# PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL PROPERTIES OF TOBACCO LEAVE (NICOTIANA TUBACUM)

**BY**


**BABALOLA SARAH BOLUWATIFE**

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**JULY, 2025.**

## CERTIFICATION

This is certify that this project is the original work carried out and reported by **BABALOLA SARAH BOLUWATIFE** with matric number **HND/23/SLT/FT/0578** to the Department of Science Laboratory Technology, Microbiology unit, Institute of Applied Sciences (IAS) Kwara State Polytechnic Ilorin and it has been approved In partial fulfilment of the requirements of the Award of Higher National Diploma (HND) In Science Laboratory Technology

_____ _____

**MR. YAHAYA, GOGATA MOHAMMED** **DATE**
**(Project supervisor)**

_____ _____

**MRS. AHMED T.** **DATE**
**(HOU MICROBIOLOGY)**

_____ _____

**DR. USMAN A.** **DATE**
**(Head of Department)**

_____ _____

**External Examiner** **DATE**

## DEDICATION

I dedicate this project to God Almighty, the source of my strength, wisdom, and inspiration. Without His grace, this journey would not have been possible.

To my beloved parents, whose unwavering love, prayers, and sacrifices laid the foundation for my success, thank you for being my rock.

To my siblings and family members, for their constant encouragement and belief in me, your support meant more than words can express.

To all my friends and mentors who stood by me through the ups and downs of this academic journey, I say thank you. Your words, presence, and motivation carried me through.

Lastly, I dedicate this work to every student striving to overcome challenges and fulfill their dreams may this serve as a reminder that perseverance and faith always lead to victory.

## ACKNOWLEDGEMENT

First and foremost, I return all glory to God for His grace, strength, and wisdom throughout the journey of this project. Without His guidance, this would not have been possible.

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This project is a result of not just my effort, but the collective support of so many people. I'm truly grateful.

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## ABSTRACT

The medicinal importance of Tobacco leaves with antimicrobial Nicotine and antioxidant are found in the tobacco leaves and alkanoids and polyphenol are the major compound found in tobacco leaves (bioactive compounds, pharmacological effects).

Background

Tobacco leaves have been used for centuries in traditional medicine, despite their notorious association with smoking related diseases.

This study aim to investigate the medicinal properties of tobacco leaves, exploring their potential benefits and applications.

As tobacco leaves contain diverse bioactive compounds with anti-inflammatory, antioxidant and antimicrobial properties, showing potential in treating various diseases.

Tobacco leaves possess significant medicinal value warranting further research into their therapeutic applications.

# CHAPTER ONE

## 1.0 INTRODUCTION

*N. tabacum* is considered native to South and Central America, perhaps as far north as Mexico, and had already been in wide use across the New World by the time the Spanish discovered the Americas (Candolle, 2010). Some historians have concluded that the plant was originally chewed or taken as snuff in South America, and it was the Europeans who began the practice of smoking it, although in North America, apparently from Mexico north to California and Canada, the Aztecs and native Americans had an ancient history of smoking the plant (Candolle, 2010). The first reports of dried leaves being smoked come from scouts sent by Christopher Columbus into the interior of Cuba.

It has been suggested that no form of tobacco had been present in pre-Columbian Paraguay or La Plata district, Uruguay; rather, *N. tabacum* was introduced here by the Spaniards (Candolle, 2010). The species is not native to Brazil (Candolle, 2011; Forzza et al, 2010), but other *Nicotiana* species endemic to Brazil were used in the same fashion.

*N. tabacum* was introduced from the Americas to Europe by the Spaniards shortly after the discovery of America (Pammel, 1911), perhaps as early as 1518 (Alvina and Madulid, 2009), and was certainly observed growing in Portugal by 1560 (Candolle, 2010). Some accounts credit Jean Nicot, the French ambassador to Portugal, as introducing the plant to France, where it was originally grown as an ornamental and medicinal plant.

Historical evidence suggests the species was introduced from Europe to the Middle East at the beginning of the 17$^{th}$ century. The first written record of its use in Persia [now Iran] was in 1626, though it was apparently present in India by 1605, perhaps

by European introduction as well (Candolle, 2010). The species soon became widely cultivated across the region as a high-demand cultural commodity, especially for use in the hookah.

Introduction of the species to Asia is somewhat uncertain, but repeated introductions may have occurred by various European explorers who brought seeds with them. The species was certainly introduced by way of the 1565-1815 Spanish Manila-Acapulco galleon trade route between Mexico and the Philippines (Alvina and Madulid, 2009) in order to establish tobacco plantations in the Spanish colony. The Portuguese may have introduced the species to their Asian colonies in the late 16$^{th}$ century; it was reportedly spread to Japan by the Portuguese at the beginning of the 17$^{th}$ century (Candolle, 2010). The species' introduction to China was not before 1700 and could have easily been through the frequent communications between Chinese traders and Manila-based Spaniards and Filipinos, or direct encounters with European explorers.

## 1.2 BOTANY

Tobacco (Nicotiana tabacum) is an annual herbaceous plant that belongs to the Solanaceae (nightshade) family. It is native to the Americas and has been cultivated for centuries, primarily for its leaves which are used in various tobacco products.

The tobacco plant has a well-developed taproot system and sturdy, erect stems that can reach up to 2 meters in height. The leaves are the most important part of the plant, and they are large, broad, and sticky, typically green or pale yellow in color. The leaves have a prominent midrib and are petiolate (attached to the stem by a petiole).

The flowers of the tobacco plant are tubular and funnel-shaped, with colors ranging from pink to red or white. The plant undergoes a distinct life cycle, with vegetative and reproductive stages, before reaching maturity (Laws, 2010).

## 1.3 MORPHOLOGY

Plants are an essential part of the ecosystem. Every life on the earth is directly or indirectly dependent on plants. Among the different parts of a plant, the leaf is the most essential. Primarily, leaves have two functions: photosynthesis and transpiration. In some plants, it takes up the responsibility of reproduction also. Let‘s learn more about the morphology of leaves, parts of a leaf, its types and modifications (Kress *et al.*, 2003).

Tobacco, Nicotiana tabacum, is an herbaceous annual or perennial plant in the family Solanaceae grown for its leaves. The tobacco plant has a thick, hairy stem and large, simple leaves which are oval in shape. The tobacco plant produces white, cream, pink or red flowers which grow in large clusters, are tubular in appearance and can reach 3.5-5.5 cm (1.25 -2 in) in length. Tobacco may reach 1.2-1.8 m (4-6 ft) in height and as is usually grown as an annual, surviving only one growing season. Tobacco may also be referred to as Virginia tobacco or cultivated tobacco and originates from South America. The cultivated tobacco plant normally grows to one or two feet high. The five flower petals are contained within a Corolla and can be colored white, yellow, pink, or red. The tobacco fruit measures at 1.5 mm to 2 mm, and consists of a capsule containing two seeds.The leaves, however, are the most economically important part of the plant. The leaf blades are enormous, often growing to 20 inches long and 10 inches wide. The leaf shape can be ovate (egg-shaped), obcordate (heartshaped) or elliptic (oval, but with a small point at one end.)

## 1.4 PHYSIOLOGY

The leaves of *Nicotiana* plants produce leaf wax toxins on their surface in high concentrations to inhibit blue mould and fungal infections, and *N. tabacum* has also been studied for its production of phytoalexins as a major defense mechanism in response to microbial infections

1.4.1 Leaf Development and Structure**:** Tobacco leaves develop from the plant's apical meristem, which is responsible for the continuous growth and expansion of the leaf. As the leaves mature, they become larger, thicker, and more resinous due to the development of glandular trichomes on the leaf surface.

The leaf's internal structure is characterized by the presence of a well-defined midrib and secondary veins, which play a crucial role in the transport of water, nutrients, and photosynthates throughout the leaf. The leaf's mesophyll tissue is rich in chloroplasts, enabling efficient photosynthesis.

1.4.2 Photosynthesis and Metabolism**:** Tobacco leaves are highly efficient at photosynthesis, converting light energy, carbon dioxide, and water into glucose and other organic compounds. This process is facilitated by the leaf's high chlorophyll content and the abundance of chloroplasts in the mesophyll cells.

The metabolic pathways in tobacco leaves are complex, involving the synthesis of a wide range of secondary metabolites, including alkaloids, terpenoids, flavonoids, and phenolic compounds. These compounds contribute to the plant's defense mechanisms and play a role in its medicinal properties.

**1.4.3 Phytochemical Synthesis and Accumulation:** Tobacco leaves are notable for their high content of the alkaloid nicotine, which is synthesized in the roots and translocated to the leaves. Nicotine is the primary psychoactive and addictive

compound in tobacco, but it has also been investigated for its potential therapeutic applications.

In addition to nicotine, tobacco leaves accumulate a variety of other biologically active phytochemicals, such as nornicotine, anabasine, anatabine, cembrenediol, labdanediol, quercetin, kaempferol, rutin, chlorogenic acid, and caffeic acid. These compounds contribute to the plant's antimicrobial, anti-inflammatory, and antioxidant properties (Forzza, 2010).

## 1.5 LIFE CYCLE

Tobacco is an annual herbaceous plant that undergoes a well-defined life cycle, consisting of several distinct stages.

**Seed Germination and Seedling Establishment:** The life cycle of tobacco begins with the germination of seeds. The small, oval-shaped seeds require warm, moist conditions to germinate, typically taking 7-14 days to emerge from the soil. As the seedlings develop, they form a rosette of leaves close to the ground and develop a taproot system.

**Vegetative Growth:** After the initial seedling stage, the tobacco plant transitions into the vegetative growth phase. During this phase, the plant undergoes rapid leaf and stem development, with the leaves becoming larger, thicker, and more resinous due to the proliferation of glandular trichomes on the leaf surface.

**Flowering and Reproductive Stage:** As the plant matures, it enters the reproductive stage, characterized by the development of flowering stems and the production of flowers. The flowers are typically arranged in clusters and can be white, pink, or purple in color. Following pollination, the flowers develop into seed capsules, which eventually split open to release the small, oval-shaped seeds, thus completing the life cycle (Britton, 2011).

## 1.6 ECONOMIC IMPORTANT

Poppy extracts have traditionally been used to relax smooth muscle tone, making them potentially useful in the treatment of diarrhea and abdominal cramping. The extract has been used as a sedative analgesic and antitussive. Poppy seed oil is used as a vehicle for chemotherapy delivery and to diagnose fistulae. However, there are no clinical trials to support these uses. Morphine is prepared from the opium poppy.

The chemistry of the genus Papaver is well known. When the unripened seed capsule is scored, a milky latex exudes. The dried latex is known as opium, which contains more than 30 alkaloids. The most important of these alkaloids are morphine (20%), noscapine (5%), codeine (2%), papaverine (2%), and thebaine (1%). Codeine is the most widely used opium alkaloid and is obtained from natural sources or through the methylation of morphine or synthetic transformation of the baine.

Because of the medicinal importance of morphine derivatives, efforts have been made to identify a species of Papaver that contains high levels of a suitable starting compound for the commercial synthesis of codeine. In some varieties of P. bracteatum, thebaine constitutes 98% of the total alkaloid content. 22 Commercially, thebaine may be readily converted to codeine, oxycodone, hydrocodone, or dihydrocodeine. P. bracteatum may become the species of choice as a legal source of alkaloid precursors. Poppy seed oil, used as a vehicle for pharmacological substances as well as oil-based paints, varnishes, soaps and liniments contains saturated palmitic and stearic acids and oleic, linoleic, alpha-linolenic, and other unsaturated fatty acids. Poppy seeds and their oil contain only minuscule amounts of opium alkaloids (Alvina and Madulid, 2010).

## 1.7 EFFECT

**When taken by mouth:** Tree tobacco is Likely Unsafe when taken by mouth. Tree tobacco contains a chemical called anabasine. This chemical is poisonous. Poisoning

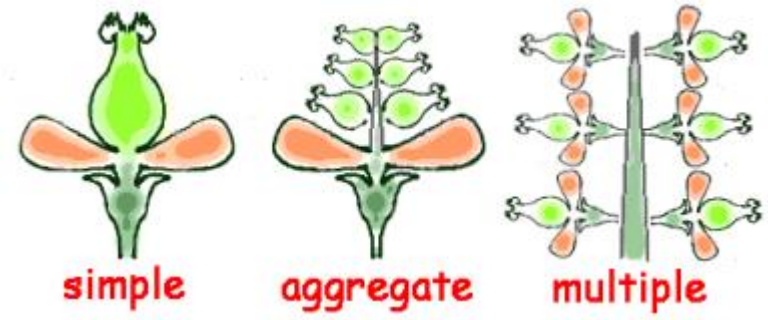
might cause the heart to stop beating, brain damage, severe muscle weakness and spasms, severe vomiting, breathing problems, seizures, high blood pressure, and death.

**When applied to the skin:** There isn't enough reliable information to know if tree tobacco is safe or what the side effects might

## 1.8CLASSIFICATION

According to the above points, we can classify fruits into types of fruits

- Simple
- Aggregate
- Composite




### i) Simple fruit

These fruits develop from the monocarpellary ovary or multicarpellary syncarpous ovary. Only one fruit is formed by the gynoecium. Simple fruits are of two types

**a) Fleshy Fruits:** In fleshy fruits, the fruit wall is differentiated into epicarp, mesocarp, and endocarp. These fruits develop from superior or inferior syncarpous gynoecium.

**b) Dry Fruits:** The pericarp of simple dry fruits is usually quite dry and hard. It is not differentiated into the three layers of epicarp, mesocarp and endocarp. In some dry fruits, this pericarp is broken down and the seeds are scattered or dispersed. These fruits are dehiscent fruits.

**ii) Aggregate Fruits**

These are the fruits that develop from the multicarpellary apocarpous ovary. It becomes a fruitlet because each carpel is separated from one another in the apocarpous ovary. These fruits make a bunch of fruitlets which is known as etaerio.

**Etaerio of follicles:** Each fruit or etaerio is a follicle. Eg. Calotropis, Catharanthus, Magnoliaceae. In calotropis, the stigma is fused or joined in carpellary ovary and ovaries of ovules are separated. It means only two follicles are present in etaerio.

**Etaerio of achenes:** In this aggregate fruit, each fruit is an achene. Eg. Ranunculus, Strawberry, Rose, Lotus. In lotus, the thalamus becomes spongy and some achenes are embedded in it. In strawberry, the thalamus is fleshy and we can find small achenes on its surface.

**Etaerio of berries:** It is an aggregate of small berries. Eg. Polyalthia, Annona squamosa (Custard-apple). In the etaerio of Annona, all the berries are arranged densely on the thalamus.

Etaerio of drupes: In this type of fruit, many small drupes develop from different carpels. Eg. Raspberry. In this type carpel of apocarpous ovary form drupe fruit.

**iii) Muiltiple /Composite Fruits**

All composite fruits are false fruits. In these fruits, generally, there are many ovaries and other floral parts combining to form the fruit. These are of two types:

**a) Sorosis:** These fruits develop from spike, spadix or catkin inflorescence. Examples inJackfruit fruit, Kevda (screwpine). In jackfruit (Kathal) pistillate flowers are developed around the peduncle. In fruit formation, the pericarp becomes spongy and fused.

**b) Sycosis:** These fruits develop from hypanthodium inflorescence. Receptacle becomes hollow and has a pore. Numerous small scales surround the pore. Eg. Ficus species Peepal (Britton, 2011).

## 1.9 MODE OF PRODUCTION

Using a transdisciplinary clinical approach, we conducted in-depth interviews with the tabaquero applying the systematizing expert interview method, in order to map modes of preparation and administration, indications, contraindications, effects, risks, adverse effects, and systemic aspects of tobacco-based remedies (Britton, 2011).

## CHARACTERISTICS

Wood aromatic, leaves exstipulate, floral parts usually numerous, free spirally arranged; stamens with distinctive enlarged and flat connective; Gynoecium multipistilate, apocarpous.

### A. Vegetative characters

Habit and habitat: Trees, shrubs or lianas. Artabotrys climbs by means of hooks. Oil ducts present in the bark, leaves and perianth leaves.

Terrestrial and perennial. Evergreen, deciduous, cultivated as well as wild.

Root: Tap, deep and extensively branched. Stem: Erect, branched, solid, woody, sometimes woody climbers. Leaves – Simple, entire, alternate, exstipulate, distichous, gland dotted (Alvina and Madulid, 2010).

## B. Floral characters:

Inflorescence: Often solitary, axillary, sometimes cauliflourous in groups.

Flower: Actinomorphic but zygomorphic in Monodora due to difference in size of petals, hermaphrodite, unisexual in Stelechocarpus, complete, trimerous, hypogynous, perigynous (Eupomatia) spirocyclic, often aromatic.

# CHAPTER TWO

## 2.0 BIO SYSTEMATIC AND TAXONOMY

Tobacco (Nicotiana tabacum) is an annual herbaceous plant that belongs to the Solanaceae or nightshade family. It can grow up to 2 meters tall and has large, broad leaves that are the primary source of its medicinal and industrial uses.

The leaves of the tobacco plant are simple, alternate, and can grow up to 50 cm in length. They have a thick, waxy texture and are usually dark green in color. The plant produces small, tubular flowers that are typically white, pink, or purple.

## 2.1 Medicinal Compounds:

The tobacco leaf contains several biologically active compounds of medicinal importance:

Nicotine - This is the primary psychoactive alkaloid in tobacco, which has stimulant properties and potential therapeutic effects. It can be used in smoking cessation and to treat certain neurological disorders.

Anabasine - Another alkaloid found in tobacco that has insecticidal and antihelminthic (anti-worm) properties.

Nornicotine - An alkaloid with demonstrated anti-cancer and neuroprotective potential.

Tobacco-Specific Nitrosamines - These compounds, while carcinogenic in high doses, have shown antimicrobial and anti-inflammatory effects at lower concentrations.

Polyphenols - Tobacco leaves contain antioxidant compounds like chlorogenic acid and rutin that provide health benefits.

## 2.2 Medicinal Applications:

The medicinal compounds found in tobacco leaves have been explored for use in various therapeutic applications, including:

Smoking cessation

Cognitive enhancement

Treatment of neurological disorders (e.g., Alzheimer's, Parkinson's, Tourette's)

Antimicrobial and anti-inflammatory treatments

Insecticide and antihelminthic (anti-worm) applications

## 2.3 HABITAT

*N. tabacum* has been reportedly grown in a wide range of habitats including forests, plains, mountains, wetlands, savannahs, dry valleys, and apparently even on a volcano. In North America, the species occurs in the uplands of the following regions: arid west, Atlantic and Gulf coastal plains, eastern mountains and piedmont, the Great Plains, and Northcentral, and northeast, while in Hawaii and parts of the Midwest, the species has been known to occur in some wetland habitats, although it usually occurs in non-wetlands (USDA-NRCS, 2014).

In Antioquia, Colombia the species occurs in premontane forests (Vascular Plants of Antioquia, 2014). In Bolivia, the species grows in rainforests, semi-deciduous and montane forests, savannahs, and dry valleys (Bolivia Checklist, 2014). In Ecuador, the species was reportedly growing on the slope of a volcano (Candolle, 1885), and more recently has been reported to occur across the Galapagos, Andean, and Amazonian regions (Vascular Plants of Ecuador, 2014).

# CHAPTER THREE

# METHODOLOGY

## 3.1 EXPERIMENTAL SITE

The experimental was carried out at a Microbiology laboratory, Department of Science Laboratory Technology Kwara State Polytechnic, Ilorin.

## 3.2 COLLECTION OF PLANT MATERIALS

Leaves were collected from the Tobacco leave (*Nicotiana tubacum*) plant at Kwara State Polytechnic, Ilorin no Nigeria. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to climate dust and other foreign particles and to clean the leaves throughly. It was dried under shade at room temperature and grinded into powder. The powdered samples were sealed in a polythene bags until the time of extraction.

## 3.3 PREPARATION OF LEAVE EXTRACTS

Two solvents were used in the preparation of leaf extracts (methanol and distilled water).

Two amber bottles were used with coach containing 20grams of the grinded plants material, 200ml of each solvents was added. It was shaken and left to soak for 5days, during the period of 5days it was shaken twice daily. Thereafter, it was filtered using Whatman No. I filter paper. The solvents was placed in a water bath and leave to evaporate to make the final volume one-fifth of the original volume. It was stored in airtight bottles for further studies (Sahira and Cathrine, 2015)

## 3.4 TEST MICRO-ORGANISMS

Three pathogenic bacteria, *viz*., *staphylococcus aureus, salmonella typhi,* and *klebsiella pneumonia* were used during the present study and were obtained from Micro biology laboratory of the department of Microbiology at Kwara State Polytechnic, Ilorin, the cultures were sub-cultured and maintained on nutrients agar slants and stored at 4$^{o}$C.

## 3.5 INOCULUM PREPARATION

For standardizing the inoculums, the test organisms were sub-culture on nutrients agar plates and incubated overnight, colony material from this overnight culture of the test organisms was taken with the aid of sterilized wire loop and transferred into a tube containing 5.oml of normal saline until the turbidity was matched with 0.5 McFarland standards (McFarland, 1907)

## 3.6 PHYTOCHEMICALS ANALYSIS

Phytochemical test were done to find the presence of the active chemical constituents such as Alkaloids, Glycoside, Terpenoids, Flavonoids, Phenol, Saponins and Tannins by the following proceedure.

### 3.6.1 Test for alkaloids (Meyer's test)

The extracts of Tobacco leave (*Nicotiana tubacum*)

was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent12. The samples were then observed for the presence of turbidity or yellow precipitation (Trease and Evan, 2009).

### 3.6.2 Test for glycoside

Tp the solution of the extract in Glacial acetic acid, few drops of Ferric chloride and concentrated sulphure acid are added, and reddish brown collouration was observed at the junction of two layers and the bluish green colour in the upper layer (Chessbrough, 2000,).

### 3.6.3 Test for terpenoids

4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chlorofone.

Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoids (chessbrough, 2000)

### 3.6.4 Test for flavonoid

4mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was adedd and red colour was observed for flavonoids orange colour for flavonoids (chessbrough, 2000,)

### 3.6.5 Test for reducing sugars

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red percipitate (Trease and Evan, 2009)

### 3.6.6 Test for phenolic compounds (ferric chloride Test)

300 mg of extracts was diluted in 5 ml of distilled water and filtered to the filtrate $5^0/_0$ Ferric chloride was added and observed for dark green colour formation (Trease and Evan, 2009)

### 3.6.7 Test for tannins

To 0.5 ml of extracts solution, 1 ml of water and 1-2 drops of ferric chloride solution was added Blue colour was observed for garlic tannins (Trease and Evan, 2009).

### 3.6.8 Test for saponins

2g of the powered sample was boiled in 20 ml of distilled water in a water bath 10ml of the filterable was mixed with 5 ml of distilled water shaken vigorously for a stable persistent broth. The following was mixed 3 drops of olive oil and shaken vigorously and then observed for the formation of emulsion (Trease and Evan, 2009).

## 3.7 PREPARATION OF EXTRACTS IMPREGNATED PAPER DISCS

A paper puncher was used to punched out 100 Discs of 6mm diameter from Whatman no. 1 filter paper, the discs were then sterilized by autoclaving at 121$^o$C for 15 minutes and then allowed to cool. Ten bijou bottles were used, three (3) for the aqueous extracts another three (3) for Methanolic extracts the remaining two is for control both positive and negative.

0.1gram of extracts was dissolved in 1ml of DSMO (Dimethyl suffoxide) which is equivalent to 100,000ug/ml to which 100 discs were added and shaken to equilibrium so that, each discs absorbed 0.001g equivalent to 1000ug/discs.

0.5g of extracts was dissolved in 1ml of DMSO which is equivalents to 50,000ug/ml to which 100 disc were added with the help of shaking at equilibrium each disc absorted 0.0005g equivalents to 500ug/disc.

0.5g of extracts was dissolved in 1ml of DMSO which is equivalents to 50,000ug/ml to which 100 disc were added with the help of shaking at equilibrium each disc absorted 0.0005g equivalents to 500ug/disc.

0.5g of extracts was dissolved in 1ml of DMSO which is equivalents to 12.500ug/ml to which 100 disc were added with the help of shaking at equilibrium,, each disc absorted 0.0012g which is equivalents to 125ug/ml. these were stored and kept for further use.

The positive control used was Ampiclox and it was dissolved with 1ml of DMSO after which 100 discs was added. The negative control was used 1ml of DMSO (Bonev *et al.,* 2008)

## 3.8 DETERMINATION OF ANTIBACTERIA ACTIVITY

The antibacterial activity of the leaf extracts was determined using agar disc diffusion method; the known procedure by Kirby-Bauer was adopted. Four (4) nutrients agar plate was used for each bacteria inoculums. Two (2) for the

aqueous extracts: one of the it was divided into four parts (each for different concentration of the extracts) the others were divided into two (one side for the positive control and the other for negative control). The same was done for the Methanolic extracts. Nutrients agar was inoculated with the given microorganisms by spreading the bacteria inoculums on the media by the use of sterile swap stick. The extracts impregnated paper discs containing different concentration of the neem extracts (100,000ug, 50,000ug, 25,000ug, and 12,5000ug) was picked with sterile forceps, it was placed firmly on the surface of inoculated plates, two control were used these are: the positives control disc of Ampiclox (500mg) and a negative control disc (with DMSO). Both disc were then allowed for pre-difussion time of 15minutes and they were then inverted and incubated at 37$^{o}$C for 24hours and the diameter of the zone of inhibition formed was measured after incubation with the aid of meter rule to determine the effectiveness of the extracts on the test organisms (Bonev *et al., 2008*)

# CHAPTER FOUR

# RESULT AND DISCUSSION

## 4.1 PHYSICAL APPERANCE OF THE EXTRACTS RECOVERED.

**Table 1: physical properties of leaf extracts of Tobacco leave (*Nicotiana tubacum*) extracts**

| Extracts | Wight of Sample Used | Volume of solvent used | Weight of extracts recovered | Colour | Odour | Texture |
|---|---|---|---|---|---|---|
| Aqueous | 09g | 100ml | 3.19g | Dark | pungent | Creamy |
| Methanolic | 09g | 100ml | 2.6g | Greenish Black | Pungent | Hard |

The result showed the weight of plants sample used, the volume of the solvent, the volume of extracts recovered, the colour, odour and texture.

## 4.2 PHYTOCHEMICAL SCREENING

The result for phytochemical screening has shown in Table 2 shows that alkaloids, saponins, flavonoids, tannins, and phenol are present in both methanolic and aqueous extracts glycoside is present in the Methanolic extracts but absent in the Aqueous extract while terpenoids is absent in both extract.

**Table 2: qualitatives phytochemical analysis of Tobacco leave (*Nicotiana tubacum*)**

| Extracts/phytochemical | Ethanol | Methanol |
|---|---|---|
| Alkaloids | + | + |
| Flavonoids | - | + |
| Glycosides | + | – |
| Phenol | - | – |
| Saponins | + | + |
| Tannins | + | – |
| Terpenoids | + | + |

## 4.3 ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACTS OF Tobacco leave (Nicotiana tubacum)

Table 3: Sensitivity test of Methanolic extracts against the test organism (zone of inhibition)

| Isolates | 1000ug/dis | 500ug/dis | 250ug/dis | 125ug/dis | Ampeol |
|---|---|---|---|---|---|
| | D.M.S. | | | | |
| | C c | c | c | x | O |
| | | | | | 5mg/dis |
| *Staphylococcus* | 27 | 08 | 08 | 07 | 00 |
| *Yaureus* | 08 | 00 | 02 | 40 | 00 |
| *Klebsiella* | 10 | 10 | 00 | 32 | 00 |
| *Pneumonia* | 00 | 01 | 05 | 00 | 00 |
| *Salmonella* | 10 | 10 | 00 | 03 | 00 |
| *Typhi* | 00 | 01 | - | - | 01 |

Based on the experiment carried out on the Methanolic extracts of water leaf against bacteria inoculum was observed that there was high inhibitory activity on *staphylococcus aureus* 27mm at concentration of 1,000ug/disc, 9mm at 500ug.disc, 8mm at 250ug/disc, 7mm at 125ug/disc. The positive control was 25mm and the negative control is 0. The act extracts is higher than the positive control.

*For klebsiella pneumonia* the highest is 8mm at the concentration of 1000ug/disc, 8mm at 500ug/disc, 0 at 250ug/disc and 0 at 125ug/disc. The negative is 0 and the positive 40mm, the positive is higher than the extracts.

*For salmonella typhi* the highest is at 1000ug/disc and 500ug/disc with 10mm, followed by 5mm at 250ug/disc. There was no activity at 125ug/disc. The negative is 0 while the positive is 32mm.

## 4.4 ANTIBACTERIAL ACTIVITY OF AQEOUS EXTRACT OF TOBACCO LEAVE (*Nicotiana tubacum*)

Table 4: sensitivity test of aqueous extract against the test organism (zone of inhibition)

| Isolates | 1000ug/disc D.M.S.O | 500ug/disc | 250/disc | 125ug/disc | Ampiclo V 5mg/disc |
|---|---|---|---|---|---|
| *Staphylococcus Us aureus* | 1000 | 08 | 06 | 05 | 25 |
| *Klebsiella Pneumonia* | 1800 | 10 | 00 | 00 | 45 |
| *Salmonella* Typhi | 1000 | 06 | 00 | 00 | 25 |

Interpretation

Based on the experiment carried out on the aqueous extract of Tobacco leave(*Nicotiana tubacum*)

against bacteria inoculums 18mm was observed that there was high inhibitory activity on *klebsiella*

*pneumonia* 18mm at the concentration of 1000ug/disc, 10mm at 500ug/disc, 0 at 250ug/disc and 0 at 125ug/disc. The negative 0 is the positive 45mm.the positive is higher than the extract.

For *salmonella typhi* the highest inhibition is at 1000ug/disc with 10mm and 6mm at 500uh/disc. There was no activity at 250ug/disc and 125ug/disc, the negative is 0 while the positive is 25mm.

**4.5 DISCUSION**

The result of phytochemicals in the present investigation showed that the plant leaves contain components like tannins, saponins, phenol, flavonoids, glycosides. The antibacterial activity of Methanolic extract of *waterleaf* showed maximum *zone* of inhibition (27mm) against *staphylococcus aureus,* followed by *salmonella typhi* (10mm) and *klebsiella pneumonia* (18mm) against *klebsiella spp,* followed by *staphylococcus aureus ans salmonia typhi* with 10mm. the methanol and aqueous extract showed considerable activity against the bacterial inoculum, the methanol extract was more active than the standard against *staphylococcus aureus,* previuos study cinducted by (Gueddeur *et al .2002*) *suggests that the essential oil of O, majorana* possess antibacterial activity. The

work conducted by (Farooqi and Sreeramu, 2004) reveals that the leaves of majoram have antibacterial acitivity against *Escherichia Coli, pseudomonas aeroginosa, staphylococcus aureus* and *salmonella typhi,* similary antibacterial activity of ethanol, chlorofon and water extract of *Marrubium vulgare,* was further assessed against, *salmonella typhi, staphylococcus aureus, Escherichia coli* and *pseudomonas aeruginosa,* were recorded (AL-Bakri *et al., 2006*).

The presence of these phytochemical components may be responsible for the observed antimicrobial activity of the plant leaf extract. This findings conforms to the report of (Anyanwu and Dawet, 2005) in which similar constituents was found to exhibits antiprotozoal and antibacterial activities, Flavonoids has also been reported to have greater potential benefit to human health (Jouad *et al., 2001*). Imran Khan *et al., 2010* studied that phytochemicals analysis of water leaves by using different solvent such as petroleum ether, chloroforms, methanol show the pressure of triterpenes, glycoside and fatty acids. Other phytochemicals studied in this analysis were absent in all extract of leaves Antibacterial activity of water leaf was analysed by previous workers showed that the chloroform extract of leaves possess significant activity, than petroleum other and methanol extract. Himal paudel *et al.,* 2008 reported that the ethanol extract of water leaf whole plant shows presence of flavonoids and tannins only similarly the extract of water leaf is active against *Ecoli* followed by *staphylococcus aureus,* Earlier

observation done by (Srinivasan *et al.,* 2001) also showed the antifungal and antibacteria activity of water leaf.

# CHAPTER FOUR

# CONCLUSION AND RECOMMENDATION

## 4.0 CONCLUSION OF TOBACCO LEAVE (*Nicotiana tubacum*)

## 5.1 CONCLUSION

Large-scale cultivation of tobacco and the amount of waste from the tobacco processing industry is huge, and improper treatment may lead to nicotine poisoning, environmental pollution, and biomass waste. With the discovery of many bioactive substances in tobacco, tobacco waste can become a very useful new raw material. Compounds present in tobacco exhibit important biological activities including resistance to numerous diseases, regulation of human health, sterilization, and pest control. Such a wide range of application scenarios gives a high potential value to the by-products of the tobacco processing industry. During the development of new tobacco related products, the first consideration is the harm, which nornicotine can cause to the human body, in order to avoid toxic and side effects. Secondly, the influence of intestinal flora on tobacco bioactive substances should be considered for health products from tobacco waste, and tobacco fermentation products will be a potential research direction. Finally, the influence of the extraction method on the results is critical in the industry.

The unique morphological characteristics and phytochemical composition of tobacco leaves contribute to their medicinal importance and potential therapeutic applications. While the use of tobacco products is well-known for its harmful health effects, the responsible and controlled use of tobacco-derived compounds could lead to the development of new and effective therapeutic agents.

## 5.2 RECOMMENDATION

- ✓ Further studies should be carried out with other pathogenic bacteria in evaluate the antibacterial activity.
- ✓ Further studies should be carried out on other parts of Tobacco leave
- ✓ to determine the presence of photochemical in them
- ✓ Further studies should be carried out with the other solvent to know their effectiveness.

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