also have notable growing. Its growth has spread from Asia to include areas of Africa (including Nigeria, Madagascar, Tanzania, Ethiopia), as well as Central and South America, including Peru, Costa Rica, and the Caribbean Islands (Purseglove *et al*. , 1981).

Driven by its great economic, gastronomic, and therapeutic importance, turmeric is grown in several levels all around to. Though Asia still accounts for most of commercial output, growing demand has driven farming in unconventional areas of Africa and Latin America—usually for local markets and Applications of herbal medicine (Gupta *et al*. , 2013)

To sum up, turmeric's range of use has grown from its Asian beginnings to a global presence, with India as the main hub. Its flexibility makes it a often grown and sold out therapeutic spice.

2.3.3. Ethno-Medicinal Uses of *Curcuma longa* (Tumeric)

Because of its therapeutic qualities, turmeric (*Curcuma longa*) has long been used in traditional medical systems like Ayurveda, Siddha, and Traditional Chinese Medicine (Prasad and Aggarwal, 2011). Turmeric has been used as a medicinal spice for a variety of illnesses throughout history. It is used as an anti-inflammatory treatment for skin conditions, wound healing, arthritis, and digestive issues in Indian ethnomedicine (Aggarwal *et al.,* 2007). According to Ravindran *et al*. (2007), it is frequently used topically as a paste for wounds, burns, and skin infections. It can also be taken in milk or herbal formulations to treat respiratory conditions like colds.

Turmeric is used in Traditional Chinese Medicine to treat irregular menstruation, relieve abdominal pain, and increase blood circulation (Liu *et al.,* 2013). Similarly, turmeric is used as a general tonic and for liver and stomach issues in traditional African practices (Akinmoladun *et al*., 2015). Its bioactive compound curcumin, which has anti-inflammatory, antimicrobial, antioxidant, and hepatoprotective properties, is primarily responsible for its ethnomedical applications (Gupta *et al*., 2013). Numerous contemporary scientific studies have been based on these traditional applications, and the majority of them have confirmed turmeric's potential as a medicine.

As a multifunctional healing agent for inflammatory conditions, digestive health, skin care, and overall wellness, turmeric is widely used in ethnomedicine throughout Asia, Africa, and beyond. Modern integrative medicine is still influenced by its traditional uses.

2.4. *Panax spp.* (Ginseng)

2.4.1. Description of *Panax spp.* (Ginseng)

Ginseng is a popular medicinal plant that actually refers to a few different species in the Panax genus. The two most well-known types are Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius*) (Attele *et al.,* 1999). Interestingly, the name "Panax" comes from a Greek word meaning "all-healing," which shows just how highly people have regarded this plant for centuries (Christensen, 2009).

This herb grows slowly and can reach about 30 to 60 centimeters in height. Its most prized part is the root, which is thick and fleshy. The plant also has a stem with leaves made up of three to five leaflets (Yun, 2001). Farmers usually wait 4 to 6 years before harvesting the roots because older ones are believed to be more powerful (Bhat *et al.,* 2014).

Ginseng roots are pale yellow and have a forked shape that kind of looks like a human figure. They taste bitter-sweet and are packed with ginsenosides—these are the main compounds that give ginseng its health benefits (Kennedy *et al.,* 2001). Besides ginsenosides, the roots also contain other useful substances like polysaccharides, peptides, and polyacetylenes.

The plant thrives in cool, shaded environments with well-drained soil. Asian ginseng is mostly grown in places like Korea, China, and Russia, while American ginseng is native to North America, especially the U.S. and Canada (Ratan *et al.,* 2021).

Beyond its healing properties, ginseng is also culturally significant and economically valuable. In traditional Asian medicine, it's often used to boost energy and promote long life. Today, it's traded all over the world.

Ginseng is a slow-growing herb with unique, fork-shaped roots full of bioactive compounds. It's grown mainly in Asia and North America and remains one of the most famous and widely used medicinal plants globally.

2.4.2. Distribution of *Panax spp.* (Ginseng)

Ginseng mostly comprises two species, Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius*), as well as other related species like Panax notoginseng and Panax japonicus (Attele *et al.*, 1999). Particularly in Asia and North America, its natural habitat is limited to the chilly temperate climates of the Northern Hemisphere. Originally from Korea, Northeast China, and the Russian Far East, *Panax ginseng* has long been farmed as a prized medicinal plant (Christensen, 2009). (Yun, 2001) Korea is the top producer; its ginseng is of great quality; China and Russia come behind. By contrast, Panaxquinquefolius hails from eastern North America, including areas of Canada such Ontario and British Columbia and the United States including Wisconsin and Appalachia. According to Ratan *et al.*, 2021, this species has been grown as well as wild-harvested; a sizable proportion of the output from America and Canada is exported to Asian markets.

Other species have more local relevance. Indigenous to Japan, Panaxjaponicus is extensively grown in Yunnan, China; both are significant in regional therapeutic techniques but have a diminishing part in world trade (Kennedy *et al.*, 2001). In reaction to rising global need for herbal supplements, growth has spread beyond these indigenous areas to other chilly nations such Germany and New Zealand (Bhat *et al.*, 2014). Ginseng is mostly produced in East Asia and North America, with Korea, China, and the U. S. /Canada as the main producers. Its cultivation to new areas has been motivated by its capacity to prosper in temperate environments

and great economic value, thus making it a commonly traded.

2.4.3. Ethno-Medicinal Uses of *Panax spp.* (Ginseng)

Ginseng has traditionally been utilized as an adaptogen, a chemical increasing the body's general nonspecific resistance to several stresses. Its customary uses include treating tiredness and weakness, cognitive decline and memory loss, sexual dysfunction and infertility, immunodeficiency and convalescence, as well as cardiovascular and metabolic issues.

Ginseng is a well-known medicinal plant that's been used for centuries in traditional healing systems across Asia, North America, and Indigenous cultures. The most popular types are Asian ginseng (Panax ginseng) and American ginseng (Panaxquinquefolius), both known for their ability to boost energy, improve health, and promote longevity (Attele *et al.,* 1999).

In Traditional Chinese Medicine, Asian ginseng is considered an adaptogen basically, something that helps the body handle stress. It's often used to treat fatigue, low appetite, and weak immunity. People also take it to improve mental focus, sexual health, and overall vitality (Yun, 2001). In Korean traditional medicine, ginseng is called the "life root" and is used to build stamina, reduce stress, and help people recover from illness (Ratan *et al.,* 2021).

American ginseng has its own history in Native American medicine. Tribes used it to treat headaches, digestive issues, fevers, and as a general health booster (Bhat *et al.,* 2014). Today, it's still popular for its cooling and calming effects, which are considered gentler than Asian ginseng.

In Southeast Asia, another type called Panax notoginseng is used to stop bleeding, improve circulation, and reduce inflammation (Kennedy *et al.,* 2001).

Across cultures, ginseng is known for helping restore energy, strengthen the immune system, support mental health, and improve sexual function. These traditional uses have inspired modern research, which has confirmed many of its benefits especially its antioxidant and immune-boosting effects.

In short, ginseng is more than just a root. It's a powerful herbal remedy that's deeply rooted in traditional medicine and still widely used today to promote health and longevity.

Future studies should emphasize standardized extracts, mechanistic biomarkers, and integration with conventional treatments.

2.5. Vaccinium myrtillus (Bilberry)

2.5.1. Description of *Vaccinium myrtillus* (Bilberry)

Bilberry (*Vaccinium myrtillus*) is a perennial shrub from the family Ericaceae, Native to Europe, parts of Asia, and North America, and it is strongly connected to blueberry (Vacciniumcorymbosum) (MazzaandMiniati, 1993). It is usually found in acid, low nutrient soils like those in heaths, forests, and mountanous areas (Kučera *et al.,* 2021).

With slender, angular, green stems, the shrub usually stands 15–60 cm high. Its leaves are little, oval, and serrated along the edges. Spring sees the solitary, bell-shaped, pinkish-white blooms of blueberry (Howard *et al.,* 2012). With red-purple flesh, the dark blue to almost black berries are 5 to 9 mm in size. Unlike cultivated blueberries, bilberries have pigments (anthocyanins) throughout the fruit, which gives the flesh its distinctive dark color (Mazza and Miniati, 1993).

It is rich in anthocyanins, flavonoids, tannins, and vitamins C and E, all of which give bilberries their strong antioxidant properties Nile and Park, 2014. They have traditionally been gathered for

medical reasons and as nourishment. Fresh, dried, or processed into jams, juices, wines, and extracts the berries are eaten.

In conclusion, bilberry is a nutrient-dense, anthocyanin-rich wild berry with recognizable deep-blue berries that has a long history of usage in both traditional diets and herbal medicine across Europe and beyond.

2.5.2. Distribution of *Vaccinium myrtillus* (Bilberry)

Bilberry is a circumboreal/holarctic shrub having a core range in Europeandwide-ranging extensions into northern and temperate to subarctic Northern Hemisphere.Disjunctdistributions in Greenland and western North America (Negrușier, 2024; Martău *et al.,* 2023; USDA FEIS, n.d.) as well as central Asia (incl. Siberia, Caucasus, Japan).

Recent research emphasizes range dynamics and habitat reactions: blueberry can grow in subalpine areas under land-use change and warming,and exhibits plastic phenological responses to altitude and disturbance (Zeidler *et al.,* 2024; Kubov *et al.,* 2024).

From a regional angle, Nordic nationspossess some of the biggest continuous bilberry resources globally; national forest inventory - based projectionsreveal very high annual wild yields, highlighting its ecological and financialrelevance in Finland and Sweden (Miina *et al.,* 2021; Vaneková *et al.,* 2022).

In essence, V. myrtillus is a commonunderstorey species foundinboreal and montane Europe with a wider holarctic distribution, ecologically focused on chilly climates and Particularly in subalpine regions, acidic soils and presently showing significantreactions to climate and land-use change (Negrușier, 2024; Zeidler *et al.,* 2024).

2.5.3. Ethno-Medicinal uses of *Vaccinium myrtillus* (Bilberry)

Across Europe and some regions of Asia, bilberry (*Vaccinium myrtillus*) has traditionally been used for medicine over a long time. Its wild fruit and leaves have been used as folk cures for a variety of conditions (Mazza and Miniati, 1993). Traditionally, Bilberry prepared by indigenous and rural people as infusions, decoctions, syrups, jams or poultices; and use of the leaves as teas or extracts. (Vaneková *et al.,* 2022)

Historically, bilberry fruit has been employed to cure dysentery and diarrhoea because of its astringent tannins that lessen intestinal inflammation and fluid loss (MazzaandMiniati, 1993; Häkkinen *et al.,* 1999). In folk medicine, the fruit and leaf preparations are also used to treat gastrointestinal problems and as mild disinfectants for scratches and eye irritations (Kučera *et al.,* 2021).

One well-known classic use is for cardiovascular and eyesight. Bilberry extracts were formerly said to improve night vision and strengthen capillaries; these uses resulted in wider interest and commercial extracts targeting retinal and microvascular circulation (Mazza and Miniati, 1993; Nile and Park, 2014). Results from ethnomedical practice also cites blueberry for unrinary tract problems, gout, and as a general tonic, reflecting its believed anti-inflammatory and antioxidant actions (Vaneková *et al.,* 2022).

Bilberry are ascribed its purported therapeutic effects in traditional medicine (antioxidant, anti-inflammatory, vasoprotective, and astringent). Many of its folk uses depend on these phytochemicals, which have also inspired recent scientific research (Häkkinen *et al.,* 1999; Kučera *et al.,* 2021).

Although conventional use is prevalent and ingrained culturally, modern reviews emphasize that traditional claims vary regionally and have different preparation, dosage, and application. Some traditional uses (e.g., vision improvement and vascular protection) have supporting experimental data, but clinical evidence is mixed and more high-quality human trials are needed to validate numerous ethnomedical assertions (Nile and Park, 2014; Vaneková *et al.,* 2022).

For culinary and modest medical use, safety profiles are often good; yet, standardization of extracts and instructions on interactions (e.g., with anticoagulant medications) still need work. regions where one should proceed with care (Kučera *et al.,* 2021).

As an astringent, anti-diarrhoeal, wound healer, and circulatory/vision tonic, blueberry has a long-standing position in European ethnomedicine. Because of high anthocyanin and tannin content, its conventional applications are biologically plausible; still, conversion of folk medicine into evidence-based treatment necessitates standardized preparations and additional clinical studies (MazzaandMiniati, 1993; Vaneková *et al.,* 2022).

2.6. Comparative Analysis of Medicinal Plants: *Allium sativum* (Garlic), Turmeric, Ginseng, and Bilberry

Each of the four plants that comprise Nature Gift® garlic, turmeric, ginseng, and bilberry brings special advantages, but they also have significant things in common. They all support brain health, combat oxidative stress, and lessen inflammation. Because of these overlapping characteristics, they are particularly helpful in treating complex conditions like anxiety and depression, where a variety of biological and lifestyle factors are involved.

2.6.1. Constituents of Phytochemistry

Every plant has a wealth of potent natural substances:

- Sulfur compounds like S-allylcysteine and allicin give garlic (*Allium sativum*) its potency. In addition to combating infections, these also shield the brain from stress and may elevate mood by affecting dopamine and serotonin levels (Ademiluyi *et al.,* 2016; Sohn *et al.,* 2016).
- Curcumin and turmerones are the primary components responsible for the effects of turmeric (*Curcuma longa*), a golden spice. According to Ng *et al*. (2017) and Lopresti *et al*. (2022), these substances lessen inflammation, counteract free radicals, and promote the development of new neural connections—a process that is frequently interfered with in depression.
- Ginsenosides, which are found in ginseng (*Panax spp.*) roots, aid in the body's ability to cope with stress. They enhance protective proteins like BDNF in the brain and promote energy, memory, and calmness (Wang *et al.,* 2017; Lopresti *et al.,* 2019).
- Bilberry (*Vaccinium myrtillus*): This antioxidant is particularly noteworthy due to its dark purple anthocyanins. According to preliminary research, they may enhance mood and memory, protect the nervous system, and improve circulation (Ghosh *et al.,* 2017; Whyte *et al.,* 2020).

2.6.2. Pharmacological Similarities and Dissimilarities

Each of the four plants has a distinct specialty, but they all combat inflammation and oxidative stress:

- Garlic's benefits for heart and immune health are most well-known.
- The best evidence for improving mood is found with turmeric.
- Ginseng increases resilience and aids in stress management.
- Bilberry primarily improves circulation and cognitive function, but it also has positive effects on mood.

Because of these distinctions, they work well together.

2.6.3. Polyherbal Formulations' Potential for Synergy

Nature Gift® employs a "teamwork approach" by combining these plants. Rather than depending on a single herb, it blends: ginseng for energy and resilience; bilberry for improved mood and cognition; and garlic and turmeric for reducing stress and inflammation.

The way that contemporary medicine frequently combines treatments to address various aspects of the same issue is reflected in this synergistic effect. Additionally, it reflects the wisdom of traditional medicine, which favors herb mixtures over individual remedies (Uwaya *et al.*, 2025)

Table 2.6.1: A Comparative Overview of Medicinal Properties

Plant	Key Compounds	Main Health Roles	Mental Health Links	Evidence Strength
Garlic	Allicin, S-allylcysteine	Cardiovascular protection, antimicrobial, antioxidant	May boost serotonin and dopamine, antidepressant-like effects in animals	Moderate (animal strong, human limited)
Turmeric	Curcumin, turmerones	Anti-inflammatory, antioxidant, immune support	Improves mood, increases BDNF, meta-analyses show benefit in depression	Strong (animal strong, human growing)
Ginseng	Ginsenosides (Rb1, Rg1, Rd, Re)	Adaptogen, stress resistance, cognitive support	Enhances resilience, modulates brain chemicals, supports neurogenesis	Moderate-to-strong (animal strong, human emerging)
Bilberry	Anthocyanins, flavonols	Vascular support, antioxidant, eye and brain health	Reduces depressive-like behavior in animals, improves mood in small trials	Moderate (animal strong, human limited)

Plate 1: Allium Sativum (Ejo *et al*., 2021)

Plate 2: Tumeric (Prasad and Aggarwal, 2011)

Plate 3: Ginseng (Yuan *et al.*, 2016)

Plate 4: Bilberry (Kowalska *et al.*, 2019)

2.7. OVERVIEW OF DEPRESSION

Common, chronic, and recurring mental health illness known clinically as major depressive disorder(MDD), depression is distinguished by guilt. Or persistent sorrow, exhaustion, diminished interest or delight, bad focus, and lowself-worth (WHO, 2023). One of the primary contributors to the global disease load is it; it impacts individuals of all ages.

Epidemiological studies indicate that depression impactsmorethan280 million people worldwide, with women showing a larger prevalence than men (Malhiand Mann, 2018; WHO, 2023).The illness results from a complicated interplay of biological, psychological, and social influences. Among biological factors are genetic predisposition, neurotransmitter imbalances (serotonin, dopamine, norepinephrine), hormonal dysregulation, and structural changes in the brain. Psychosocial stressors like trauma, long-term illness, unemployment, or social isolation (Friedrich, 2017) are also crucial triggers.

Depression runs from mild to severe clinically. Because in its most extreme form it can cause suicidal ideas and actions, this is a major public health problem. WHO estimates that almost 700,000 individuals per year die by suicide, and depression is a major risk factor (WHO, 2023).

Treatment for depression demands a multimodal approach including psychotherapies (interpersonal therapy, cognitive-behavioral therapy), pharmacotherapy (antidepressants like SSRIs and SNRIs), and lifestyle interventions (exercise, good sleep hygiene, social support) are examples of evidence-based treatments (Malhiand Mann, 2018).

Depression is a complicated disease with major consequences on social, financial, and personal areas. Given its widespread occurrence, the fact that it is often underestimated and undertreated

underlines the necessity of greater awareness, early treatment, and access to mental health care (Friedrich, 2017; WHO, 2023).

Recent West African research on herbal treatments for depression have emphasized the application of traditional plants to treat oxidative stress and neurotransmitter imbalances (Uwaya *et al.,* 2025).

2.7.1. Types of Depression

1. Major Depressive Disorder (MDD): The most prevalent form, distinguished by ongoing melancholy, loss of interest, exhaustion, sleep and appetite changes, and poor functioning (Malhiand Mann, 2018).

2. Persistent Depressive Disorder (Dysthymia): A chronic form spanning two years or more, with less intense but ongoing symptoms affecting daily life.

3. In those with bipolar disorder, bipolar depression presents itself as bouts of depression interspersed with periods of mania or hypomania (Grande *et al.*, 2016).

4. Seasonal Affective Disorder (SAD): Usually seen in autumn/winter because of less sunshine exposure, this kind of depression is connected with seasonal changes (Melrose, 2015).

5. Some women suffer from postpartum depression after delivery, with sadness, worry, and tiredness that obstruct infant care (O'Hara and McCabe, 2013).

6. Premenstrual dysphoric disorder (PMDD): This is marked by mood swings, irritability, and depression in the luteal phase of the menstrual cycle. It define a severe form of premenstrual syndrome (Yonkers *et al.*, 2008).

7. Usually mood-congruent (MalhiandMann,2018), psychotic depression is major depression accompanied by psychotic symptoms including hallucinations or delusions.

8. Atypical depression is distinguished by hypersomnia (Simmons *et al.,* 2016), mood reactivity

(brief mood improvement in reaction to positive events), more appetite, and weight gain.

9. Situational Depression (Adjustment Disorder with Depressed Mood) is a brief depressive reaction triggered by major stressful life events such loss, trauma, or significant change (Friedrich, 2017)..

2.7.2. Causes of Depression

Malhi and Mann (2018) believe that depression is a multi-factorial illness resulting from the interaction of biological, psychological, and societal influences.

1. Biology's Causes

- Genetics: According to family and twin studies (Sullivan *et al.,* 2000), several susceptibility genes have been implicated in 30–40% heritability.
- Neurotransmitter Imbalance Dysregulation of serotonin, dopamine, and norepinephrineis strongly related to depression symptoms (Friedrich, 2017).
- Hormonal Changes: Depression can result from thyroid problems, postpartum
- hormonal changes, and hypothalamic-pituitary-adrenal (HPA) axis dysfunction (Pariante and Lightman, 2008).
- Brain Structure and Function: Decreased hippocampal volume and altered prefrontal cortex activity are rather often observed (Drevets *et al.,* 2008).

2. Psychological Factors

- Low self-esteem, high neuroticism, and faulty coping techniques all personality traits raise vulnerability (Kendler *et al.,* 2006).
- Factors leading to start and recurrence include hopelessness, excessive rumination, and pessimistic thought patterns (Beck, 2008).

3. **Social and Ecological Considerations**

- **Early life adversity:** Heim and Nemeroff (2001) find that childhood trauma, neglect, and loss significantly raise the chance of later-life depression.
- **Chronic Stressors:** Among the strong triggers are poverty, discrimination, unemployment, and relationship conflicts (Friedrich, 2017).
- **Social Isolation:** Loneliness and a lack of social support make one more vulnerable (Cacioppo *et al.,* 2010).

4. Lifestyle and Medical Aspects

- **Chronic Conditions:** Depression is strongly related with diabetes, cardiovascular disease, and chronic pain syndromes, according Whooley and Wong (2013).
- Substance misuse worsens depression and causes it as well, according to Boden and Fergusson (2011).
- **Lifestyle Factors:** Jacka *et al*. (2010) say that bad eating patterns, lack of exercise, and erratic sleep patterns are all contributors.

2.7.3. Treatment of Depression

A multimodal and tailored strategy including a mix of pharmacological, psychological, and lifestyle treatments is needed for depression (Malhiand Mann, 2018).

1. **Pharmacological Methods:**

Antidepressants: First-line therapies include SSRIs (Selective Serotonin Reuptake Inhibitors) such fluoxetine and sertraline because of their safety and tolerability (Cipriani *et al.,* 2018). Serotonin-Norepinephrine Reuptake Inhibitors Particularly in extreme melancholy, like venlafaxine works very effectively.

MAOIs and tricyclic antidepressants (TCAs) are set aside for treatment of resistant patients because of side effects (Friedrich, 2017). New medicines Ketamine and esketamine offer quick alleviation for treatment of resistant depression (Wilkinson *et al.*, 2017).

2. **Psychotherapy:**

Cognitive-behavioral therapy (CBT): (Beck, 2008) emphasizes on reorganizing negative thought patterns. Interpersonal Therapy (IPT) aim sat relationship stressors and role changes. Mindfulness-Based Cognitive Therapy (MBCT) avoids relapse especially in repeated depression (Kuyken *et al.*, 2016).

3. **Lifestyles and Social Interventions**

- Normal exercise relieves depressive symptoms and improves treatment response (Schuch *et al.*, 2016).
- Diet and Sleep: Positive effects from nutritional strategies and sleep hygiene on mood regulation (Jacka *et al.*, 2010).
- Social Support: Improves treatment adherence and increases resilience (Cacioppo *et al.*, 2010).

4. **Other Therapies: Neurostimulation and Electroconvulsive Therapy (ECT)**

Particularly for psychotic characteristics, efficient for major or treatment-resistant depression (UK ECT Review Group, 2003). Non-invasive choice for patients who are insensitive to medication is repetitive transcranial magnetic stimulation (rTMS). Vagus Nerve Stimulation (VNS) is under consideration in chronic, refractory situations.

5. **Integrated and Emerging Approaches Combined Treatment:**

For mild-to-severe depression, antidepressants plus psychotherapy are more effective than either alone (Cuijpers *et al.*, 2014).

- Digital Therapy: Online CBT and mobile health initiatives are becoming more easily available as digital therapies.
- Personalized medicine: Genetic analysis and biomarker investigations are opening the door for individualized therapies (Malhiandamp; Mann, 2018).
- Herbal Alternatives:

  Through adaptogenic effects, polyherbal treatments have shown potentials in controlling stress and anxiety in areas such as West Africa (Uwaya *et al.*, 2025).

2.8. OVERVIEW OF ANXIETY

Anxiety is a complex emotional state marked by tension, anxiety, unease, and physical arousal feelings. Although some degree of anxiety is natural and normal, helping people get ready for challenges - pathological anxiety results when these reactions are excessive, long-lasting, and affect Anxiety disorders taken together constitute among the most common psychiatric illnesses globally, with an estimated worldwide lifetime prevalence. Often co-occurring with depression, substance misuse problems, and other medical illnesses, they impose great economic, social, and health loads of 16 - 29% (Bandelow and Michaelis, 2015).

From a neurobiological standpoint, anxiety involves altered neurotransmitter activity, including serotonin, dopamine - hyperactivation of the amygdala, and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. GABA, norepinephrine systems (Nutt, 2001).

Anxiety can psychologically show itself as extreme worry, avoidance behaviors, impaired concentration, and hypervigilance. Environmentally, trauma, negative childhood experiences, and chronic stress all greatly add to the onset and maintenance of anxiety disorders (McLaughlin *et al*., 2010).

2.8.1. Types of Anxiety Disorders

Several main anxiety disorder categories are distinguished by the DSM-5-TR (American Psychiatric Association [APA], 2022). They are:

- **Generalized Anxiety Disorder (GAD):** Continual and disproportionate concern across a range of subjects lasting at least six months, combined with symptoms including restlessness, Sleep problems, irritability, trouble focusing, and muscle stiffness are among Baldwin *et al.,* 2014.
- **Panic Disorder:** Recurrent, unanticipated panic attacks - sudden surges of extreme anxiety or pain accompanied with physical symptoms like palpitations, chest discomfort, lightheadedness, and breath shortness (Roy-Byrne *et al.,* 2006).
- **Social Anxiety Disorder:** An extreme worry of social or performance contexts where embarrassment or criticism is likely (Stein and Stein, 2008).
- **Specific Phobias**: Excessive, illogical dread of particular items or circumstances (APA, 2022).
- **Seperation Anxiety Disorder:** Intense worry about losing attachment figures (Shear *et al.,* 2006).

DSM-5-TR groups conditions such as obsessive-compulsive disorder (OCD) and post-traumatic stress disorder (PTSD) individually even if they share characteristics with anxiety disorders. Nevertheless, Comorbidity with anxiety disorders is high (Kessler *et al.,* 2005).

2.8.2. Causes of Anxiety

Anxiety disorders arise from a multifactorial interaction of biological, psychological, and environmental elements:

1. **Biological elements:**
   - Twin and family research point to a heritability of 30–40% (Hettema *et al.,* 2001).
   - Dysregulated fear reactions result in abnormalities in serotonergic, noradrenergic, and GABAergic systems, among others (Nutt, 2001).
   - *Hyperactivity in the amygdala and insular cortex with decreased prefrontal cortex* regulatory control has been linked (Etkinand Wager, 2007).
2. **Psychological Factors:**
   - Clark and Beck, 2010 found maladaptive thinking patterns, catastrophizing, and attentional bias toward danger indicators.
   - Personality qualities include neuroticism and behavioral constraint in youth (Biederman *et al.,* 2001).
3. **Social and Environmental Factors:**
   - Trauma and stress include bullying, neglect, childhood abuse, and unfavorable life events (McLaughlin *et al.,* 2010).
   - Financial insecurity, joblessness, and personal conflict are chronic stressors.
   - Cultural and social influences molding symptom expression and coping.

2.8.3. Treatment of Anxiety

Treatment is multidisciplinary by combining psychological, pharmacological, and complementary techniques:

1. **Psychological treatments:**
   - Cognitive Behavioral Therapy (CBT) is the Gold standard involving cognitive restructuring, relaxation training, and exposure therapy. It is proven in its effectiveness for anxiety disorders (Hofmann *et al.*, 2012).
   - Acceptance and commitment therapy (ACT) and mindfulness-based stress reduction (MBSR) encourage non-judgmental awareness (Khoury *et al.,* 2013).
2. **Pharmacological Interventions:**
   - First-line medicines include SSRIs (e.g., sertraline, escitalopram) and SNRIs (e.g., venlafaxine, duloxetine) (Baldwin *et al.*, 2014).
   - For short-term comfort, benzodiazepines (such lorazepam) have dependence risk (Bandelow *et al.*, 2017).
   - Adjunctive: Buspirone, pregabalin, and several unusual antipsychotics (Bystritsky *et al.*, 2013).
3. **Guidelines-Based Care:**

International guidelines (e.g., NICE, APA) advise graduated care: low-intensity therapy for mild anxiety, CBT and/or SSRIs/SNRIs for moderate-to-severe cases, and expert treatments for treatment-resistant disorders.

4. **Approach Complementary and Lifestyle:**

Exercise, sleep hygiene, cutting caffeine, and herbal treatments including kava and polyherbal combinations seem to have some advantages (Stubbs *et al.*, 2017).

CHAPTER THREE

MATERIALS AND METHOD

3.1. Apparatus and Equipment Used

The equipment used includes the following:

Analytical weighing balance (Ohaus Corp., Pine Brook, NJ, USA, China), beaker (50 ml), conical flask (500 ml), hand gloves, cotton wool, stopwatch/timer, mortar and pestle, measuring cylinder (100 ml), transparent bowl, wooden stool, masking tape, mouse cage, needle and syringe (1 ml, 2 ml), oral gastric tube (1 ml), universal bottle (10 ml), elevated plus maze, and a water bath (HH-S6, China).

3.2. Chemicals and Drugs Used

The following chemicals and drugs were used for this study:

Fluoxetine (20 mg/kg), Diazepam (10 mg/kg), Distilled Water, Nature Gift® Supplement.

3.3. Procurement of Nature Gift® Supplement

The Nature Gift® supplement (Batch No: 0001, NAFDAC REG NO: A7-4333L, PRD: 02/10/2024, BB: 02/10/2026) was obtained from Nature's Renaissance International Limited (90 Allen Avenue, Ikeja, Lagos, Nigeria) and manufactured by UTAD HERBAL RESOURCES LIMITED (Fredineri Street, Ugbor Village, Benin City, Edo State, Nigeria).

3.4. Extraction

The nature gift® supplement was extracted using maceration. Nature Gift® supplement 30 g was soaked in 500 ml of distilled water and allowed to macerate for 72 hours. The mixture was filtered through muslin cloth and Whatman No. 1 filter paper and concentrated over a water bath. The extract was stored in a refrigerator at four degrees Celsius before use.

3.5. Experimental Animals

Seventy (70) healthy mice (20-30 g) were obtained from a commercial animal house in Ibadan, Oyo State, Nigeria. The mice were housed at the Phytomedicine Animal Unit, Department of Plant Botany and Biology (PBB), Faculty of Life Sciences, University of Benin, under standard laboratory conditions with a 12-hour light/dark cycle. The mice were acclimatised for two weeks and well fed with standard animal pellets and water. All procedures adhered to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals. This study was approved by the Science Laboratory Technology Research Ethical Committee with reference number.

3.6. Experimental Design

The antidepressant activity of the Nature's Gift® supplement was evaluated using the forced swim test (FST) and tail suspension test (TST), two widely used models to screen for antidepressant-like effects (Castagné *et al.*, 2011; Uwaya *et al.*, 2025).

3.6.1. Forced Swim Test (FST): Twenty-five mice weighing 20-30 grams were allotted into five groups with five animals in each. Group 1 received distilled water at 10 ml/kg; groups 2, 3 and 4 received 50 mg/kg, 100 mg/kg, and 200 mg/kg of Nature Gift extract, respectively, while

Group 5 received 20 mg/kg of the standard drug, fluoxetine, orally. One hour after administration of Nature's Gift® and fluoxetine, respectively, the mice were placed in an inescapable transparent cylinder (height 40 cm, diameter 20 cm) filled with water (depth 30 cm) at 25°C. The animals were allowed to swim for 5 minutes, and the time of immobility (a sign of despair-like behaviour) was recorded. A reduction in immobility time after treatment is indicative of antidepressant-like activity (Koek *et al.,* 2018; Unal and Canbeyli, 2019).

3.6.2.Tail Suspension Test (TST): Twenty-five mice weighing 20-30 grams were allotted into five

groups with five animals in each. Group 1 received distilled water (10 ml/kg); groups 2–4 received Nature Gift® extract at 50, 100 and 200 mg/kg, respectively; and group 5 received the standard drug fluoxetine (20 mg/kg) orally. One hour after administration of Nature gift® and fluoxetine, the mice were suspended from the edge of a shelf 60 cm above the table top by their tails using adhesive tape, and the time of immobility (immobility being indicative of a depressive state) was recorded for 5 minutes for each animal. The test was conducted in a noise-free room to minimise external stressors (Koek*et al.,* 2018; Unal and Canbeyli, 2019).

3.7. Anxiolytic Activity

The elevated plus maze (EPM) test was employed to assess the anxiolytic effects of the extract (Mechiel and De Boer, 2003).

3.7.1.Elevated Plus Maze (EPM): The maze consists of two open arms and two closed arms, elevated 50 cm above the floor. Twenty mice weighing 20-30 grams were randomly divided into five groups with four animals in each. Group 1 received distilled water (10 ml/kg); groups 2–4 received Nature Gift® extract (50, 100 and 200 mg/kg, respectively); and group 5 received the standard drug diazepam (10 mg/kg). One hour after the administration of Nature's Gift® extract anddiazepam, the animals were placed in the centre of the maze and allowed to explore for 5 minutes. The percentage of time spent in the open arms and the time of entries into the open arms were used as indicators of anxiety. Increased exploration of the open arms is considered a sign of anxiolytic-like behaviour (Mechiel and De Boer, 2003).

3.8. Statistical Analysis

The mean ± SEM was used to express the data. Graph-Pad Prism 9.0 was used for statistical analysis. Statistical significance was set at $p < 0.05$, and differences between groups were compared using Tukey's post hoc test after one-way Analysis of Variance (ANOVA) (Field, 2013).

CHAPTER FOUR

RESULTS

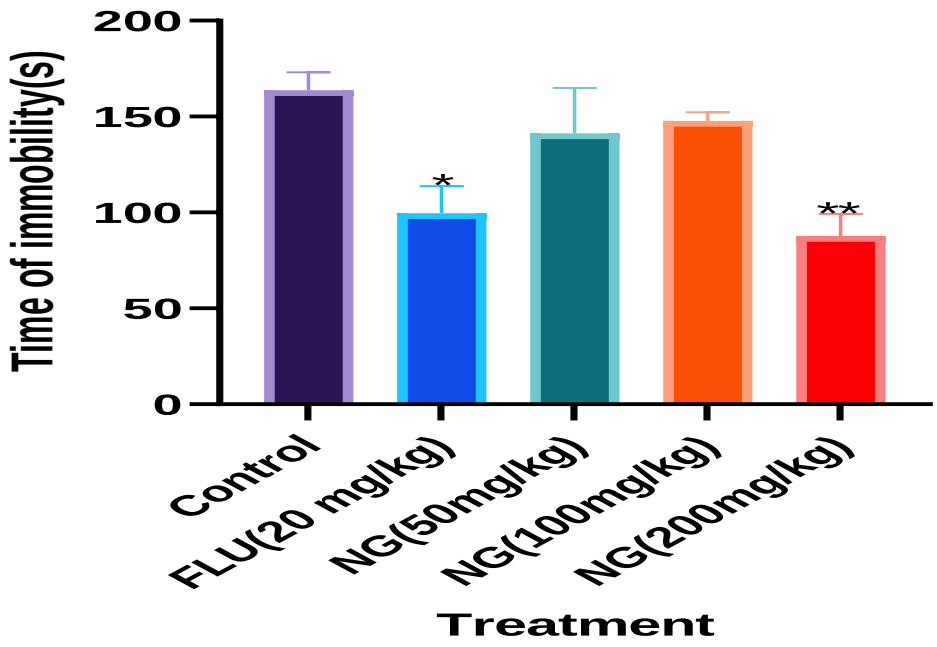

Figure 1: Effect of Nature's Gift® on immobility time in the Forced Swim Test (FST).

Nature's Gift® extract at 200 mg/kg and fluoxetine (20 mg/kg) reduced immobility time in the force swimming test when compared with control (*p < 0.05; **p<0.01).Values are represented as Mean ± SEM, n=5 per group.NG: Nature's Gift® extract; FLU: Fluoxetine

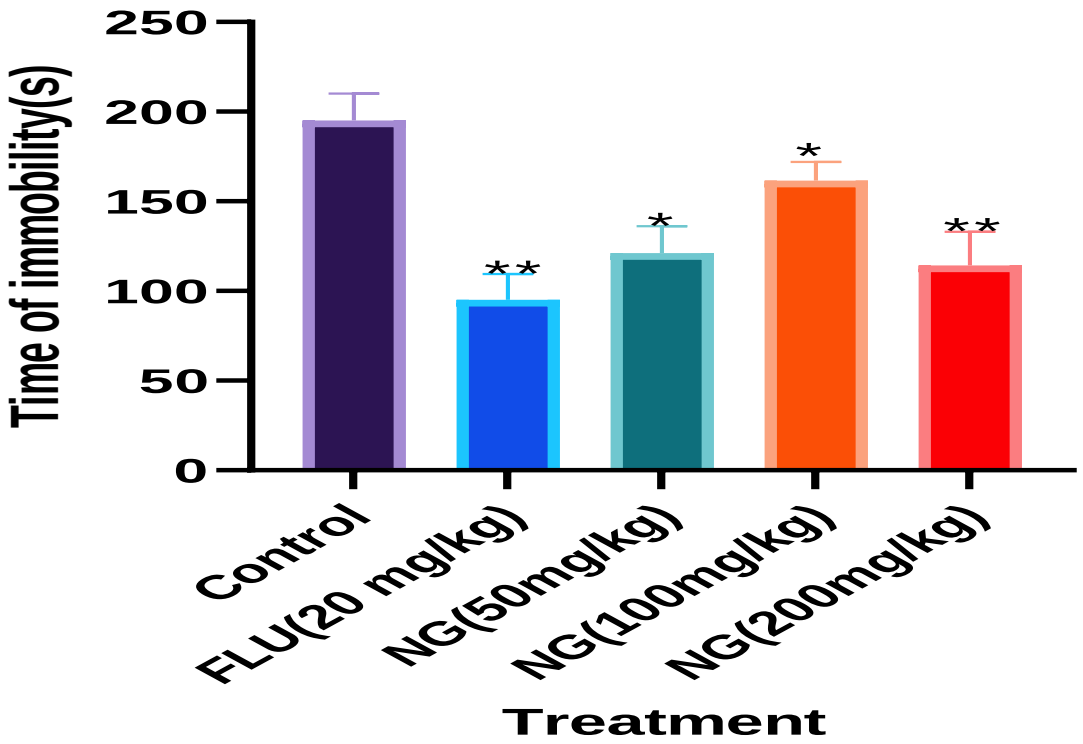

Figure 2: Effect of Nature's Gift® on immobility time in the Tail Suspension Test (TST).

Nature's Gift® extract (50, 100 and 200 mg/kg) and fluoxetine (20 mg/kg) reduced immobility time in the tail suspension test when compared with control (*p < 0.05; **p<0.01).Values are represented as Mean ± SEM, n=5 per group.NG: Nature's Gift® extract; FLU: Fluoxetine

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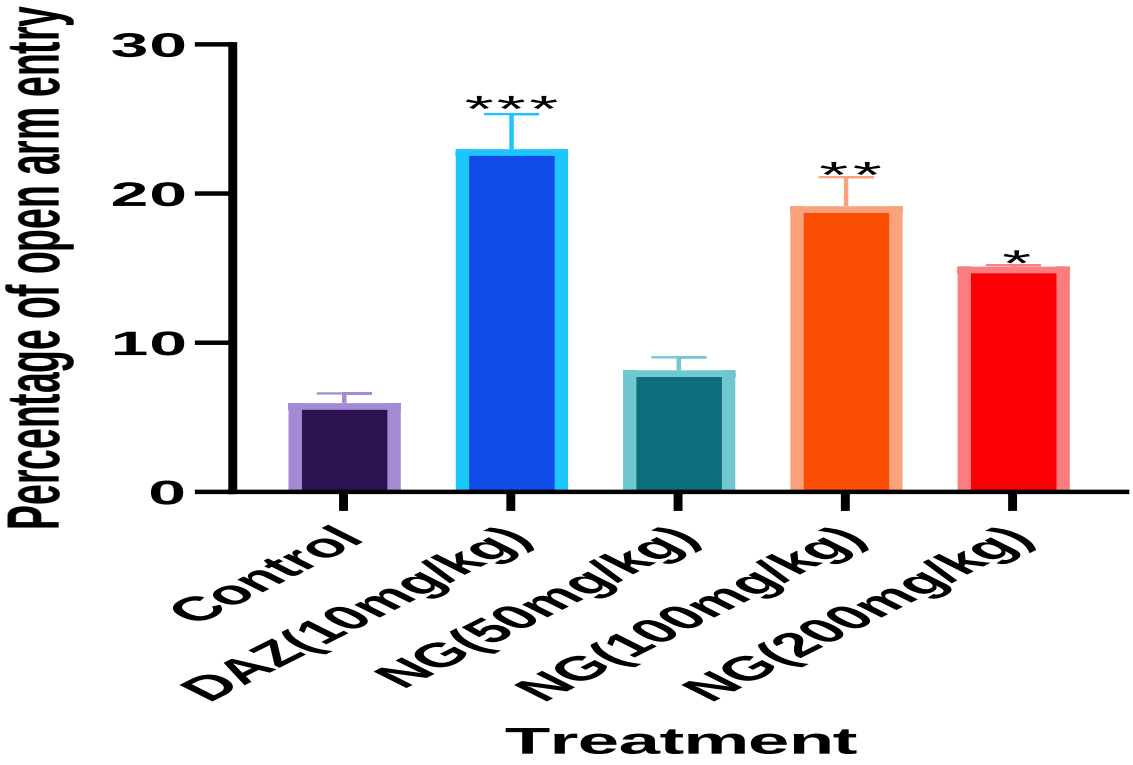

Figure 3: Effect of Nature's Gift® on open-arm exploration in the Elevated Plus Maze (EPM)

Nature's Gift® extract (100 and 200 mg/kg) and diazepam (10 mg/kg) increased the percentage time (%) spent in the open arm when compared with control (*p < 0.05; **p<0.01).Values are represented as Mean ± SEM, n=5 per group.NG: Nature's Gift® extract; FLU: Fluoxetine

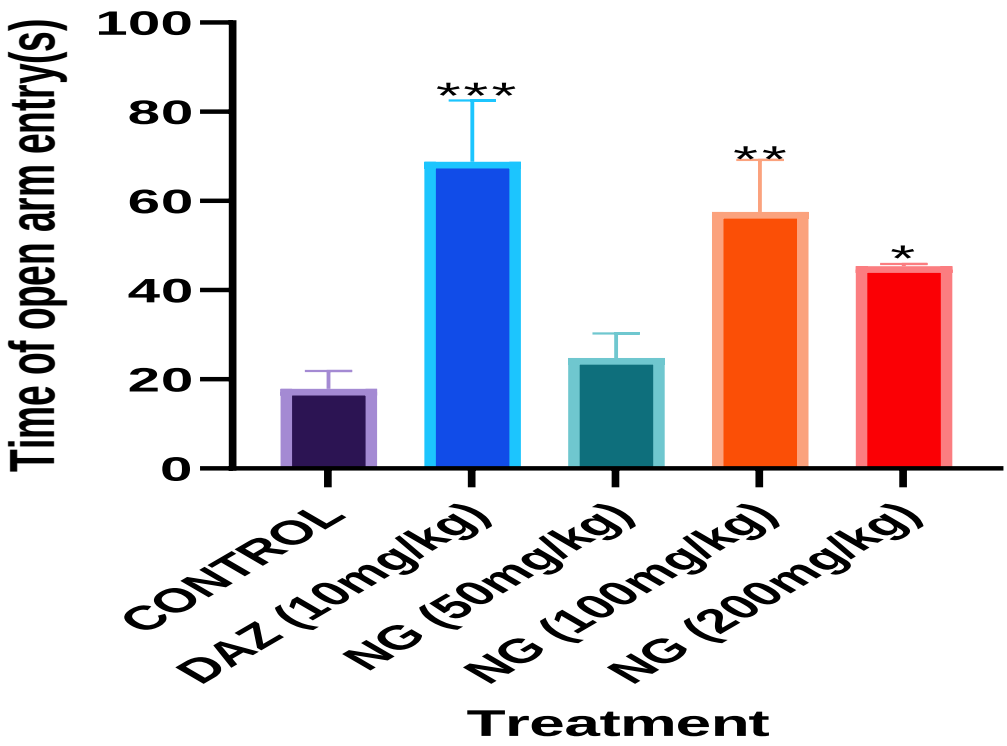

Figure 4: Effect of Nature's Gift® and Diazepam on the time spend in the open arms of the EPM

Nature's Gift® extract (100 and 200 mg/kg) and diazepam (10 mg/kg) increased the time(s) spent in the open arm when compared with control (*p < 0.05; **p<0.01).Values are represented as Mean ± SEM, n=5 per group.NG: Nature's Gift® extract; FLU: Fluoxetine

CHAPTER FIVE

5.1. DISCUSSION OF FINDINGS

Common methodologies used to evaluate health interventions for anxiety- and depression-related behaviours in patients include the forced swimming endurance test, the tail suspension test, and the elevated plus maze (EPM) (Uwaya *et al.*, 2024; Tran and Gellner, 2023). The forced swimming test, tail suspension test, and elevated plus maze (EPM) are the principal animal models employed to evaluate potential antidepressant and anxiolytic compounds (Uwaya *et al.*, 2024). The forced swimming test and tail suspension test assess rodents' vulnerability to negative mood states and reflect an animal's sense of helplessness (Uwaya *et al.*, 2024). This study showed that the Nature's Gift® extract at 200 mg/kg and fluoxetine at 20 mg/kg significantly reduced immobility time, as shown in Fig. 1, compared to the control group. Furthermore, Nature's Gift® extract at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg, in conjunction with fluoxetine at 20 mg/kg, significantly reduced immobility time in the forced swimming test compared to the control, as illustrated in Fig. 2. The effectiveness of Nature's Gift® extract in reducing immobility duration in both the forced swimming endurance test and the tail suspension test suggests that the combination exhibits antidepressant and antistress-like properties. Fluoxetine, used as the benchmark, is classified as a Selective Serotonin Reuptake Inhibitor (SSRI), commonly prescribed for the treatment of depression. It functions by obstructing the reuptake of serotonin into presynaptic neurones via the inhibition of the reuptake transporter protein in the presynaptic terminal (Robertson *et al.*, 2019; Cao *et al.*, 2019).

The elevated plus maze (EPM) is a widely utilised technique for assessing anxiety-related behaviours in rodents (de Figueiredo Cerqueira *et al.*, 2023). This model comprises a plus-

shaped elevated maze with two open arms and two enclosed arms, intended to evaluate the intrinsic conflict between rodents' fear of open spaces and their exploratory behaviour (Arabo *et al.,* 2014). The exploratory behaviour of an animal in the EPM is generally defined by several metrics, concerning the frequency of entries and the duration spent on the two arm types (Arantes *et al.,* 2013). This study demonstrated that Nature's Gift® extract at doses of 100 mg/kg and 200 mg/kg, in conjunction with diazepam (10 mg/kg), significantly increased the duration spent in the open arm compared to the control, as illustrated in Figs. 3 and 4. Diazepam, regarded as the benchmark medication, is a rapid-acting and enduring benzodiazepine commonly prescribed for anxiety disorders, acute seizures, severe muscle spasms, and spasticity linked to neurological conditions (Calcaterra and Barrow, 2014). The Nature's Gift® extract increases the frequency of open arm entries, indicating its anxiolytic effects.

The behavioural outcomes from the Forced Swim Test (FST), Tail Suspension Test (TST), and Elevated Plus Maze (EPM) indicated that Nature's Gift significantly enhanced mood and anxiety-related behaviours in mice. The treated groups exhibited markedly diminished immobility durations in both the Forced Swim Test (FST) and Tail Suspension Test (TST), alongside heightened exploration of open arms in the Elevated Plus Maze (EPM). Depression and anxiety are among the most prevalent disorders impacting the central nervous system (CNS). They are frequently linked to disturbances in neurotransmitter function particularly serotonin, dopamine, and noradrenaline alongside oxidative stress and inflammation within the brain (Ménard *et al.,* 2017). The favourable behavioural modifications noted in the mice administered with Nature's Gift indicate that the supplement possesses both antidepressant and anxiolytic characteristics, potentially aiding in the restoration of chemical equilibrium and the mitigation of oxidative or inflammatory stress in the brain. Nature’s Gift, a natural supplement composed of

botanical ingredients including garlic (*Allium sativum*), turmeric (*Curcuma longa*), ginseng (*Panax species*), and bilberry (*Vaccinium myrtillus).*

Garlic (*Allium sativum*) is a principal element of Nature's Gift and is extensively acknowledged for its antioxidant and neuroprotective properties. Organosulfur compounds in garlic, including allicin and S-allylcysteine, have demonstrated neuroprotective effects by neutralising free radicals and modulating neurotransmitter systems (Bayan *et al.,* 2014). These actions may indirectly facilitate mood regulation and explain the noted decrease in depressive-like behaviour among the treated mice.

Turmeric (*Curcuma longa*), a significant component, comprises curcumin – a bioactive compound exhibiting potent anti-inflammatory and antioxidant properties. Curcumin has been documented to elevate serotonin and dopamine levels in the brain while also enhancing the expression of brain-derived neurotrophic factor (BDNF), which facilitates neuronal growth and plasticity (Ng *et al.,* 2017). Numerous preclinical and clinical investigations indicate that curcumin may ameliorate symptoms of depression and anxiety by augmenting neurochemical pathways (Lopresti *et al.,* 2015). The treated mice exhibit enhanced mobility and diminished anxiety, corroborating the findings of the current study.

The adaptogenic characteristics of ginseng (*Panax species*) help the body manage stress more effectively. The active constituents, known as ginsenosides, have demonstrated the ability to modulate the hypothalamic-pituitary-adrenal (HPA) axis, elevate neurotransmitter concentrations, and facilitate neurogenesis in the hippocampus (Wang *et al.,* 2017). These effects collectively enhance stress resilience and stabilise moods. The antidepressant-like response observed in the treated mice correlates with previous findings that ginseng extracts can diminish

behavioural despair in analogous experimental models. Bilberry (*Vaccinium myrtillus*), abundant in anthocyanins and other flavonoids, offers supplementary antioxidant protection that may enhance the efficacy of the other components in the supplement. Anthocyanins enhance cerebral blood circulation and safeguard neural tissue from oxidative harm, which are essential elements in alleviating anxiety and augmenting cognitive performance. The synergistic presence of these antioxidants in Nature's Gift® likely amplified its overall impact on mood and behaviour.

The findings indicate that Nature's Gift® may operate its antidepressant and anxiolytic effects via various complementary mechanisms, including the reduction of oxidative stress, regulation of inflammatory responses, modulation of neurotransmitter equilibrium, and enhancement of neuroplasticity. This multifaceted approach reflects the intricate nature of depression and anxiety, which frequently necessitate interventions that tackle both biochemical and psychological aspects (Ménard *et al.,* 2016). Despite the encouraging findings, certain limitations must be recognised. The study failed to assess biochemical markers, including serotonin levels, BDNF expression, or oxidative stress parameters, which would have offered more direct evidence of the underlying mechanisms. The study was conducted over a brief duration, and the long-term safety or efficacy of the supplement was not evaluated. Evaluating each component of the supplement independently would elucidate their distinct contributions to the observed effects. Notwithstanding these constraints, the findings present promising initial evidence that Nature's Gift® may possess genuine therapeutic potential in the management of mood-related disorders. The combination of natural compounds, each possessing established antioxidant and neuroprotective attributes, offers a comprehensive method for enhancing emotional well-being without the adverse effects linked to traditional medications..

5.2. CONCLUSION

This research demonstrates that Nature's Gift® Supplement possesses antidepressant and anxiolytic effects. It also suggests that Nature's Gift® supplement may improve emotional well-being, especially concerning mood disorders.

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