THE ANTITUSSIVE AND EXPECTORANT PROPERTIES OF D3 ORGANIC® SUPPLEMENT

BY

God's greatness Osas ODION (Miss)

LSC2007323

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this work " THE ANTITUSSIVE AND THE EXPECTORANT PROPERTIES OF D3 ORGANIC® SUPPLEMENT" was carried out by God's greatness Osas ODION (Miss), of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, for the award of Bachelor Degree (B.Sc.). in Science Laboratory Technology, of the University of Benin.

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DEDICATION

This work is dedicated to God Almighty, the giver of knowledge, understanding and good health for his never ending grace and mercies, and to my parents for unconditional love and support throughout the period of my study.

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First and foremost, I am profoundly grateful to God Almighty for His divine guidance and grace.

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To my beloved parents for unwavering love and support throughout my university education. And sacrifices and encouragement have been the bedrock of my success and I am forever grateful.

To my siblings, sister OZ, Bros God’s will, sister gift and my brother in law bros GP and To my friends my GEE Kristopher, Helen ,A friend that turned into a sister, valentina my manchi ,thelmooo my love AKA Best option Rep, Debby my babe, isioma Luciana my lover, Sarah baby, Happy, Racheal baby, blessing my lolo, Olamiposi my man, ,Sandra, success, D-14 and all of my course mates who have in one way or the other contributed to the success of this work.

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ABSTRACT

D3 Organic® is a supplement composed of various plant materials, including Desmodium gangeticum, Eclipta alba, Garcinia kola, Ocimum sanctum, Curcuma longa, and Tetracarpidium conophorum. The aim was to determine the antitussive and expectorant properties of the D3 Organic® supplement . In an antitussive study, 25 mice were allotted to 5 groups of 5 mice in each group. Group 1 is the control, group 2 is dihydrocodeine (25 mg/kg), and groups 3–5 are D3 Organic® supplement extract at 25, 50 and 100 mg/kg, respectively. One hour after oral drug administration, all the mice were exposed to 25% NH_4OH (ammonium hydroxide) to induce cough. In the expectorant, 30 mice were allotted in 6 groups of 5 mice in each group. Group 1 is the control, group 2 is bromohexane (15 mg/kg), and groups 3–5 are D3 Organic® supplement extract at 25, 50 and 100 mg/kg, respectively. Group 4 was given Sodium Chromoglycate (NaCr) at a dose of 50 mg/kg. All treatments were administered orally for five days, except Sodium Chromoglycate. In the last 30 minutes after Intraperitoneal administration of sodium chromoglycate, 5 mg/kg of ammonium chloride was given to each of the mice orally. Phenol red at a dosage of 500 mg/kg was administered intraperitoneally (IP) 30 minutes after the other drug administration. The mice were sacrificed, and the tracheal length of 2 cm was removed. Each piece of trachea was kept in 2 mL of normal saline for 30 minutes, and 0.1 mL of 1 M NaOH solution was added to the saline, and the absorbance at 460 nm was measured using a spectrophotometer.D3 Organic® supplement (25, 50 and 100 mg/kg) and dihydrocodeine (25 mg/kg) reduced the number of cough bouts when compared to the control ($p<0.05$) for ammonia-induced cough in mice.D3 Organic® supplement (25, 50 and 100 mg/kg) and bromohexane (15 mg/kg) increased phenol red secretions when compared to control ($p<0.05$). The experimental results demonstrate that the aqueous leaf extract of D3 Organic® possesses significant antitussive and expectorant properties,

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

D3 Organic® is a supplement composed of various plant materials, including Desmodium gangeticum, Eclipta alba, Garcinia kola, Ocimum sanctum, Curcuma longa, and Tetracarpidium conophorum (George, 2025). Plants have long been integral to human life, serving as sources of both food and medicine (Dafam *et al*., 2016). Medicinal plants, also known as medicinal herbs, have been used since prehistoric times in traditional medicine to treat and prevent numerous diseases (Dafam *et al*., 2016). Several plants, such as Desmodium gangeticum and Eclipta alba, are valued in herbalism for their therapeutic properties (Upadhyay *et al*., 2011; Molligoda *et al*., 2023). These plants are rich sources of bioactive compounds useful in drug development. For example, Eclipta alba is highly regarded in various ethnomedical traditions, including Ayurveda and Chinese medicine, for its liver-protective, hair-regenerative, and antimicrobial effects (Molligoda *et al*., 2023).

A cough is a natural reflex that clears the airways of mucus, irritants, or foreign particles. It is a common symptom triggered by infections (such as the common cold or flu), allergies, asthma, or exposure to irritants like smoke or dust. Coughing serves as a defence mechanism to maintain clear airways and normal breathing (Irwin *et al*., 2018). Coughs are typically classified as acute or chronic. Acute cough lasts less than three weeks and is often caused by respiratory infections, such as viral upper respiratory tract infections. Chronic cough persists for more than eight weeks and may be associated with conditions like chronic bronchitis, asthma, gastro-oesophageal reflux disease (GERD), or post-nasal drip (Irwin *et al*., 2018).

An expectorant is a medication or substance that helps loosen and thin mucus in the airways, making it easier to cough up and clear from the lungs, bronchi, and trachea. By reducing mucus thickness and stickiness, expectorants improve airway clearance and relieve symptoms of respiratory conditions like the common cold, bronchitis, or infections that produce excessive phlegm (Kardos and Kardos, 2021).

1.2. Aim of study

To determine the antitussive and expectorant properties of D3 Organic® supplement

1.3. Objective of study

- To evaluate the antitussive properties of the D3 Organic® supplement using the ammonium hydroxide-induced cough model.
- To evaluate the expectorant properties of D3 Organic® supplement using phenol dye-secretion method.

CHAPTER TWO

LITERATURE REVIEW

2.1. *DESMODIUM gangeticum*(SALPARNI)

2.1.1 DESCRIPTION OF *DESMODIUM gangeticum*

Desmodiumgangeticum commonly known as Salparni, is a perennial undershrub in the Fabaceae (legume) family. It typically attains a height of 60–130 cm, with angular, often pubescent (hairy) branches. (Mohan *et al* ., 2021). The plant bears simple, unifoliate leaves, ovate-oblong to lanceolate in shape, measuring approximately 3–14 cm long × 2–7 cm wide, glabrous on the upper surface and somewhat hairy beneath . Inflorescences are terminal or axillary racemes, with flowers ranging in color from purple to white . The fruits are moniliform (beaded) pods, typically 6–8-segmented with hooked hairs that adhere to animals, aiding in seed dispersal; the individual seeds are kidney-shaped and compressed .(Mohan *et al* ., 2021).

2.1.2. DISTRIBUTION OF *DESMODIUM gangeticum*

The Desmodiumgangeticum, a perennial erect or ascending under-shrub growing to 60–130 cm, is distributed across the warmer regions of India (Mohan *et al*., 2021). Its native range extends across tropical Africa, Sri Lanka, southern China, Southeast Asia (including Malaysia, Malesia, the Philippines, and New Guinea), as well as Australia and Taiwan (Mishra *et al.*,2023; Vasani *et al*., 2022). It is also found throughout the Asian tropics, including India, the Gangetic plains, lower Himalayan foothills, Thailand, Myanmar, Indochina, China, and Taiwan, with additional populations in tropical Africa and Australia (Singh *et al*., 2019).

2.1.3. ETHNOMEDICINAL USES OF *DESMODIUM gangeticum*

The Desmodiumgangeticum(Fabaceae), known in Ayurveda as Shalparni, is traditionally employed as a tonic, febrifuge, digestive aid, anticatarrhal, antiemetic, and for treating inflammatory chest conditions (Rastogi *et al*., 2011). Pharmacological profiling has validated a wide range of bioactivities such as anti-inflammatory, antioxidant, cardioprotective, antidiabetic, antiulcer, antiamnesic, antiviral, antileishmanial, immunomodulatory, and hepatoprotective properties (Rastogi *et al*., 2011). Additionally, contemporary scoping reviews report antinociceptive, antibacterial, anti-inflammatory, cardioprotective, and antiamnesic effects, and note preliminary clinical uses for hypertension, bronchitis, and gout, albeit with limited trial robustness (Vasani*et al*., 2022).

Plate 1: *desmodiumgangeticum leaves.* **Source:** (Rastogi *et al*., 2011).

2.2.*ECLIPTAalba*(FALSE DAISY)

2.2.1. DESCRIPTION OF *ECLIPTA alba*

Ecliptaalba also know as (Ecliptaprostrata), commonly called false daisy, is an annual or perennial herb of the family Asteraceae. Characterized by its prostrate or ascending growth habit, the plant is much-branched with opposite lanceolate leaves and small white flower heads. The stems are slender, often purplish, and root at the nodes in moist soils. The leaves are sessile, rough-textured, and covered with fine hairs (Timalsina and Devkota, 2021).The plant's fruit is a blackish achene lacking a prominent pappus, a key diagnostic trait distinguishing it from related taxa (Vimala and Hans, 2023). Ecliptaalba grows abundantly in moist, disturbed habitats such as rice fields, irrigation channels, riverbanks, and swampy lowlands, making it a common weed in tropical and subtropical regions. With strong adaptability, it is widely distributed across Asia, Africa, the Americas, and Australasia (Timalsina and Devkota, 2021).Leaf Morphology of Eclipta alba leaves are sessile and narrowly lanceolate with a scabrid surface due to stiff hairs. When crushed, they emit a slightly bitter odor, indicative of coumestans and essential oils (Jahan *et al*., 2014).Floral Biology of Theplant's are described as small white flower heads that attract various pollinators, including bees and small flies. However, it is largely autogamous, relying on self-pollination. This reproductive strategy enhances its ability to spread successfully in disturbed habitats (Timalsina and Devkota, 2021).

2.2.2. DISTRIBUTION OF *ECLIPTA ALBA*

Eclipta alba is a cosmopolitan herb found in tropical and subtropical regions worldwide. Native to Asia, Africa, and South America, it has naturalized in North America, Europe, and Australasia

(Li *et al*., 2019). The species thrives in moist areas, including Abundant in South Asia such as, India, Nepal, Bangladesh, Sri Lanka, and Myanmar, often in rice fields, riverbanks, and wetlands (Timalsina and Devkota, 2021).It also Widely seen distributed in southern and central provinces, used in folk medicine and traditional Chinese formulations (Li *et al*., 2019). it recorded in Tropical Africa Found in countries like Nigeria, Uganda, and Kenya, where it grows as a ruderal weed and medicinal herb (Jahan *et al*., 2014). Americas Recorded in Central and South America, the United States, and the Caribbean islands, largely spread through agricultural and horticultural activities (Timalsina and Devkota,2021).Adaptability and Colonization of Eclipta alba's is that it's wide distribution is attributed to its ability to thrive in disturbed, moist soils and reproduce through seeds and vegetative rooting at nodes, making it an aggressive colonizer in wetland ecosystems (Vimala and Hans, 2023).

2.2.3. ETHNOMEDICINAL USES OF *ECLIPTA alb*

Eclipta alba also commonly known as Bhringraj or False Daisy, has been widely used in traditional medicine systems like Ayurveda, Siddha, and folk healing across Asia, Africa, and South America. The plant is traditionally used for liver ailments, skin infections, promoting hair growth, wound healing, and respiratory disorders.Ethnobotanical surveys have documented its use in treating fever, asthma, gastrointestinal issues, and splenomegaly, as well as topical applications for cuts and wounds (Prajapati *et al*., 2010). In Ayurveda, E. alba is particularly renowned for its hepatoprotective properties, used to treat jaundice, liver cirrhosis, and hepatitis (Saxena *et al*., 2010). This is supported by phytochemicals like wedelolactone, luteolin, and apigenin, which exhibit hepatoprotective and antiviral effects (Wu *et al*., 2012).The plant is also valued for its antidiabetic properties, with leaf extracts shown to reduce blood glucose and HbA1c levels in animal models, validating its traditional use in diabetes management (Singh *et al*., 2014). Additionally, studies highlight its neuroprotective potential, where saponins and flavonoids improve cognition and reduce seizure activity, aligning with its use as a nerve tonic (Guenné *et al*., 2019).Ayurvedic literature further emphasizes its role in treating conditions like anemia, cough, worm infestations and hair-related disorders, while also noting its rejuvenative and anti-aging properties (Yadav and Huddar, 2024). Ethnomedicinal documentation from tropical regions also highlights its external use for wound healing, burns, and hemorrhage control. (Sharma *et al*., 2020).

Plate 2:*Eclipta alba* **Source:** (Sharma *et al*., 2020).

2.3. *GARCINIA kola* (BITTER KOLA)

2.3.1. Description of *Garcinia kola*

Garcinia kola Heckel, commonly known as bitter kola, is a medium-sized evergreen tree native to the moist lowland forests of West and Central Africa, where it grows wild and is cultivated on a small scale (Agyili*etal*., 2006). The tree can reach 12–15 m in height, although it can grow up to 25–30 m in undisturbed forests. Its straight cylindrical bole has a fluted trunk and smooth dark brown bark that exudes a sticky yellowish latex when cut (Tauchen*etal*., 2023).The leaves are simple, opposite, and elliptic to obovate, with a leathery texture and glossy green color, measuring up to 20 × 6 cm. The small, greenish-white flowers are borne in terminal clusters, and the plant is predominantly dioecious, producing separate male and female individuals (Manourová*etal*., 2019).The fruits are fleshy, globose berries, 5–7 cm in diameter, turning yellow to orange-red when ripe, and containing 2–4 large brown seeds covered by an orange pulp. The seeds are flattened and bitter, and are the most widely used part of the plant (Agyili*etal*., 2006; Tauchen*etal*., 2023).Garcinia kola thrives in humid, well-drained soils and is often found in secondary forests, forest edges, and home gardens. Beyond its medicinal importance, it contributes to agroforestry systems, providing shade and serving as a culturally significant tree in many West African societies (Agyili*etal*., 2006).

2.3.2. DISTRIBUTION OF *GARCINIA kola*

Garcinia kola is distributed mainly across the lowland rainforests of West and Central Africa, where it grows either in the wild or in cultivated agroforestry systems. The species is found in countries such as Nigeria, Ghana, Côte d'Ivoire, Cameroon, Gabon, and the Democratic Republic of Congo, thriving in humid tropical zones with fertile soils and high rainfall (Iwu, 2014).In Nigeria, it is widespread in the southern forest regions, with about 70% of the fruits still collected from wild populations, although domestication in farmlands is becoming more common (Anegbeh*etal*., 2006). In Cameroon and Côte d'Ivoire, the tree is often retained during forest clearing and integrated into cocoa- and oil palm-based agroforestry systems, highlighting its ecological and economic importance (Komenan*etal*., 2019).However, in areas like Benin, Garcinia kola has become locally extinct in the wild due to overexploitation and habitat loss, and now persists only in cultivated or semi-cultivated environments, This distribution pattern underscores the species' ecological adaptability and the threats it faces, emphasizing the need for conservation efforts to ensure its continued availability. (Dadjo*etal*., 2020).

2.3.3. ETHNOMEDICINAL USES OF *GARCINIA kola* (bitter kola)

Garcinia kola remains a significant medicinal plant in West Africa, with recent studies validating its traditional uses The seeds are chewed to alleviate cough, bronchitis, and throat infections, which aligns with research showing bronchodilatory and anti-inflammatory effects (Manourová *et al*., 2019). Traditionally used to treat fevers and infectious diseases, the plant's antibacterial and antifungal actions are documented in pharmacological reviews (Iwu, 2014; Tauchen *et al*., 2023).Bitter kola is also used as a liver tonic in folk medicine, with reviews highlighting its hepatoprotective effects against oxidative stress (Tauchen *et al*., 2023). Additionally, The seeds and bark are used to treat diarrhea and stomach pain, reflecting long-standing ethnomedicinal practices (Iwu, 2014). It is also traditionally consumed to manage hypertension and diabetes, with recent reviews highlighting these potential benefits (Manourová *et al*., 2019).It is also traditionally consumed as a stamina booster and to manage various ailments, with pharmacological reviews highlighting its potential benefits against hypertension and diabetes (Manourová *et al*., 2019).Furthermore, bitter kola is used for fever and pain relief, supported by modern studies on the antioxidant activity of kolaviron, a biflavonoid complex found in the seeds (Tauchen *et al*., 2023).

Plate 3: *Garcinia kola* **Source:**(Manourová *et al*., 2019).

2.4. *OCIMUM sancta*(HOLY BASIL OR TULSI)

2.4.1 DESCRIPTION OF *OCIMUM sancta*

Ocimum sanctum Commonly referred to as Ocimum tenuiflorum or "Tulsi," is a highly valued aromatic shrub belonging to the Lamiaceae family. Native to India and South Asia, this perennial herb grows up to 30-90 cm tall, featuring pubescent stems and ovate leaves that are 2-5 cm long (Jamshidi and Cohen, 2017). The leaves come in two main varieties: green (Rama Tulsi) and purple (Krishna Tulsi), both emitting a strong clove-like fragrance due to their high essential oil content (Kumar *etal*., 2018). The plant produces purple tubular flowers in racemes, followed by small brown nutlets (Kumar *etal*., 2018; Upadhyay*etal*., 2021). Three distinct morphotypes are recognized: Rama Tulsi, Krishna/ShyamTulsi, and VanaTulsi, each with slightly different phytochemical compositions and aromas (Jamshidi and Cohen, 2017; Sharma *etal*., 2022). Phytochemical analysis reveals that O. sanctum is rich in essential oils like eugenol and linalool, flavonoids such as orientin and vicenin-2, phenolic acids like rosmarinic acid, and triterpenoids including ursolic and oleanolic acids (Gupta *etal*., 2020; Sharma *etal*., 2022). These compounds contribute to the plant's broad range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, cardioprotective, and adaptogenic properties (Jamshidi and Cohen, 2017; Upadhyay*etal*., 2021). In Ayurveda, Tulsi is revered as the "queen of herbs" and has been used to promote longevity, reduce stress, and treat various disease.

2.4.2. DISTRIBUTION OF OCIMUM sanctum

Ocimum sanctum (Tulsi) is a plant profoundly revered in India, where it is considered sacred and deeply woven into cultural and religious traditions (Mondal *et al.*, 2019). Its status as a cornerstone of Ayurvedic medicine underscores its immense therapeutic importance (Bhasin, 2022). The plant is classified as a potent adaptogen, meaning it helps the body resist stressors, which is a key reason for its widespread cultivation and global use (Chandorkar *et al*., 2022).The significant pharmacological effects of Tulsi are driven by its complex phytochemistry, which includes a unique profile of bioactive compounds such as flavonoids, polyphenols, and volatile oils like eugenol (Jamshidi and Cohen, 2017). This robust scientific evidence supporting its health benefits has solidified its role as a premier medicinal herb and catalyzed its commercial cultivation and distribution far beyond its native range to meet international demand (Cohen, 2022).

2.4.3. ETHNOMEDICINAL USES OF OCIMUM sancta

Ocimum sancta (Holy Basil or Tulsi) is a plant profoundly revered in India, where it is deeply woven into cultural and religious traditions (Mondal *et al*., 2019) and is considered a foundational herb within Ayurvedic medicine (Bhasin, 2022). Its ethnomedicinal uses are extensive, as it is traditionally employed as a holistic adaptogen to help the body cope with stress and to support immune function (Chandorkar *et al*., 2022; Cohen, 2022). According to Jamshidi and Cohen (2017), Tulsi is used for treating respiratory conditions like asthma, colds, and coughs, while Cohen (2022) also notes its use in managing metabolic health by regulating blood glucose and cholesterol levels. Furthermore, its applications include enhancing memory and cognitive function and alleviating anxiety (Chandorkar *et al*., 2022), as well as addressing fevers, skin infections, and wounds due to its antimicrobial and anti-inflammatory properties (Jamshidi and Cohen, 2017). The significant pharmacological effects that underpin these traditional uses are driven by its complex phytochemistry, which includes a unique profile of bioactive compounds (Jamshidi and Cohen, 2017). This robust scientific and traditional evidence has solidified its role as a premier medicinal herb and has catalyzed its commercial cultivation and distribution far beyond its native range to meet international demand (Cohen, 2022).

Plate 4: ***Ocimum sancta*** **Source: (Cohen, 2022).**

2.5. *CURCUMA longa*

2.5.1. DESCRIPTION*CURCUMA longa*

Curcuma longa L., commonly known as turmeric, is a perennial rhizomatous herb belonging to the family Zingiberaceae. It is native to South and Southeast Asia, particularly India, which remains the largest producer and exporter of turmeric worldwide (Prasad and Aggarwal, 2011). The yellow-orange rhizome of turmeric has been widely used for centuries as a culinary spice, dye, and medicinal plant in traditional medical systems such as Ayurveda, Siddha, and Traditional Chinese Medicine (Gupta *et al*., 2013). Its pharmacological activities are primarily attributed to curcuminoids, especially curcumin, which possess strong antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and anticancer properties (HewlingsandKalman, 2017). In addition to curcuminoids, turmeric contains essential oils and volatile compounds such as turmerone and zingiberene, which contribute to its therapeutic potential (Ammon and Wahl, 1991).

2.5.2. DISTRIBUTION OF *CURCUMA longa*

Curcuma longa is believed to have originated in South and Southeast Asia, particularly in the Indian subcontinent, where it has been cultivated for thousands of years (Nayak *et al*., 2016). India remains the largest producer and exporter of turmeric, accounting for over 75% of global production, with major cultivation in states such as Andhra Pradesh, Tamil Nadu, Odisha, West Bengal, and Maharashtra (Singh *et al*., 2021). Beyond India, the plant is widely distributed and cultivated across Sri Lanka, Bangladesh, Nepal, and Pakistan, where it holds both culinary and medicinal importance (Ravindranand Babu, 2016). In Southeast Asia, turmeric is extensively grown in Thailand, Indonesia, Vietnam, Myanmar, and Malaysia, where it is used in traditional medicine and as a spice (Nguyen *etal*., 2019). In China, cultivation occurs mainly in southern provinces such as Guangxi and Yunnan, where climatic conditions support large-scale production (Zhou *etal*., 2020). Similarly, in Africa, C. longa has adapted well to tropical climates and is cultivated in Nigeria, Ethiopia, and Tanzania, with increasing demand in both local and export market (Adegbite et al., 2017). Outside Asia and Africa, turmeric has also spread to the Caribbean, Central America, and South America. Brazil, in particular, has become a significant producer for the pharmaceutical and cosmetic industries (Silva *et al*., 2022). In Australia and the Pacific Islands, the plant has naturalized in warm, humid regions, with small-scale cultivation mainly for local herbal use (Williams, 2018). The wide distribution of Curcuma longa reflects its ecological adaptability, as it thrives in tropical and subtropical climates, requiring warm temperatures and high humidity, which has facilitated its expansion beyond its native range (Mishra and Tripathi, 2019).

5.3. ETHNOMEDICINAL USES OF *CURCUMA longa*

Turmeric (Curcuma longa)has been a vital component of traditional healing systems like Ayurveda, Siddha, Unani, and Traditional Chinese Medicine, valued for its anti-inflammatory, antimicrobial, hepatoprotective, and wound-healing properties (Chattopadhyay *et al*., 2016). The rhizomes are used in various forms, including powder, paste, decoction, and oil, showcasing its versatility in ethnomedicine. In Indian folk medicine, turmeric paste and decoctions are used to treat digestive issues, liver disorders, and parasitic infections (Srivastava and Mehta, 2017). It's also applied topically for cuts, burns, and skin diseases due to its wound-healing and antimicrobial properties (Mekonnen *et al*., 2019). Additionally, turmeric is consumed to manage respiratory issues, such as cough and asthma, and is believed to boost immunity (Ahmed *et al* ., 2020). In women's health, turmeric is used in post-partum care and as a uterotonic agent (Putri *et al*., 2021). It's also valued for its cosmetic benefits, including skin-lightening and anti-aging properties (Bhatt *et al*., 2018). Turmeric's significance extends beyond medicine, playing a role in cultural practice and rituals.

Plate 5:*Curcuma longa* **Source:** (Nguyen *etal*., 2019).

2.6. *TETRACARPIDIUM Cornophorum* (AFRICAN WALNUT)

2.6.1. DESCRIPTION OF *TETRACARPIDIUM Cornophorum*

Tetracarpidiumconophorum, commonly known as African walnut or Nigerian walnut, is a woody perennial climber that can grow 6 to 18 meters long, with stems reaching up to 16 cm in girth (Nwachoko and Jack, 2015). The young stems are green and hairless, turning dark grey as they mature, while the leaves are simple, ovate, and serrated, typically measuring 10 cm long and 5 cm wide, with a pointed tip (Ekwe and Ihemeje, 2013; Nwachoko and Jack, 2015).Tetracarpidiumconophorum is a monoecious climber, bearing both male and female flowers on the same plant (Janick and Paul, 2008). The male flowers are arranged in narrow panicles, while the female flowers appear near the base of the inflorescence. The flowers have distinctive features, including a stout, square-shaped style with four spreading stigmas in female flowers and numerous stamens (≈ 40) in male flowers (Janick and Paul, 2008).The fruits of Tetracarpidiumconophorum are cylindrical to ovoid capsules, measuring 6-10 cm long and 3-11 cm wide, containing round seeds about 2.5 cm in diameter (Janick and Paul, 2008). The fruit's skin transitions from green when immature to dark brown or black upon maturity . The seeds are surrounded by a thick, hard testa and have oil-rich kernels (Janickand Paul, 2008; Oyekale *et al*., 2015).Tetracarpidiumconophorum is native to West and Central Africa, particularly in humid rainforest zones (Nwachoko and Jack, 2015). It thrives in moist, fertile, well drained loam or clay loam soil, commonly found in garden edges, bottomlands, abandoned fields, and woodlands (Oyekale *et al*., 2015). The plant is widely distributed in countries such as Nigeria, Cameroon, Central African Republic, Congo, Gabon, and the Democratic Republic of Congo (Nwachoko and Jack, 2015)

2.6.2. DISTRIBUTION OF *TETRACARPIDIUMcornophorum*

Tetracarpidiumconophorum is a native climbing perennial found in the humid lowland forests of West and Central Africa (Ayoola *et al*., 2016). It is naturally distributed across countries such as Nigeria, Ghana, Cameroon, Gabon, Côte d'Ivoire, Democratic Republic of Congo, and Central African Republic, where it grows in the wild and under semi-domesticated conditions. In Nigeria, it is commonly found in the southern rainforest belt, particularly in states like Ondo, Ekiti, Ogun, Enugu, and Abia, where it thrives along farm boundaries, riverbanks, and fallow lands (Ogbuagu and Okeke, 2017). The plant is often intercropped with cocoa, kola nut, and oil palm, utilizing its twining nature to climb on taller support trees (Okonkwo *et al*., 2021). Beyond Nigeria, Tetracarpidiumconophorum is cultivated in countries like Cameroon and Gabon, valued for its food and medicinal properties. It is also found in secondary forests and village home gardens in Ghana and Côte d'Ivoire, indicating gradual domestication (Okeke and Nnamani, 2019). The species prefers humid tropical climates with well-drained loamy or sandy-clay soils and annual rainfall exceeding 1500 mm (Udeh *et al*., 2018). Experimental cultivation has also been reported in Caribbean regions due to interest in its nutraceutical and oil-rich seeds (Mensah *et al*., 2022).

2.6.3. ETHNOMEDICINAL USES OF *TETRACARPIDIUM cornophorum*

Tetracarpidiumconophorum has a rich history of use in African ethnomedicine, with various parts of the plant employed to treat a range of ailments. The plant's seeds, leaves, stem bark, and roots are utilized due to their phytochemical profile, which includes alkaloids, saponins, tannins, flavonoids, and essential oils (Onwukeme *et al*., 2017).In traditional medicine, the seeds are consumed as an aphrodisiac to enhance male fertility, libido, and sperm quality (Oghenejoboh, 2016). Additionally, decoctions of the stem or leaves are used to regulate female fertility and menstrual health. The plant's antimicrobial properties are leveraged to manage skin infections, diarrhea, and intestinal worms, with leaf and bark extracts being applied for these purposes (Olapade and Akinrinlola, 2018).The seeds are also used to address neurological issues, such as insomnia, memory loss, and mental fatigue, due to their calming and neuroprotective effects (Odoemelam *et al*., 2019). Furthermore, the plant is used to manage cardiovascular and metabolic disorders, including hypertension, high cholesterol, and diabetes mellitus, with the boiled seeds being taken to lower blood pressure and cholesterol levels (Ezealisiji *et al*., 2020).In general, Tetracarpidiumconophorum is prepared as a tonic for convalescents and used to reduce fever, fatigue, and rheumatism in I traditional healthcare practices (Chikezie *et al*., 2021). The plant's diverse uses reflect its significance in African ethnomedicine.

Plate 6:*Tetracarpidiumconophorum.* **Source:**(Ezealisiji *et al*., 2020).

2.7. OVERVIEW OF COUGH

Coughing is a vital defensive reflex that protects the respiratory system by expelling irritants, secretions, and foreign materials from the airways (Morice, 2013). The cough mechanism involves three phases: inspiration, compression with glottal closure, and explosive expiration (Song *et al*., 2021). This process is mediated by airway sensory receptors, including rapidly adapting receptors, C-fibers, and stretch receptors, which transmit signals to the brainstem cough center via the vagus nerve (Canning and Mori, 2011). Cough can be both reflexive and voluntary, indicating higher cortical involvement (Vertiganand Gibson, 2011).Coughs are classified based on duration, acute (<3 weeks), subacute (3-8 weeks), and chronic (>8 weeks) (Irwin *et al*., 2018). In adults, the most common causes of chronic cough are upper airway cough syndrome (UACS), asthma, and gastroesophageal reflux disease (GERD), forming a "pathogenic triad" (Morice et al., 2020). Other contributors include non-asthmatic eosinophilic bronchitis, ACE inhibitor therapy, and post-infectious cough (Kardos *et al*., 2020).Chronic cough is often characterized as a cough hypersensitivity syndrome (CHS), where patients experience heightened neuronal excitability, leading to coughing triggered by minimal stimuli (Song *et al*., 2021). This condition significantly impacts quality of life, causing complications such as chest pain, urinary incontinence, insomnia, and rib fractures (French *et al*., 2017).

2.7.1. CLASSIFICATION OF COUGH

2.7.1.1. ACUTE COUGH

Acute cough is defined as a cough lasting less than three weeks and is the most common type seen in primary care, often associated with self-limiting respiratory tract infections (Mello *et al*., 2020). The causes of acute cough can be categorized into infectious and non-infectious factors. Infectious causes include viral upper respiratory tract infections like the common cold, influenza, and coronavirus (Fashnerand Ericson, 2018), as well as acute bronchitis and community-acquired pneumonia (Albert, 2010; Mandell *et al*., 2019). Non-infectious causes include allergic rhinitis, irritant exposure such as pollutants or smoke (French *et al*., 2020), asthma exacerbation (Kardos *et al*., 2020), and the rare occurrence of pulmonary embolism, which can present with cough, chest pain, and dyspnea (Konstantinides *et al*., 2020).

2.7.1.2. SUB ACUTE COUGH

A sub-acute cough is characterized by its duration of 3 to 8 weeks, typically following an acute respiratory infection or another triggering event. It serves as an intermediate stage between acute cough, which lasts less than 3 weeks, and chronic cough, which persists for more than 8 weeks. The most common cause of sub-acute cough is post-infectious airway inflammation resulting from viral or bacterial respiratory tract infections. However, it can also be associated with conditions such as asthma, upper airway cough syndrome, and gastroesophageal reflux disease (Irwin and Madison, 2013; Morice *et al*., 2020). Sub-acute cough holds clinical significance as it may represent either a resolving infection or a marker for an underlying pathology that could progress to chronic cough if not properly evaluated. Effective management involves identifying the underlying cause, providing symptomatic relief, and monitoring for persistence beyond 8 weeks, at which point further diagnostic evaluation is warranted (Song *et al*., 2021).

2.7.1.3. CHRONIC COUGH

Chronic cough is often linked to asthma, chronic obstructive pulmonary disease (COPD), and bronchiectasis. In asthma, coughing is driven by airway hyperreactivity and eosinophilic inflammation (Irwin and Madison, 2013). COPD is characterized by mucus hypersecretion, impaired clearance, and airflow limitation, leading to chronic cough (Vestbo *et al*., 2013). Bronchiectasis causes coughing due to structural airway damage, impaired mucus clearance, and recurrent infections, resulting in persistent inflammation (Chalmers *et al.*, 2015).

2.7.2. CAUSES OF COUGH

Cough may arise from a wide range of underlying conditions. Infectious causes are among the most common, with viral pathogens such as rhinovirus, influenza virus, and respiratory syncytial virus often producing acute cough through transient airway inflammation, while bacterial infections including Bordetella pertussis, Mycoplasma pneumoniae, and Streptococcus pneumoniae may lead to more persistent or paroxysmal forms (Song *et al.,* 2021). Chronic respiratory disorders such as asthma, chronic obstructive pulmonary disease (COPD), and bronchiectasis are also major contributors; in asthma, cough is driven by airway hyperreactivity and inflammation, whereas in COPD it results from mucus hypersecretion and airflow obstruction, and in bronchiectasis it reflects chronic infection with associated structural airway damage (Morice *et al*., 2020). Beyond the lower airways, upper airway and gastrointestinal disorders also play important roles. Postnasal drip syndrome, also termed upper airway cough syndrome, causes cough through mucus drainage into the oropharynx, while gastroesophageal reflux disease (GERD) induces cough either by microaspiration of gastric contents or by vagally mediated reflex mechanisms (Chung *et al*., 2019). Finally, cardiovascular and medication-related causes should be considered; left ventricular heart failure can provoke cough through pulmonary congestion, and angiotensin-converting enzyme (ACE) inhibitors are a well-known pharmacological trigger of chronic dry cough, which typically resolves upon withdrawal of the drug (Dicpinigaitis, 2006).

2.7.3. TREATMENT OF COUGH

Cough treatment depends on its duration (acute, subacute, or chronic) and underlying causes

- Symptomatic relief can be achieved with antitussives (e.g., dextromethorphan) for non-productive coughs or expectorants (e.g., guaifenesin) for productive coughs to promote mucus clearance (Irwin and Madison, 2013).
- Cause-specific therapies include antibiotics for bacterial infections (Malesker et al., 2017), inhaled corticosteroids and bronchodilators for asthma (Kardos*et al*., 2020), mucolytics and bronchodilators for COPD (Wedzicha *et al*., 2017), and proton pump inhibitors for GERD-induced cough (Kahrilas *et al*., 2016).
- Non-pharmacological approaches like hydration, humidified air, and avoiding irritants can provide relief. For refractory cases, neuromodulators (e.g., gabapentin) or speech therapy may be effective (Vertigan *et al*., 2016).

2.7.4. TRADITIONAL/HERBAL TREATMENT OF COUGH

Herbal and traditional medicine have historically been central to the management of cough, especially in communities with limited access to modern pharmaceuticals or where cultural traditions favor natural therapies.(Mukherjee et al., 2017). Such remedies are appreciated for their diverse pharmacological properties, including expectorant, demulcent, anti-inflammatory, antimicrobial, and immunomodulatory effects, which aid in airway clearance and help to alleviate irritation of the respiratory tract (Mukherjee *et al*., 2017). Several medicinal plants have been traditionally used to manage cough, with scientific evidence supporting their potential benefits. These include:

2.8. OVERVIEW Of EXPECTORANT

Expectorants are agents that help remove mucus and from the respiratory tract by reducing their thickness and stickiness, making them easier to cough up, through mechanisms such as hydrating airway mucus, increasing fluid secretion, and stimulating reflexes that improve ciliary activity and mucus msecretionsovement (Dicpinigaitis *et al*., 2014; Kantar and Maffei, 2016).Expectorants are especially useful in conditions like acute bronchitis, chronic bronchitis, asthma, and chronic obstructive pulmonary disease (COPD) where mucus overproduction and impaired clearance contribute to persistent cough. By improving airway clearance, expectorants not only reduce cough frequency but also support better breathing comfort.(Kantar and Maffei, 2016).

2.8.1. TYPE OF EXPECTORANT

Expectorants are categorized into synthetic and herbal types.

2.8.1.1. SYNTHETIC EXCEPTORANT

- Guaifenesin, which reduces mucus viscosity and facilitates clearance (Dicpinigaitis, 2011).
- Potassium iodide, a mucolytic and secretagogue that increases bronchial secretions (Irwin and Madison, 2013).

2.8.1.2. HERBAL EXPECTORANTS

- Justiciaadhatoda (Vasaka), which has bronchodilatory and expectorant effects (Dhuley, 1999).
- Menthol and Eucalyptus oil, which stimulates cold receptors and enhance mucus clearance (Morris 2019)

2.8.2. CAUSES OF EXPECTORANT

Expectorants are mainly prescribed in situations where excessive airway secretions, abnormal mucus production, or impaired clearance contribute to cough and respiratory discomfort. Their use is particularly warranted when a productive cough arises from underlying respiratory diseases (Dicpinigaitis *et al*., 2014). One of the most common indications is respiratory tract infections, including acute and chronic bronchitis, pneumonia, and upper respiratory tract infections, which often cause mucus hypersecretion and airway irritation. In such cases, expectorants function by reducing mucus viscosity, enhancing clearance, and ultimately improving ventilation (Morice *et al*., 2020). They are also beneficial in chronic obstructive pulmonary disease (COPD), where thick mucus leads to airway obstruction, persistent cough, and recurrent infections. Agents such as guaifenesin, as well as herbal preparations like Hedera helix and Thymus vulgaris, have been shown to improve sputum clearance and decrease cough severity (Kardos *et al*., 2019).

2.8.3. TREATMENT WITH EXPECTORANT

Expectorants are medications that promote the expulsion of mucus from the airways, making coughs more productive and easing breathing, They are commonly prescribed for conditions marked by excessive or thick mucus, such as acute respiratory infections, chronic bronchitis, asthma, and chronic obstructive pulmonary disease (COPD).(Irwin and Madison, 2013).

2.8.3.1. PHARMACOLOGICALEXPECTORANTS:

Guaifenesin is the most widely used synthetic expectorant, increasing hydration of respiratory secretions and reducing mucus viscosity to improve mucociliary clearance, often used for symptomatic relief in acute bronchitis and upper respiratory infections (Dicpinigaitis *et al*., 2014). Potassium iodide and iodinated glycerol are less commonly used but effective in chronic bronchitis and asthma patients with mucus hypersecretion (Irwin and Madison, 2013).

2.8.3.2. HERBALEXPECTORANTS:

Hedera helix (Ivy leaf extract) exhibits mucolytic, bronchodilatory, and expectorant activity, frequently included in herbal cough syrups (Kardos *et al*., 2019). Thymus vulgaris (Thyme) provides antimicrobial and expectorant effects, improving mucus clearance and reducing cough frequency (Cwientzek *et al*., 2011). Glycyrrhizaglabra (Licorice root) is used traditionally as a soothing demulcent and expectorant in both acute and chronic cough (Wang *et al*., 2015)

2.9. INTERACTION BETWEEN COUGH AND EXPECTORANT

Expectorants loosen/thin mucus and/or increase airway secretions and mucociliary clearance, which usually makes cough more productive and, over time, can reduce cough frequency and symptoms by improving mucus removal — but they can transiently increase coughing early on and should be used with care when cough effectiveness or aspiration risk is impaired. (Ohar*et al*., 2019; Patzwaldt and Castaneda-Vega, 2024).

CHAPTER THREE

MATERIALS AND METHODS

3.1. APPARATUS AND EQUIPMENT:

Analytical weighing balance (Ohaus Corp., Pine Brook, NJ, USA), dehydrator (model: SF-4006, China), rat cages, dissecting set, beakers (50 ml and 250 ml), spectrophotometer (752+UV/VIS,Wincom,China), Water bath (HH-S6,China), measuring cylinders (100 ml and 500 ml), hand gloves, cotton wool, masking tape, 1-litre (10x10x10 cm) glass chamber, methylated spirit. The kit also includes syringes and needles (1 ml, 2 ml, and 5 ml), a stopwatch/timer, and an orogastric tube.

3.2. Chemicals and Reagents/Drugs Used

The chemicals used include ammonium hydroxide, ammonium chloride, phenol red, dihydrocodeine (DHC), bromhexine, and sodium chromoglycate.

3.3. Procurement of D3 Supplement

The D3 Organic® supplement comprises Desmodium gangeticum (50 mg), Eclipta alba (100 mg), Garcinia kola (50 mg), Ocimum sanctum (100 mg), Curcuma longa (100 mg), and Tetracarpidium cornophorum (100 mg). The supplement, batch number 0001, NAFDAC NO. A7-4335L, with a production date of 02/10/2024 and a best-before date of 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 of D3

The D3 Organic® supplementwas extracted using maceration. Thirty (30g) grams was weighed, dissolved in 500 ml of distilled water, and soaked for approximately three days (72 hours). The mixture was then filtered and concentrated over a water bath. The extract was stored in a refrigerator at 4°C prior to use.

3.5. Experimental Animals

Twenty-five 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 fed 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 a reference number: UNIBEN/FSLT/00014

3.6. Experimental Design

3.6.1. Ammonium-Induced Cough in Mice

The acute cough was studied using an ammonium-induced cough model, as described by Uwaya and Effiong, (2024), with slight modifications.

Twenty-five healthy mice of both sexes weighing 20–35 grams were divided into five groups of five mice each:

- Group 1: received 10 ml/kg of distilled water (control)
- Group 2: received 25 mg/kg of dihydrocodeine (DHC)
- Group 3: received 25 mg/kg of D3
- Group 4: received 50 mg/kg of D3

- Group 5: received 100 mg/kg of D3

The control, extract at all doses, and the standard drug dihydrocodeine were administered orally according to the weight of each mouse (20 to 35 g). One hour after administration, mice were placed in a 1-litre (10x10x10 cm) glass chamber and exposed to 0.3 ml of 25% NH_4OH for 45 seconds. Cough bouts were counted during a 5-minute exposure using a stopwatch, and the percentage of inhibition was calculated.

3.7. Expectorant Studies

Phenol Dye Secretion Method

The expectorant effect was studied using the phenol dye-secretion method as described by Uwaya and Effiong, (2024), with slight modifications.

Thirty healthy mice of both sexes weighing 20 to 35 grams were divided into six groups of five mice each:

- Group 1: received 10 ml/kg of distilled water (control)
- Group 2: received 15 mg/kg of bromhexine
- Group 3: received 25 mg/kg of D3
- Group 4: received 50 mg/kg of D3
- Group 5: received 100 mg/kg of D3
- Group 6: received 50 mg/kg of sodium chromoglycate

The control, extract, and bromhexine were administered orally for five days, except for sodium chromoglycate, which was given only on the last day. Thirty minutes before phenol red administration, sodium chromoglycate (50 mg/kg) was administered intraperitoneally. Ammonium chloride (5 mg/kg) was administered one hour after extract administration and 30 minutes after sodium chromoglycate administration. Thirty minutes later, each mouse was injected intraperitoneally with phenol red. Thirty minutes after phenol red administration, animals were sacrificed via cervical dislocation. A 2 cm length of trachea (from the thyroid cartilage to the main stem bronchi) was removed and incubated for 30 minutes in 2 ml normal saline. Then, 0.1 ml of 1M NaOH solution was added to the saline. The absorbance of phenol red released from the trachea was measured at spectrophotometer (752+UV/VIS,Wincom,China). A calibration curve (absorbance versus concentration, R^2=0.947) was plotted to extrapolate phenol red concentration.

3.8. Statistical Analysis

Results were expressed as the mean ± standard error of the mean (SEM), with n representing the number of animals per group. Data were analysed using one-way analysis of variance (ANOVA), and differences between groups were determined by the Newman-Keuls' post hoc test. All analyses were performed using GraphPad Prism (UK) software version 9. A p-value of <0.05 was considered statistically significant.

CHAPTER FOUR

RESULT

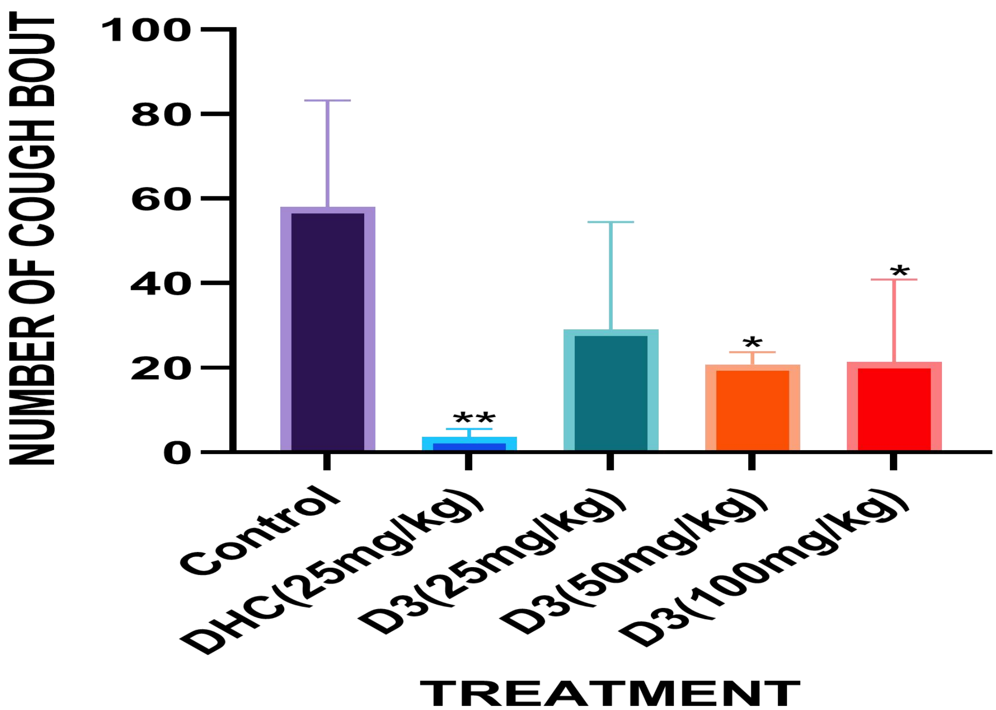

Figure 1a: The effect of administration of aqueous leaf extract of D3 on ammonia-induced cough in mice.

D3 Organic® (50, 100 mg/kg) and DHC (25 mg/kg) reduced the number of cough bouts ($P < 0.05$, $P < 0.001$) in mice with ammonia-induced cough compared to the control (DHC: Dihydrocodeine, D3). Values are represented as Mean ± SEM, n=5 per group.

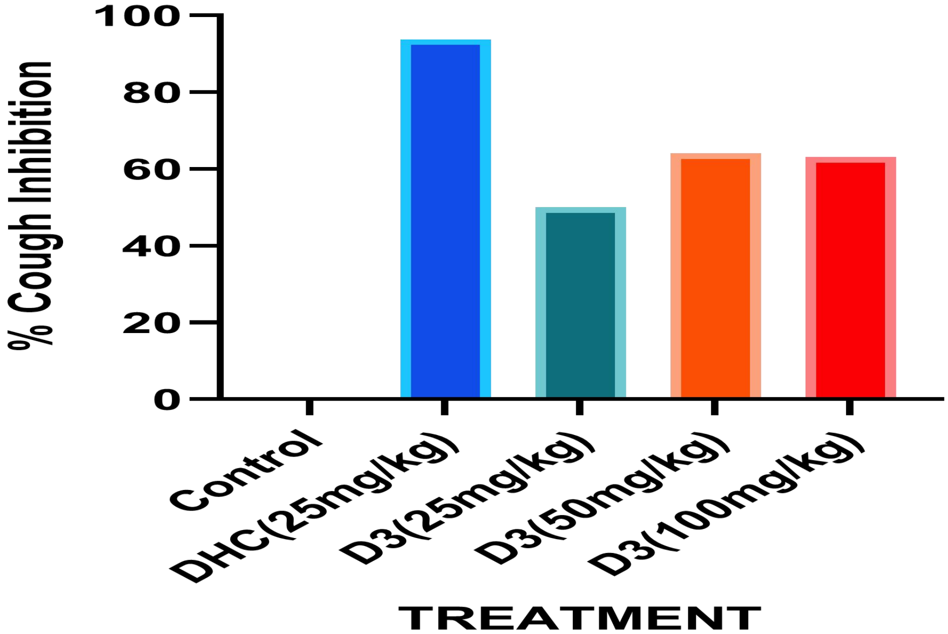

Figure 1b: The effect of administration of aqueous extract of D3 on percentage cough inhibition in ammonium-induced cough in mice.

D3 Organic® (50, 100 mg/kg) and DHC (25 mg/kg increased the percentage cough inhibition in mice when compared to the control (DHC: Dihydrocodeine, D3). Values are represented as Mean ± SEM, n=5 per group.

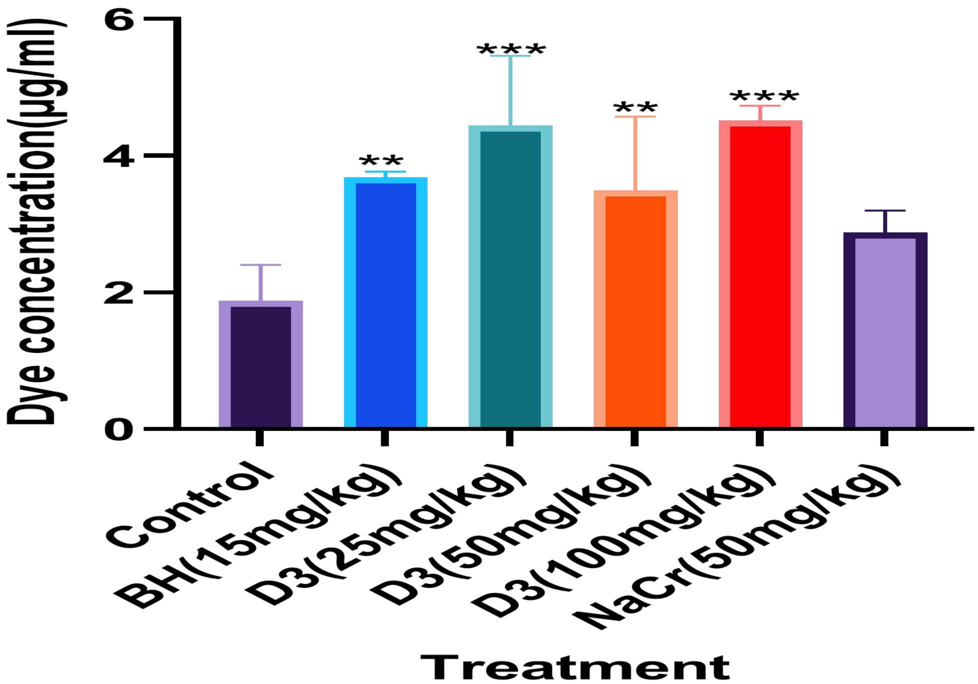

Figure 2a: Effect of aqueous extract of D3 Organic® supplement on phenol red dye secretion in mice. D3 Organic® (25, 50, and 100mg/kg) and BH (15mg/kg) increased phenol red dye secretion in the mice when compared to the control (P<0.05: P<0.01). BH: bromhexine; NaCr: sodium cromoglycate. Values are represented as Mean ± SEM, n=5 per group.

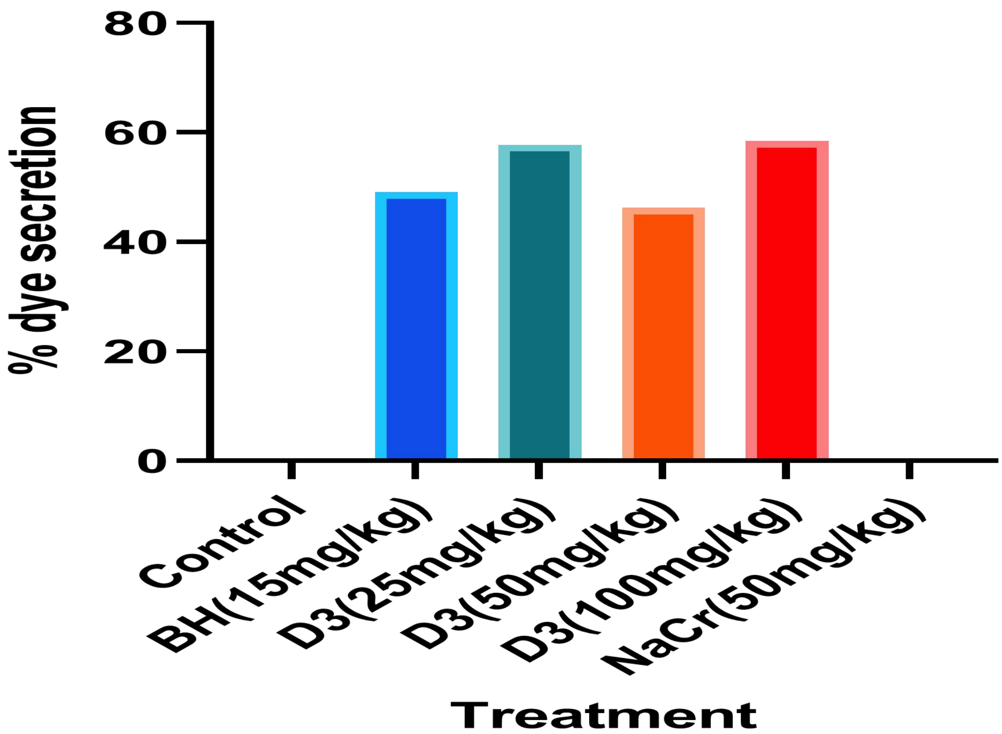

Figure 2b: Effect of aqueous extract of D3 Organic® on percentage phenol red dye secretion in mice. D3 Organic® (25, 50, and 100mg/kg) and BH (15mg/kg) increased the percentage of phenol red dye secretion in the mice when compared to the control ($P<0.05$: $P<0.01$). BH: bromhexine; NaCr: sodium cromoglycate. Values are represented as Mean ± SEM, n=5 per group.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1. DISCUSSION

The ammonia-induced cough model unequivocally establishes the antitussive properties of D3 Organic® (Figures 1A and 1B). Administration of D3 Organic® at doses of 50 and 100 mg/kg resulted in a marked reduction in the number of cough bouts, an effect comparable to the standard opioid antitussive drug, dihydrocodeine (DHC). Furthermore, the percentage cough inhibition data (Figure 1B) confirms a dose-dependent efficacy. Ammonia vapour acts as a chemical tussive agent by irritating the tracheobronchial mucosal sensory nerves (C-fibres and rapidly adapting receptors), thereby initiating the cough reflex arc (Muroi and Undem, 2018). The ability of D3 Organic® extract to suppress this reflex suggests that its bioactive constituents may exert a central action on the cough centre in the medulla oblongata (similar to DHC) or a peripheral action by desensitising the airway sensory nerves (Reynolds et al., 2022). The observed effect is statistically significant and robust across the tested doses (Figures 1A and 1B). In a complementary manner, the phenol red secretion test (Figures 2A and 2B) reveals the mucoactive potential of D3 Organic®. The extract, at all tested doses (25, 50, and 100 mg/kg), significantly increased the secretion of phenol red, a marker for airway fluid and mucin output. This effect was analogous to that of bromhexine (BH), a well-known mucolytic and expectorant agent. The phenol red method is a standard assay for evaluating tracheobronchial secretion, where an increase in dye concentration reflects enhanced mucociliary clearance (Gao *et al*., 2024). This suggests that D3 Organic® contains compounds that stimulate the secretory activity of tracheal goblet cells and submucosal glands. The ability to simultaneously inhibit the cough reflex and promote the clearance of airway secretions is a highly desirable therapeutic profile for productive coughs, as it addresses both the irritating symptom and the underlying cause by facilitating the expulsion of phlegm. The dual action of suppressing coughs and increasing mucus production may seem contradictory, but it is good for patients. A dry, non-productive cough benefits from suppression, while a productive cough benefits from improved clearance. A substance that can modulate both pathways, potentially depending on the physiological context, is of significant interest. The efficacy of D3 Organic® across these two distinct models indicates that it likely contains multiple bioactive compounds acting on different pharmacological targets. Subsequent research should concentrate on the bioassay-guided fractionation of the D3 Organic® extract to elucidate the specific chemical entities accountable for each effect (Chen and Wang, 2023). The experimental results demonstrate that the aqueous leaf extract of D3 Organic® possesses significant pharmacological activity in murine models, exhibiting both potent antitussive (cough-suppressant) and secretagogue (mucus-promoting) effects. These results offer a scientific foundation for the ethnobotanical application of this plant in the treatment of respiratory ailments.

5.2. CONCLUSION

The experimental results demonstrate that the aqueous leaf extract of D3 Organic® supplement possesses significant antitussive and expectorant properties

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