PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL EVALUATION OF *AGERATUM CONYZOIDES*

BY

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CERTIFICATION

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DEDICATION

This work is dedicated to the Almighty God, without whom I can do nothing. My Lovely and supportive Parents: Mr. and Mrs. Albert Bassey.

My Husband and Son :Mr Osamuyi Michael and Samuel Etiosa Michael.

My Siblings: Shalom, Nissi ,David and Faith.

My Niece's and Nephew: Touri, Claire, Zoe and Reghan**.**

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CHAPTER ONE

ABSTRACT

Traditional medicine due to its antimicrobial and therapeutic qualities against various diseases such as ethno-veterinary, fever, rheumatism, headache, colic, dyspepsia, uterine disorders, and pneumonia.

The objective of this study is to investigate the phytochemical constituents of the leaves of Ageratum conyzoides and determine the antimicrobial activity of Ageratum conyzoides, so as to justify its usage in Traditional Medicine Practice.

The fresh plants were collected, washed, cut into small pieces, air dried and pulverized to powder using mechanical grinder. Extraction was done by cold maceration.The methanol extract and aqueous extracts of the plant portion were subjected to phytochemical analysis using the standard method. The Aqueous and Methanol extracts of the ageratum conyzoides plant were tested for their antimicrobial activity using the cup plate method against *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilis*, *Pseudomonas aeruginosa,* and *Klebsiella pneumonia.*

The phytochemical investigation of the crude leaves extract of Ageratum conyzoides detected the presence of bio-active substances like alkaloids, flavonoid, tannin, saponin, glycosides, and phenolics and carbohydrate.

The Methanol extracts had a higher level of antibacterial activity against Staphylococcus aureus, with the zone diameter of inhibition measuring 14 mm and 18mm for Escherichia coli. The aqueous water extract's zones of inhibition against E. coli was 9.5mm and 13mm of Staphylococcus aureus.

The zones of inhibition of both the aqueous and methanol extracts were compared to that of an Analytical grade Ciprofloxacin antibiotics.

The result of this investigation suggests that Ageratum conyzoides methanol extracts may be useful in treating illnesses and infections caused by S. aureus, and E. coli. which may be due to the presence of the its chemical constituent such as alkaloids, flavonoid, tannin, saponin, glycosides and phenol, thus justifying its use by traditional medicine practitioners

Close-up view of the *Ageratum conyzoides*

CHAPTER ONE

1.0 INTRODUCTION

1.1 Medicinal Plants

Medicinal plants constitute an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80% of the rural population depends on them as primary health care (Akinyemi, 2000). Plants have been used as sources of remedies for the treatment of many diseases since ancient times and people of all continents especially Africa have this old tradition.

Despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Newman et al., 2000). However, plants used in traditional medicine are still understudied (Kirby, 1996). In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80% of the population uses medicinal plants as remedies (Kirby,1996; Hostellman and Marston, 2002).

According to the World Health Organization (WHO) the definition of traditional medicine may be summarized as the sum total of all the knowledge and practical, whether explicable or not, used in the diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing. Traditional medicine might also be considered as a solid amalgamation of dynamic medical known-how and ancestral experience. In Africa, traditional healers and remedies made from plants play an important role in the health of millions of people.

Traditional medicine has been described by the WHO as one of the surest means to achieve total health care coverage of the world's population. Numerous medicines have been derived from the knowledge of tropical forest people and clearly there will be more in the future. This alone is reason enough for any and all programs to be concerned with the conservation, development, and protection of tropical forest regions.

1.2 CHARACTERISTICS AND FEATURES OF *AGERATUM CONYZOIDES.*

The Asteraceae family consists of 1500 genera and 2500 different species (Souza et al., 2012). Ageratum is one of the genera included in the Asteraceae family and consists of 30 species (**Okunade (2002)** .*Ageratum conyzoides* is called goat-weed and has been empirically used as a drug to treat boils and fever medications(Silalahi et al 2014). A. conyzoides is a tropical plant commonly found in the western and eastern regions of the African continent, in several regions of Asia and South America. The plant is a polymorphic aromatic weed. The leaves of Ageratum conyzoides are used as wounds therapy(**Dash et al., 2011**).,7 hemorrhoids, anti-dysentery, antibacterial **Woldeyes et al. (2012)** ,8 anti-inflammatory, analgesic, antipyretic, antispasmodic, gastro protective, anti-ulcer, insecticide (Moreira et al. 2007), anti-helmintic and anti-mosquito.

1.2.1. SYNONYMS

Ageratum latifolium Car., *Ageratum cordifolium* Roxb., *Ageratum odoratum* Vilm. (Dave's Garden, 2018; Ming, 1999), *Ageratum album* Stend (Ming, 1999), Cacalia mentrasto Vell. (Ming, 1999).

1.2.2. COMMON NAMES

Hindi : Jangli pudina, uchunti; English: Goat weed, Billygoat weed,Chicken weed ; Spanish (El Salvador): Mejorana, sunsumpate (Columbia): Yerba hemostatica Portuguese: Mentrasto, Tropic ageratum African Vernacular Names West Africa (Igbo): Nri-ewu (Yoruba): Imieshu , yarnigbei.

1.2.3. TAXONOMICAL CLASSIFICATION

Kingdom : Plantae Subkingdom

Angiosperm Class:Eudicots

Order:Asterales

Family:Asteraceae

Genus:Ageratum

Species:conyzoides

Binomial name: *Ageratum conyzoides* Linn.

1.2.4. BOTANICAL DESCRIPTION

Ageratum conyzoides is a small herbaceous plant belongs to the family Asteraceae. It is softly hairy, erect, branched, annual weed up to 80-90 cm in height. It is a tropical plant used in various parts of Africa, Asia and South America for curing various diseases. The stems and leaves are covered with fine white hairs; the leaves are ovate and up to 7.5 cm long. The flowers are purple to white, less than 6 mm across and arranged in a terminal inflorescence. The fruits are achenes and easily dispersed. Because of its propagation it become a weed and causes problems for farmers and ecologists. Seeds are positively photoblastic, and viability is often lost within 12 months. It is not eaten by men because of its bad odour, like a male goat and is named goat weed or billy goat weed. The whole plant is only used for medicinal purposes and has a long history in the folk medicine of different countries (Tropilab, 2012).

1.2.5. REPRODUCTION AND PROPAGATION

The primary mode of reproduction in *Ageratum conyzoides* is sexual reproduction through seed production. The plant produces small, lightweight seeds, technically classified as achenes, which are highly adaptable to various dispersal mechanisms (Gnanavel, 2018). A mature plant can produce thousands of seeds, contributing to its rapid spread and establishment in disturbed environment.

Although less common, *Ageratum conyzoides* can propagate vegetatively. Stem fragments or root cuttings can regenerate into new plants, allowing persistence in areas where seeds may fail to establish (Sharma et al., 2019).

Dispersal Mechanisms

The species spreads through multiple mechanisms:

- **Wind Dispersal:** Lightweight seeds enable easy airborne transport.
- **Water Dispersal:** Seeds are carried by rainwater and surface runoff.
- **Human and Animal Activities:** Seeds attach to clothing, animal fur, or farming equipment, aiding long-distance dispersal (Kong et al., 2021).

The reproductive efficiency of *Ageratum conyzoides* contributes to its invasive potential. Its high seed output, dormancy, and multiple dispersal mechanisms make it a dominant species in disturbed environments. Effective management requires understanding these propagation strategies to control its spread in agricultural and ecological systems.

1.2. 6. PHYTOCHEMICAL CHARACTERISTICS

There is high variability in the secondary metabolites of *Ageratum conzyoid*e which include flavonoid, alkaloids, coumarins, essential oils, and tannin. Many of these are biologically active. The oil content varies randomly from 0.11 to 0.58% for leaves and from 0.03 to 0.18% for the roots depending on times of the year .From water distillation of the fresh flowers, the oil content was found to be 0.2% . The yield of oil from the petroleum ether extract of the seed was 26%.A large number of constituents have been identified from the GC-MS (Gas Chromatography-Mass Spectroscopy) analysis of the essential oil of Ageratum conyzoides 7-8. The largest so far, a total of 51 constituents have been reported from the analysis of an oil sample of the plant. The constituents identified include 20 monoterpenes 6.4% and 20 sequiterpenes 5.1%. The mono- and the sesquiterpenes are obtained in minute quantities trace-0.1%. The monoterpenes obtained in approximately 1% of the oil include sabinene and β-pinene, 1.6%, β-phellandrene, 1,8- cineole and limonene, 2.9%, terpinen-4-ol, 0.6%, and αterpineol, 0.5%. Ocimene which is found in trace amount in the oil from the Nigerian plant, is found to be 5.3% of the oil from the plant collected in India. α -Pinene 6.6%, eugenol 4.4% and methyleugenol 1.8% are also obtained from the Indian plant oil . The most common component of the essential oil of Ageratum conyzoides is 7-methoxy2,2-dimethylchromene (precocene-I)

Phytochemical screening of *Ageratum conyzoides* has revealed the presence of diverse bioactive compounds, including flavonoids, alkaloids, saponins, tannins, steroids, terpenoids, and essential oils (Ming et al., 2018). These compounds contribute to the plant's therapeutic properties and have been linked to antimicrobial, anti-inflammatory, and antioxidant activities.

Alkaloids

Alkaloids are nitrogen-containing secondary metabolites known for their potent pharmacological effects. Ageratum conyzoides contains pyrrolizidine alkaloids, such as lycopsamine and echinatine, which have been implicated in antimicrobial and insecticidal activities (Kariuki et al., 2020). However, some pyrrolizidine alkaloids are hepatotoxic, necessitating caution in therapeutic applications.

Flavonoids

Flavonoids in *Ageratum conyzoides* act as antioxidants and free radical scavengers, providing protective effects against oxidative stress-related diseases. Studies have shown that flavonoids, such as quercetin and kaempferol, exhibit anti-inflammatory and antimicrobial properties (Okunade, 2002). These compounds play a critical role in enhancing the plant's medicinal efficacy.

Saponins and Tannins

Saponins are glycosides with surfactant properties that contribute to the antimicrobial and immune-modulating effects of the plant. Tannins, on the other hand, exhibit astringent properties, which aid in wound healing and antimicrobial activities (Oladele et al., 2019). Both classes of compounds have been reported to enhance the pharmacological potential of *Ageratum conyzoides.*

Terpenoids and Essential Oils

Terpenoids and essential oils are vital components of *Ageratum conyzoides*, responsible for its characteristic aroma and biological activities. The essential oil extracted from the leaves and stems contains compounds such as precocene I and II, which exhibit insecticidal and antifungal properties (Singh et al., 2015). Additionally, terpenoids contribute to the plant's anti-inflammatory and analgesic effects.

The phytochemical constituents of *Ageratum conyzoides* contribute significantly to its pharmacological relevance. The presence of alkaloids, flavonoids, saponins, tannins, terpenoids, and essential oils underpins its antimicrobial, anti-inflammatory, and antioxidant activities. However, caution should be exercised due to the presence of hepatotoxic pyrrolizidine alkaloids. Further research is necessary to optimize its medicinal applications while mitigating potential toxicity risks.

1.3. ANTIMICROBIAL PROPERTIES

Antimicrobial agents are used to prevent and treat infections of various kinds. They are very important in our everyday life to prevent diseases and maintain good health,since microorganisms are ubiquitous (Okonkwo, 1988).

Essential oil of *Ageratum conyzoides* has strong toxicity against the fungi causing ringworm, Epidermophyton floccosum, Trichophyton mentagrophytes and Microsporum gypseum, with the inhibition of the mycelia being 80.28, 78.43 and 68.24%, respectively (Mishra et al., 1991). The extract of leaves, however, had no effect on the conidial germination of the fungus D. oryzae (Ganesan and Krishnaraju, 1995). Aqueous extract was tested against three Gram-positive bacteria and seven Gram-negative bacteria and evaluated by the filter paper disc diffusion method. Results showed a significant control of the growth of Alcaligenes viscolactis, Klebsiella aerogenes, Bacillus cereus and Streptococcus pyogenes (Moody et al., 2004) .

Ageratum conyzoides, commonly known as goat weed, is a tropical and subtropical plant recognized for its broad-spectrum antimicrobial properties. Its efficacy against bacteria, fungi, viruses, and parasites is attributed to its rich phytochemical profile, including alkaloids, flavonoids, tannins, saponins, and essential oils (Ogunwande et al., 2018; Ezeonu & Ejikeme, 2019)

1.3.1. Antibacterial and Antifungal Activity

The antibacterial potential of A. conyzoides has been demonstrated against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*. This activity is largely attributed to bioactive compounds such as chromenes and flavonoids, which disrupt bacterial cell membranes and inhibit enzymatic functions (Sibanda & Okoh, 2020). Additionally, its anti-fungal properties have been reported against *Candida albicans, Aspergillus niger,* and *Fusarium* species, where flavonoids and tannins contribute to fungal cell wall disruption and inhibition of spore germination (Okoye et al., 2022).

1.3.2. Antiviral and Antiparasitic Activity

Beyond bacterial and fungal infections, A. *conyzoides* exhibits antiviral properties, with studies showing inhibitory effects against *herpes simplex virus (HSV)* and *influenza virus* due to its ability to interfere with viral replication (Adegboye et al., 2021). The plant has also demonstrated antiprotozoal and anthelmintic activities, particularly against *Plasmodium falciparum* and *Trypanosoma* species, likely through disruption of parasite metabolism and neuromuscular function (Nweze & Asuzu, 2019; Mbatchou et al., 2020).

The antimicrobial mechanisms of A. conyzoides are multifaceted, involving disruption of microbial cell membranes, inhibition of protein synthesis, enzymatic interference, and interference with parasite metabolism. The synergistic action of its bioactive compounds enhances its potential as a natural antimicrobial agent.

The extensive antimicrobial properties of *Ageratum conyzoides* support its traditional medicinal use as a treatment for infectious diseases. Its broad-spectrum activity against bacteria, fungi, viruses, and parasites highlights its potential in the development of herbal-based antimicrobial therapies. However, further research is required to isolate specific bio-active compounds and assess their clinical applications.

1.3.3 OTHER MEDICINAL USES OF *AGERATUM CONYZOIDE*S.

Pharmacological properties

Ageratum conyzoides has long been known in herbal or folk medicine as a remedy for various ailments in Africa [Almagboul et al, 1985]. It has been alternative medicine for treatment of epilepsy, wounds and also as an insect repellent. The leaves are applied to burn, cut, sore and throat infection.[Adetutu,A et al, 2012]

The crude extract of the whole plant has been reported to be superior to vaseline gauze as a wound dressing material (Horie et al.1993). It has been found to have neuro-muscular blocking activity in isolated rats phrenic nerve-diaphragm and also caused greater fall in diastolic pressure compared with that of systolic pressure in anesthetized rats. It has calcium blocking activity similar to that of Verapamil Achola and (Munenge 1997).

The leaf extract has been used in the treatment of chronic pain in osteoarthrotic patients (Marques et al. 1988). Its antimicrobial and anticonvulsant activities have also been demonstrated (Whittle and Turner.; 1981). The methanolic extract of the whole plant also has antimicrobial activity (Almagboul et a1985) . Aqueous extract of the leaves has been reported to prevent coagulation of the whole blood while causing precipitation of some blood materials. Bleeding time was also decreased in this assay (Akah 1988).

1.4. 0. PREVIOUS STUDY CARRIED OUT *ON AGERATUM CONYZOIDES*

Traditionally, *Ageratum conyzoides* has been used in various parts of the world like Africa, Asia and South America as folk medicine. The whole plant produces volatile strong smelled oil which also possesses various biological activities. It is used for wound dressing, curing various skin diseases, ophthalmic, colic, ulcers treatment, as purgative and febrifuge. The decoction or infusion of the herb is given in stomach ailments such asdiarrhoea, dysentery, intestinal colic, flatulence, rheumatism fever, and gynaecological diseases. Other folk remedies include anti-itch, sleeping sickness, and mouthwash for toothache, antitussive, vermifuge, tonic and killing lice. The leaves are used for application on cuts, sores, anti-inflammatory agent, homeostatic, insecticide, skin diseases, ringworm infection, antidote to snake poison, malarial fever, anti-tetanus, uterine problems, prolapse of anus, swollen piles, throat infection, painful gums, abscess for early suppuration, wound healing and leucorrhoea and infant diarrhoea. It has been reported to have nematocidal activity and potential used in controlling pests.

The plant has an anti enteralgic and antipyretic, for cuts as a wound dressing. In India, it is used for leprosy treatment and as an oil lotion for purulent ophthalmic. Besides these, it is used for preparing local hair lotion in Manipur, India for treating dandruff. In some parts of Africa, the plant is used for headaches, dyspnoea, mental and infectious diseases. The leaves crushed in water are applied intra-vaginally for uterine troubles and also given as emetic. In Central Africa, the plant is applied for treating burnt wounds, while in Kenya it is used as anti-asthmatic, antispasmodic and for homeostatic effects traditionally. In Brazil, the leaves of this plant are served as anti-inflammatory, analgesic and anti-diarrhoea. The plant is also particularly used for treatment of gynecological diseases in Vietnam. The plant also has a number of magical and superstitious attributes, like against snake bites. In western part of Nigeria, it is believed that incantations help against witches and bad medicine. In Congo, the leaf sap is believed to improve luck of card players. The leaf of the plant is reported to have hematopoietic potentials which could possibly cure anaemia and further reported to have gastroprotective activity.

1.5. CHARACTERISTICS AND FEATURES OF ORGANISMS USED.

Staphylococcus aureus:

Staphylococcus aureus is a Gram-positive, spherical or cocci-shaped, non-spore forming, non-motile, facultative anaerobe that is part of the family Staphylococcaceae (Holt et al., 1994; Le Loir, Baron, & Gautier, 2003).

It is an opportunistic pathogen that is ubiquitous in nature and is found in air, dust, water, surfaces, and in the natural flora of skin and mucous membrane of humans and animals (Argudín, Mendoza, & Rodicio, 2010; Hennekinne, De Buyser, & Dragacci, 2012; Le Loir et al., 2003).

It can grow between 7 and 48 °C, with the optimum being 37 °C and at pH values between 4 and 10, with the optimum being pH 6 to 7 (Hennekinne et al., 2012).

Staphylococcus aureus can be identified by its ability to produce free or bound coagulase and ferment mannitol, producing acid but no gas (Okonkwo and Hugbo ,1988).

Escherichia coli:

Escherichia coli are Gram-negative, short rod-shaped bacteria and members of the family Enterobacteriaceae. They are usually motile with peritrichous flagella, possessing fimbriae, and are facultative anaerobes and capable of fermentative and respiratory metabolism.

***Bacillus subtilis*:**

Bacillus subtilis is a Gram-positive, rod-shaped, catalase-positive bacterium commonly found in soil and the gastrointestinal tract of ruminants and humans. It is widely studied as a model organism in bacterial research due to its ability to form endospores, its genetic tractability, and its wide array of industrial applications (Barbe et al., 2009).*B. subtilis* is a facultative aerobe that can form oval, heat-resistant endospores, which allow it to survive extreme environmental conditions such as desiccation, heat, and UV radiation (Errington, 2003). The bacterium also exhibits motility through peritrichous flagella and is capable of biofilm formation, enhancing its resistance to environmental stress (Vlamakis et al., 2013). *Bacillus subtilis* belongs to the phylum *Firmicutes*, class *Bacilli*, order *Bacillales*, and family *Bacillaceae* (Earl et al., 2008). Although it shares evolutionary characteristics with other *Bacillus* species, it is uniquely known for its effective sporulation and genetic competence (Kunst et al., 1997).

Due to its rapid growth and capacity to secrete enzymes, *B. subtilis* is utilized extensively in biotechnology. It serves as a microbial cell factory for producing antibiotics (such as bacitracin), enzymes (including amylases and proteases), and bio-based polymers (Schallmey, Singh & Ward, 2004). Additionally, its antagonistic effects against plant pathogens make it valuable as a biofertilizer and biopesticide in agriculture (Borriss, 2011).Recent studies have highlighted the probiotic potential of *B. subtilis*, especially in promoting gut health and modulating immune responses. It has been used in oral probiotic formulations to improve gastrointestinal health and reduce the incidence of infections like *Clostridium difficile*-associated diarrhea (Hong et al., 2005).*B. subtilis* produces several antimicrobial peptides, including subtilin, surfactin, and bacilysin, which inhibit the growth of pathogenic bacteria and fungi (Stein, 2005). These antimicrobial compounds are being studied for their potential applications in the pharmaceutical industry as well as in food preservation .*Bacillus subtilis* is a crucial bacterium with diverse applications in biotechnology, medicine, and agriculture. Its resilience to extreme conditions, production of beneficial compounds, and importance as a genetic model organism make it a key focus of scientific research and industrial use.

Klebsiella pneumonia

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated bacterium that typically inhabits the human respiratory and gastrointestinal tracts. It is a facultative anaerobe, capable of fermenting lactose to produce acid and gas (Madigan et al., 2018). It is a common cause of hospital-acquired infections, including pneumonia, urinary tract infections, and bloodstream infections. The bacterium is also known for its resistance to multiple antibiotics, particularly through the production of extended-spectrum beta-lactamases (ESBLs) (Podschun and Ullmann, 1998).

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a versatile, motile, Gram-negative bacterium known for its ability to thrive in a wide range of environments, including soil, water, and hospital settings. It is oxidase-positive and forms a characteristic blue-green pigment due to the production of pyocyanin (Gibbons, 2009). The bacterium is an opportunistic pathogen, causing infections in immunocompromised individuals, particularly in burn wounds, cystic fibrosis patients, and those with indwelling medical devices (Bodey et al., 2008). Its resistance to antibiotics and disinfectants is of significant clinical concern.

ANTI-FUNGAL AGENTS USED

Aspergillus niger

Aspergillus niger is a ubiquitous, filamentous fungus that is primarily involved in the decomposition of organic matter in the environment. It is a potent producer of enzymes and organic acids, notably citric acid, which is widely used in industry (Singh et al., 2009). Although generally non-pathogenic, *A. niger* can cause infections in immunocompromised individuals, particularly in the lungs, leading to aspergillosis (Latge, 1999). The species is also known for its ability to produce mycotoxins, such as ochratoxins, under certain conditions.

CANDIDA ALBICANS

Candida albicans is a dimorphic, yeast-like fungus that can switch between a unicellular yeast form and a multicellular filamentous form, contributing to its pathogenicity (Alcamo, 2009). It is a common commensal organism of the human gastrointestinal tract, mouth, and vagina but can cause infections under immunocompromised conditions, leading to conditions such as oral thrush, vaginal candidiasis, and systemic candidiasis (Brown et al., 2012). *C. albicans* is also known for its ability to form biofilms, which contribute to its resistance to antifungal therapies (Nobile et al., 2012).

PENICILLIUM NOTATUM

Penicillium notatum is a species of fungus famous for being the original source of the antibiotic penicillin, discovered by Alexander Fleming in 1928 (Fleming, 1929). It is a filamentous mold that produces characteristic greenish conidia. *P. notatum* is commonly found in soil, decaying organic matter, and indoor environments. The production of penicillin has revolutionized medicine, though strains of *Penicillium* are also known to produce other bioactive metabolites (Elliott et al., 2009). Its role in antibiotic resistance, especially to other molds, has been a subject of ongoing research.

1.6. SCREENING APPROACHES

The current trend of screening and developing substances from plants follow the process below;

i The initial screening of crude plant extract to determine the activity against some standard microbial agents.

ii. If the extract is found to possess a significant level of antimicrobial activity, the phytochemical analysis is carried out to identify the components present in the extract.

iii. The separation and isolation of the active components responsible for activity is carried out.

iv. The type of activity, whether bacteriostatic (that is , merely inhibiting the growth of the organisms) or bactericidal (that is , actually killing the organism) is determined by finding the minimum inhibitory concentration (MIC) or Maximum inhibitory concentration (MBC) where applicable.

v. Other tests such as the effect of pH , inoculum size, media component and temperature of the activity of the compound, are carried out.

vi. Other sophisticated chemical assays such as UV-spectrophotometry, IR spectrophotometrycan be used to elucidate the chemical structure of the compound, and its mode of action may be investigated.

vii. Chemical modification of the structure can be carried out in order to improve on the spectrum of activity and potency (Ayinde, 2001).

1.7. RATIONALE OF THE STUDY

Background Information and Rationale

Medicinal plant based natural products from medicinal plants have long been a major source of therapeutic medicine . *Ageratum conyzoides* commonly known as "Goatweed" or "Billy Goat Weed," is used extensively in traditional medicine to treat inflammatory diseases, wounds, skin infections, and gastrointestinal issues.

Despite its extensive ethnomedicinal applications, scientific validation of its bioactive compounds and antimicrobial efficacy remains limited. This study seeks to bridge that gap by conducting a preliminary phytochemical screening and antimicrobial activity assay of *Ageratum conyzoides*.

Phytochemical Analysis Rationale

Medicinal plants exert their pharmacological effects due to the presence of bioactive secondary metabolites such as alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic compounds. These compounds have been reported to possess antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties. Conducting a preliminary phytochemical analysis will help identify the presence and nature of these bioactive constituents, providing a scientific basis for the plant's medicinal potential.Furthermore, knowledge of the plant's chemical profile supports its potential standardization, quality control, and pharmaceutical development. Identifying these phytochemicals is essential for further pharmacological studies and possible drug formulation.

Antimicrobial Activity Rationale

The increasing prevalence of antibiotic-resistant pathogens poses a significant challenge to modern medicine, necessitating the search for novel antimicrobial agents. *Ageratum conyzoides* has been traditionally used for the treatment of microbial infections, but its antimicrobial efficacy against specific pathogens needs to be scientifically established.

This study investigates the inhibitory effects of *Ageratum conyzoides* extracts against common bacterial and fungal pathogens, potentially identifying it as a source of new antimicrobial compounds. The results could contribute to the development of alternative antimicrobial therapies, particularly in regions with limited access to conventional antibiotics.

Significance of the Study

1. Scientific Validation: The study will provide empirical evidence supporting the traditional use of *Ageratum conyzoides* in treating microbial infections.
2. Antimicrobial Drug Discovery: Findings may lead to the identification of bioactive compounds with potential pharmaceutical applications.
3. Combating Antibiotic Resistance: By exploring natural alternatives, the study contributes to efforts in addressing the global challenge of antibiotic resistance.

4. Foundation for Further Research**:** The results will serve as a basis for future in-depth pharmacological and toxicological studies.

By integrating preliminary phytochemical screening and antimicrobial testing, this study will establish a scientific basis for the traditional uses of *Ageratum conyzoides*. The findings will contribute to the field of ethnopharmacology, phytomedicine, and pharmaceutical sciences, highlighting the plant's potential in modern drug development.

1.8. RELEVANCE OF THE STUDY

This study will be relevant to Students, Teachers, Lecturers, Researchers, Healthcare professionals, Pharmaceutical companies, Traditional medicine practitioners, Ministries of Health and other relevant government Agencies; as it opens up new areas of therapeutic interest.

1.9 OBJECTIVES OF THE STUDY

This study aims to achieve the following;

- To investigate the phytochemical constituents present in in the leaves of *Ageratum conyzoides.*
- To evaluate the antimicrobial activities of the aqueous and *methanol* extract of the leaves of *Ageratum conyzoides* and thus validate its use in traditional medicine practice for the treatment of infections.
- To compare the level of antimicrobial activity of the aqueous extract to the methanol extract of the leaves of *Ageratum conyzoides.*
- To compare the observed antibacterial activity of the *Ageratum conyzoides with* an analytical grade antibiotics Ciprofloxacin Hydrochloride.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 MATERIALS

Plant Material

Ageratum conyzoides **(**whole plant, leaves, or aerial parts) was used as the plant material for this study.

Glassware

Beakers, test tubes, and conical flasks, Measuring cylinders and pipettes Pasteur pipette, Macerating jar, Glass rods,Petri dishes,Universal bottles,Micro-pipettes .

Equipment's:

Rotary evaporator,Weighing balance ,Water bath Incubator, refrigerator ,hot- air oven, Autoclave, Tripod stand, Bunsen burner, Mortar and pestle, test tube racks, freeze drying machine, bowls ,Aluminium foil paper, test tube holder, electric grinding machine, and wire gauze.

- **Solvents for Extraction**

Methanol – Used in methanol extraction of bio-active compounds.

Distilled water – Used in aqueous extraction of phytochemicals.

Chemicals and Reagents for Phytochemical Screening

The following reagents were used to test for the presence of phytochemicals in the plant extract:

Alkaloids Dragendorff's reagent ,Mayer's reagent , Wagner's reagent, Hager reagent was used.

Flavonoid

Lead acetate solution – Forms a yellow precipitate in the presence of flavonoids.

Tannins

Ferric chloride ($FeCl_3$) solution – Forms a blue-black or green coloration if tannins are present.

Gelatin solution – Used to detect hydrolyzable tannins.

Saponins

Distilled water – For froth test (formation of persistent froth indicates saponins).

Phenolic Compounds

Ferric chloride ($FeCl_3$) solution – Forms a deep blue or green color with phenolics.

Steroids and Terpenoids

Sulfuric acid (H_2SO_4) and acetic anhydride – Used in the Liebermann-Burchard test for steroids.

Chloroform and sulfuric acid (Salkowski test) – Identifies terpenoids.

Glycosides

Keller-Killiani reagent (glacial acetic acid + $FeCl_3$ + H_2SO_4) – Tests for cardiac glycosides.

Legal's test (sodium nitroprusside + NaOH) – Detects cyanogenic glycosides.

All the reagents were gotten from Pharmacognosy laboratory, Pharmaceutical research Laboratory and pyrex scientific and chemical supply company

Culture Media for Antimicrobial Assay

Nutrient agar – General-purpose medium for bacterial growth.

Mueller-Hinton agar – Standard medium for antimicrobial susceptibility testing.

Sabouraud dextrose agar – Selective medium for fungal growth.

Antimicrobial Agents (Positive Controls) and Anti-fungal Agents

Standard antibiotics (Analytical grade Ciprofloxacin) – Used as positive control.and ketoconazole(Anti-fungal agent) were used as reference antimicrobial agents.

Microorganisms for Antimicrobial Analysis

- **Gram-positive bacteria** (*Staphylococcus aureus*, *Bacillus subtilis*)
- **Gram-negative bacteria** (*Escherichia coli*, *Pseudomonas aeruginosa*)
- **Fungal strains** (*Candida albicans, Aspergillus niger, and Penicillium notatum)* were the Microorganisms used in the study and they were gotten from the Microbiology Department of the University of Benin Teaching Hospital.

2.2. METHODS

2.2.1.Collection and identification of plant materials:

The fresh sample of plant *Ageratum conyzoides* Linn. Were collected from University of Benin and Medical road In Benin City, Edo state. The plant was validated by Dr Osas the Head Of Department of Pharmacognosy,Faculty of Pharmacy, university of Benin.

2.2.2Preparation of the plant material

The plant was washed properly under the tap water to remove the adhering dust particles. Then air-dry at room temperature for five (5) weeks to get the crispy texture and constant weight. Finally, grind the dried plant to make powder by using a grinding machine. The powdered drug was weighed using a weighing balance and stored in sterile air-tight labelled container at room temperature.

2.3. 2.Extraction of plant material

Five hundred grams (500 g) of the powdered plant was weighed with electric weighing balance (Gerhardt, England) and transferred into 10 L glass extraction jar. The extraction was carried out by maceration for 72h at room temperature (25±2°C) using 99.9% methanol (15000 ml) as solvent with intermittent agitation for maximum extraction of phytochemicals. The liquid extract was filtered using Whatman No. 1 filter paper.

filter paper. and concentrated in a rotary evaporator (Lab-tech Ltd., England) at 40oC. The extract was weighed, transferred into a clean dry glass container and stored at -4°C for further analysis. [Hussain et al..2014].

2.2.3 PRELIMINARY PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical analysis of *Ageratum conyzoides* was conducted using standard procedures as described by Trease and Evans (1989) and Sofowora (1993). For each test, 200 mg of the powdered plant material was utilized.

1. Test for Alkaloids

Mayer's Test: 200 mg of the powdered sample was mixed with and boiled with 10ml of distilled water and heated gently over a water bath for 30 minutes. After cooling, the mixture was filtered.2ml each of the filtrate was added to four (4) test tubes. few drops of Mayer's reagent were added to the filtrate. The formation of a cream-colored precipitate indicated the presence of alkaloids.

Dragendorff's Test: Similarly, to another portion of the filtrate, a few drops of Dragendorff's reagent were added. The appearance of an orange-red precipitate confirmed the presence of alkaloids.

Wagners test: few drops of Wagner's reagent was added to another test tube containing 2ml of the extract. The formation of a reddish brown precipitate indicate the presence of alkaloids.

Since proteins give similar results as alkaloids with the above reagents, a confirmatory test for alkaloids was done as follows;A methanol extract of the sample was concentrated to dryness ,and redissolved in dilute tetraoxosulphate (iv) acid so as to denature any proteins present before testing with the alkaloidal reagent as shown above. The result was recorded as shown in table 1.

2. Test for Flavonoids

Alkaline Reagent Test: To another portion of the ethanolic extract, a few drops of sodium hydroxide solution were added. The formation of a yellow color, which turned colorless upon the addition of dilute acid, confirmed the presence of flavonoids. The result was recorded as shown in table 2.

3. Test for Tannins

Ferric Chloride Test: 200 mg of the powdered sample was boiled in 10 ml of distilled water and then filtered. To 2 ml of the filtrate, a few drops of 5% ferric chloride solution were added. A blue-black or greenish-black coloration indicated the presence of tannins.

4. Test for Saponins

Froth Test: 200 mg of the powdered sample was shaken vigorously with 5 ml of distilled water in a test tube. The formation of a stable froth that persisted for about 15 minutes indicated the presence of saponins.

5. Test for Steroids

Salkowski's Test: 200 mg of the powdered sample was mixed with 2 ml of chloroform, and then 2 ml of concentrated sulfuric acid was carefully added to form a layer. A red coloration at the interface indicated the presence of steroids.

6. Test for Terpenoids

Lieberman-Burchard Test: 200 mg of the powdered sample was mixed with 2 ml of acetic anhydride and heated gently. After cooling, 1 ml of concentrated sulfuric acid was added along the sides of the test tube. The formation of a green coloration indicated the presence of terpenoids.

7. Test for Phenols

Ferric Chloride Test: 200 mg of the powdered sample was boiled with 5 ml of distilled water and filtered. To 2 ml of the filtrate, a few drops of 5% ferric chloride solution were added. The appearance of a deep blue or green coloration indicated the presence of phenolic compounds.

8. Test for Glycosides

Keller-Kiliani Test: 200 mg of the powdered sample was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This mixture was then carefully underlaid with 1 ml of concentrated sulfuric acid. The formation of a brown ring at the interface indicated the presence of cardiac glycosides.

9. Test for Anthraquinones

Borntrager's Test: 200 mg of the powdered sample was shaken with 10 ml of benzene and then filtered. To 5 ml of the filtrate, 5 ml of 10% ammonia solution was added and the mixture was shaken. A pink, red, or violet coloration in the ammoniacal (lower) phase indicated the presence of free anthraquine.

The Salkowski test was performed to detect the presence of steroids and terpenoids in the extract.

10. Test for carbohydrate.

Molisch test

The **Molisch test** is a qualitative test used to detect the presence of **carbohydrates** in a sample. The procedure was carried out as follows.

200 mg of the powdered plant material was **boiled with 10 ml of distilled water** for 5minutes.The solution was then **filtered** to obtain a clear filtrate.

Molisch Test Procedure:

2mls of the filtrate was transferred into a clean test tube.
2 drops of molisch reagent (alpha- naphthol in ethanol) were added to the filtrate and mixed well. 2mls of concentrated sulfuric was carefully added along the walls of the test tub to form separate layer. The test tub was observed for the formation of a violet or purple ring at the interface of the two layers.

Test for reducing sugar

Fehling's Test

Preparation of Extract: 2g of the powdered plant material was boiled with 5 mL of distilled water for 5 minutes. The solution was then filtered, and the clear filtrate was used for the test.

Fehling's Test Procedure:2 mL of the filtrate was mixed with 1 mL of Fehling's solution A and 1 mL of Fehling's solution B in a test tube. The mixture was heated in a boiling water bath for 2–5 minutes. The appearance of a brick-red precipitate indicated the presence of reducing sugars.

2.2. 4. EXTRACTION OF PLANT MATERIAL

Methanol extraction

Five hundred grams (500 g) of the powdered plant was weighed with electronic weighing balance (Gerhardt, England) and transferred into 10 L glass extraction jar. The extraction was carried out by maceration for 72h at room temperature (25±2°C) using 99.9% methanol (1000 ml) as solvent with intermittent agitation for maximum extraction of phytochemicals. The liquid extract was filtered using Whatman No. 1 filter paper. and concentrated in a rotary evaporator (Lab-tech Ltd., England) at 40oC. The extract was weighed, transferred into a clean dry glass container and stored at -4°C for further analysis. [Hussain et al..2014].

Aqueous Extraction

1000 mL volume of distilled water was introduced into a big glass jar containing 600 g of powdered *Ageratum conyzoides*. The mixture was thoroughly stirred to ensure uniform dispersion and subjected to maceration for 72 hours. Agitation was performed periodically to enhance aqueous extraction. After maceration, the extract was filtered through a funnel lined with Whatman No. 1 filter paper to separate the filtrate from residual biomass. The resulting filtrate was subsequently concentrated using a lyophilizer to obtain a dry aqueous extract. The lyophilized extract was weighed using an electronic weighing balance, stored in a sterile, airtight glass container, and preserved under refrigeration at 4°C to maintain bioactive potency (Perez *et al*., 1990).

2.2.5 Preparation of different concentration of plant Extracts and Standard antibiotic Samples.

i. The methanol extract of the leaves of *Ageratum conyzoides* was a thick viscous syrup form with a dark colour. The total weight was 13.64g.

ii. The aqueous extract of the leaves of *Ageratum conyzoides* was a thick gummy solid black in colour. It's total weight was 22.63g

iii. The standard antibiotic sample used was Ciprofloxacin hydrochloride.

Concentrations of Plant Extract prepared.

The methanol extract was reconstituted by dissolving 2 g of extract in 4 ml of distilled water, yielding a final concentration of 500 mg/ml.

The aqueous extract was similarly prepared by dissolving 2 g of extract in 6 ml of distilled water to obtain a concentration of 250 mg/ml (Sofowora, 1993).

Preparation of Nutrient Medium

Nutrient agar, nutrient broth, and Sabouraud agar were prepared in accordance with the manufacturer's guidelines and sterilized at 121°C for 15 minutes using an autoclave (Perez *et al.*,1990).All laboratory techniques and procedures adhered strictly to standard microbiological practices.

Bacteria and Fungi Suspension Preparation

Sterile media, including nutrient agar, Sabouraud dextrose agar, and broth, were prepared following standard microbiological procedures. A sterile inoculating loop was used to extract bacteria and fungi samples from pure subcultures aseptically. Each colony was suspended in 4 mL of sterile water, and vortex mixing was performed to ensure even distribution. The turbidity of bacterial suspensions was standardized using McFarland's solution to approximate a bacterial density of 1×10^8 CFU (Odangowei *et al.*, 2019).

Inoculation of Microbial Isolates

Sterile swab stick was immersed in a 4 mL suspension of the test microorganisms, including *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*. The swab was carefully rotated against the inner tube wall to remove excess fluid before being used for inoculation. The plates were rotated at 60 degrees each after each streaking motion to ensure uniform distribution of the inoculum across the agar surface.

Inoculation of Microbial Isolates

A sterile swab stick was immersed in 4 ml of bacterial suspensions and used to inoculate agar plates using the streaking technique (Bauer *et al.*, 1966).

Cup Plate Method

The media were sterilized at 120°C for 15 minutes and poured into Petri dishes to solidify (Srinivasan et al.,2001). Microbial suspensions were spread across the agar surface to create a uniform lawn of growth (Rios *et al.*, 1988). Sterile cork borers (10 mm) were used to create wells in the solidified agar. The base was sealed to prevent leakage (Bauer et al.,1966). A sterile pipette was used to add a fixed volume of extracts into the wells. A control well containing only the solvent was included. Ciprofloxacin and ketoconazole were placed in separate wells as reference antibiotics and antifungal agents (serving as positive control). The Plates were incubated at 35-37°C for 24 hours for bacteria and at 25-28°C for 48-72 hours for fungi (CLSI, 2008). The inhibition zones (clear areas where microbial growth was prevented) were measured using a using a caliper or ruler.(Cowan, 1999).

CHAPTER THREE

3.0 RESULTS

3.1 Preliminary Phytochemical Analysis

Alkaloids, tannins, terpenoids, cardiac glycoside, saponins, steroids,were found in Ageratum conyzoides aqueous crude leaves extract [Table 1]. While Alkaloid,Tannin, Flavonoid, saponin, phenol,terpenoids, Steriods, Carbohydrate, Protein,cardiac glycosides were found in the methanol extract of the leaves of Ageratum conyzoides [The results are represented in Table 1].

Alkaloids: The Formation of a reddish-brown precipitate (Wagner's test) indicated the presence of alkaloids.

Tannins: Appearance of a blue-black or greenish-black color (Ferric chloride test) indicated the presence of tannin.

Phenols: Formation of a blue or green color (Ferric chloride test) indicated the presence of phenol in both the aqueous and methanol extract.

Carbohydrates (Molisch test): Development of a purple or violet ring at the interface indicates the presence of carbohydrates.

Proteins: Appearance of a **violet color** (Biuret test) indicates the presence of protein in the methanol extract.

Glycosides: Development of a reddish-brown color (Borntrager's test).

Steroids: Appearance of a reddish-brown or blue-green color (Salkowski test) in both the methanol and aqueous extract indicated the presence of steroids.

Saponins: Formation of a stable froth (Froth test) indicated the presence of saponin in the aqueous extract.

Terpenoids: Development of a reddish-brown color (Salkowski test) in both the aqueous and methanol extract.

Table 1. Results of phytochemical screening of plant extracts viz, methanol and aqueous water.

S/N	NAME OF TEST	AQUEOUS EXTRACT	METHANOL EXTRACT
1	Alkaloid	+	+
2	Tannin	+	+
3	Flavonoids	–	+
4	Saponins	+	–
5	Carbohydrates	+	+
6	Proteins	–	+
7	Phenols	+	+
8	Terpenoids	+	+
9	Glycoside	+	+
10	Steroid	+	+

KEYS

+ ; Constituent Present,

– ; Constituent not present.

3.2 Antimicrobial activity

Table 2 : Antibacterial activity of *Ageratum conyzoides* Extracts (Zone of inhibition in mm)

S/N	BACTERIA SPECIES	AQUEOUS EXTRACT(mm)	METHANOL EXTRACT(mm)	POSITIVE CONTROL(CIPROFLOXACIN) (mm)
1	Escherichia coli	9.5	14	16
2	Staphylococcus aureus	13	18	30
3	Pseudomonas aeruginosa	NZ	16	40
4	Bacillus subtilis	11	20	35
5	Klebsiella pneumonia	NZ	NZ	30

KEY:

NZ : No inhibitory zone diameter(mm) observed.

Table 3: Anti-fungal activity of *Ageratum conyzoides* Extracts (Zone of inhibition in mm)

S/N	FUNGI SPECIES	AQUEOUS EXTRACT(mm)	METHANOL EXTRACT(mm)	CONTROL(KETOCONAZOLE) (mm)
1	***Aspergillus niger***	**NZ**	**8**	**24**
2	***Candida albicans***	**8**	**12**	**30**
3	***Penicillium notatum***	**NZ**	**10**	**28**

KEY:

NZ : No inhibitory zone diameter (mm) observed.

CHAPTER FOUR

4.0 DISCUSSION

Phytochemical screening

Medicinal plants have been the main source for the formation of herbal drugs in the pharmaceutical area due to availability of bioactive markers. The presence of tannin, flavonoid, steroid, proteins, and glycosides in various extracts is responsible for antimicrobial properties of plant. So that *A. conyzoides* extract possess remarkable anti-inflammatory, and anti-bacterial properties. The results obtained in this study revealed the antimicrobial efficacy of methanol extracts.

The preliminary phytochemical screening of the leaves of *Ageratum conyzoides* extracts revealed the presence of various bioactive compounds, including alkaloids, tannins, carbohydrates, phenols, terpenoids, glycosides, and steroids in both aqueous and methanol extracts. Flavonoids and proteins were detected only in the methanol extract, whereas saponins were found exclusively in the aqueous extract. This variation in phytochemical composition suggests that different solvent systems selectively extract different classes of bioactive compounds based on their polarity (Edeoga et al., 2005).

The presence of alkaloids, tannins, flavonoids, and phenols is particularly significant, as these secondary metabolites have been reported to exhibit antimicrobial properties (Cowan, 1999). Alkaloids are known to interfere with microbial metabolism, while tannins have been shown to inhibit bacterial growth by precipitating proteins and forming complexes with microbial enzymes (Haslam, 1996). The presence of flavonoids in the methanol extract aligns with previous studies that have demonstrated their antioxidant and antimicrobial potential (Harborne, 1998). Saponins, detected only in the aqueous extract, are known for their ability to disrupt microbial membranes, contributing to their antimicrobial effects (Sparg et al., 2004).

The antimicrobial activity assay against the five bacterial species demonstrated that the methanol extract exhibited greater inhibition compared to the aqueous extract. The zones of inhibition ranged from 9.5mm to 14mm for the aqueous extract and 14mm to 20mm for the methanol extract, compared to the positive control (ciprofloxacin), which produced inhibition zones ranging from 16mm to 40mm.

Among the tested bacteria, *Staphylococcus aureus* and *Bacillus subtilis* showed the highest susceptibility to both extracts, with inhibition zones of **13mm and 11mm** (aqueous extract) and **18mm and 20mm** (methanol extract), respectively. These results suggest that the plant contains bio-active compounds effective against Gram-positive bacteria, which aligns with previous studies indicating that flavonoids, tannins, and phenols contribute to antibacterial activity against *Staphylococcus. aureus* and *Bacillus subtilis* (Ghosh et al., 2008).

The methanol extract exhibited moderate activity against *Escherichia coli* (**14mm**) and *Pseudomonas aeruginosa* (**16mm**), while the aqueous extract showed weaker activity against *E. coli* (**9.5mm**) and no activity against *P. aeruginosa*. This suggests that non-polar or semi-polar compounds in *Ageratum conyzoides* may be responsible for its antibacterial effects, as these are better extracted by methanol solvent than water (Nostro et al., 2000).

Klebsiella pneumonia was resistant to both extracts, indicating that the plant may not contain compounds capable of significantly inhibiting this pathogen. This resistance could be due to the bacterial outer membrane's protective mechanisms, as *Klebsiella spp.* are known to exhibit high antibiotic resistance due to their ability to produce extended-spectrum beta-lactamases (ESBLs) (Paterson & Bonomo, 2005).

Overall, the results confirm that *Ageratum conyzoides* possesses antibacterial properties, particularly against Gram-positive bacteria, which is consistent with earlier reports on its traditional use in the treatment of bacterial infections (Okunade, 2002).

The anti-fungal activity results showed that the methanol extract exhibited better inhibition zones against fungal species compared to the aqueous extract. *Candida albicans* was the most susceptible, with inhibition zones of **8mm (aqueous extract) and 12mm (methanol extract)**, while *Penicillium notatum* exhibited moderate susceptibility (**10mm inhibition** with methanol extract and no activity with aqueous extract). *Aspergillus niger* was resistant to the aqueous extract but had a **weak inhibition zone of 8mm with the methanol extract**.

The stronger activity of the methanol extract suggests that the active anti-fungal compounds in *Ageratum conyzoides* are more soluble in organic solvents than in water (Aboaba et al., 2011). Flavonoids, phenol, and tannins which were predominantly detected in the methanol extract, have been associated with anti-fungal properties, possibly by disrupting the fungal cell membranes and interfering with ergosterol biosynthesis (Singh & Kumar, 2011).

However, compared to the positive control (ketoconazole), which exhibited inhibition zones ranging from **24mm to 30mm**, both extracts showed significantly lower activity. This suggests that although *Ageratum conyzoides* has anti-fungal properties, its potency may not be sufficient for clinical application without further purification and concentration of its active compounds.

The findings of this study validate the traditional use of *Ageratum conyzoides* in herbal medicine for treating microbial infections (Okwu & Igara, 2009). The significant antibacterial activity against *S. aureus* and *B. subtilis* supports its ethnomedicinal application in wound healing and skin infections. The moderate anti-fungal effects against *C. albicans* also suggest potential for use in treating fungal infections, though its efficacy is lower than that of conventional anti-fungal drugs.

The observed differences in antimicrobial activity between the aqueous and methanol extracts highlight the importance of solvent selection in phytochemical extractions. Methanol proved to be a more effective solvent, likely due to its ability to dissolve both polar and non-polar bio-active compounds (Eloff, 1998).

CHAPTER FIVE

5.0 CONCLUSION

The result of this study confirms that *Ageratum conyzoides* contains important

Bioactive compounds and has antimicrobial properties. The Phytochemical screening showed the presence of alkaloids, tannins, flavonoids, phenols, glycosides, and terpenoids in the plant which are known to have medicinal benefits.

The methanol extract contained more phytochemicals than the aqueous extract, suggesting that methanol is better at extracting active compounds. The antibacterial test showed that both extracts were effective against *Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis*, with the methanol extract showing stronger activity. However, neither extract worked against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, possibly due to resistance (Poole, 2011).

The antifungal test showed moderate activity against *Candida albicans, Aspergillus niger,* and *Penicillium notatum*, with the methanol extract again performing better (Okunade, 2002).

These findings support the traditional use of *Ageratum conyzoides* in treating bacterial and fungal infections, especially against Gram-positive bacteria and common fungal pathogens. The stronger effect of the methanol extract highlights the importance of choosing the right solvent to get the most active ingredients (Eloff, 1998). Future research should focus on isolating these compounds and exploring their potential as new antimicrobial agents, especially with increasing antibiotic resistance worldwide (WHO, 2021).

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