ANTIMICROBIAL EFFECT OF *Nicotiana tabacum* (TOBACCO) LEAF EXTRACT ON *Staphylococcus aureus* and *Escherichia coli*

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Abstract: The present study evaluates the phytochemical composition of ethanolic extract and antimicrobial property of ethanolic and aqueous extracts of *Nicotiana tabacum.* The antimicrobial effects of *Nicotiana tabacum* (tobacco) leaf extract on microbial isolates *(Staphylococcus aureus* and *Escherichia coli)* was evaluated using using agar well diffusion method. Also, the Phytochemical screening of the plant was done using standard chemical methods. Phytochemical screening of ethanol extract of *N. tabaci* detected the presence of alkaloids, tannins, saponins, flavonoids and cyanogenic glycosides. The antimicrobial effects of tobacco leaves using ethanol and water showed that the extract inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* by a diameter of 2.8 and 3.6cm respectively while water extract inhibited the organisms by a diameter of 1.2 and 1.4cm respectively. At a concentraton of l.OOmg/ml of *Nicotiana tabacum* leaf extract *Staphylococcus aureus,* an inhibition of 2.3cm was observed while on *Escherichia coli,* the same concentration of the extract inhibited 2.8cm The mineral estimate in *N. tabaci* leaf using atomic absorption spectrophotometer (AAS) showed that Fe is 648.53mg/kg, Mg, 640.33mg/kg, Na, 7021.30mg/kg, K, 3128.63mg/kg, Ca, 17,551.33mg/kg, and Zn, 46.30mg/kg. The result of this study validates the use of tobacco leaf extract as snuff in treatment of cold and catarrh as it is commonly used by the elderly in Eastern Nigeria and can also be used in eliminating infections caused by gram positive and gram negative bacteria.

Keywords: antimicrobial, *Nicotiana tabacum,* phytochemical, pathogens

Introduction

In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant (WHO, 2001).Therefore, there is needed to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful and active ingredients which can serve as sources and templates for the synthesis of new antimicrobial drugs (Pretorius *et al.,* 2003; Moreillium *et al.,* 2005). The use of medicinal plant is the most ancient

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approach of healing known (Iwu, 1993). Its uses in treating various forms of ancient diseases both microbial and non-microbial origin, date back to prehistory. An impressive number of modern drugs have been isolated from medicinal plants like Artemisinin and taxol, many of which are based on their use in traditional medicine (Sofowora, 1993; Adesanya, 2005). Traditional medicine has been known for centuries in many parts of the world with deep sociocultural influence, synthesis of many modern drugs are based on this form of medicine (Sofowara, 1993) Reputed efficacies of plants use in this practice are reported to have been experienced and passed down from one generation to the other (Rukangira, 2001).

Nicotiana tabacum (tobacco) is a perennial herbaceous plant. It is found only in cultivation, It grows to heights between 1 to 2 metres. N. tabacum is a native of tropical and subtropical America but it is now commercially cultivated worldwide (Rakesh et al., 2013). Other varieties are cultivated as ornamental plants or grow as a weed. *Nicotiana tabacum* Linné is a robust annual little branched herb up to 2.5 m (8.2 ft) high with large green leaves and long trumpet shaped white-pinkish flowers. All parts are sticky, covered with short viscid-glandular hairs, which exude a yellow secretion containing nicotine (Rakesh et al., 2013).

Nicotiana tabacum possesses various pharmacological activities which have been reviewed (Rakesh et al., 2013). However, it is imperative that more clinical and pharmacological studies should be conducted to investigate the unexploited potential of this plant (Greer and Poulson, 1983). In addition, it had a wide variety of uses for physical complaints, such as venomous bites and stings, internal and external parasites, and the symptomatic relief of pain, which justifies its wide use and appreciation by traditional practitioners all over the world (Haber, 1994). It has many practical folklore traditional medicinal uses even though a vast population are dependent on it as a result of addiction due to its caffeine content (Sairam et al., 2003). It is understood that if used in positive ways it had the power to heal and protect; but if abused, it also had the power to harm (Giannopoulou, 2003).

Tobacco leaf contains several pyridine alkaloids, the principal one being a liquid alkaloid, nicotine. Other alkaloids present include nicoteine, nicotimine, anabaine anatalline and nornicotine. It also contains a high percetntage of organic acids. Leaves also contain glucosides, tahacinin, tahacilin and iso-quercitrin, 1-quinic, chlorogenic, caffeic and oxalic acids. They also contain terpenic and carcinogenic substances (Rakesh et al., 2013).

In Nigeria, many people treat different forms of infections using

therapeutic remedies made from plants. It has been shown that traditional medicines have genuine utility and about 80% of the rural population depend on them as a source of primary health care (Rakangira 2001; Olukoya *et al.*, 1993). This has been attributed to their easy accessibility, availability and relatively low cost of production compared to conventional pharmaceutical products (Nwachukwu *et al.*, 2010) Many traditional medicine practitioners in Nigeria use variety of plants to treat different kinds of microbial infections such as abscesses wound and skin infections gonorrhea, ulcers, dysentery and typhoid fever Olukoya *et al.*, 1993; Akinyeni *et al.*, 2005).

Methanol extract of *Picralima nitida* seed and *Musa paradisiaca* stalk and peel inhibited the growth of *Aspergillus niger, Aspergillus oryzae* and *Rhizopus stolonifer* (Okorondu, 2011; Okorondu *et al.*, 2012). Ethanol extract of *Moringa oleifera* and *Jatropha curcas* leaves inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* (Okorondu *et al.*, 2013).

Medicinal plants contain physiologically active principles which over the years have been exploited in traditional medical practice for the treatment of various ailments (Adebanjo *et al.*, 1983). Okigbo and Emoghene (2004) reported the use of plant extract from *Ocimum gratissimum* and *Azadiracta indica* as a substitute for chemical pesticide for control of sigatolka disease of banana.Ali *et al* (1988) had earlier demonstrated that citrus wastes are a potential source of biologically active principles. They tested C. reticulate seed extracts against some fungal species namely *fusariumsolani, Helminthosporium sativum,Aspergillus flauus* and*Aspergillus niger.* All except *Aspergillusflavus* and *H. Sativum* were strongly inhibited by extracts from C. Reticulate seed.Leaf extracts of *Chromolaema odorata*(L.) with salt are used to scent aromatic baths (Liogier, 1990). Extracts of this plant have been shown to inhibit or kill *Neisseria gonorrhea* (Laceres *et al.*, 1995) and to accelerate blood clothing (Triratana *et al* 1991). The leaves of *caganuscajan* (L.) millsp.., can be used for toothache, mouth wash, sore gums, child delivery and dysentery (Duke, 1981, Okigbo and Omadamiro, 2007).

Enterotoxigenic *E. coli* is a common cause of traveler's diarrhea and also most common cause of urinary tract infection (Jawetz *et al.*, 1989). *Salmonella typhi* has been reported to cause Salmonella gastroententis in humans and several Virulent Serovars of *Salmonella typhi* has been reported to cause Salmonella gastroenteritis in humans and several virulent serovars of *Salmonella typhi* cause typhoid fever (Prescott *et al.*, 1996). The prevalence of microbial resistance to existing antimicrobial drugs especially the beta-lactam antibiotics and therefore underscores the need for the continuous search for new antimicrobials (Olorundare *et al.*,

1998). The consequence of drug resistance implies that new drugs, both synthetic and natural, must be bought to treat disease for which known drugs are no longer useful (Okigbo and Omodamiro, 2007). One of the avenues for such a search is to screen local medicinal plants for likely antimicrobial activities (Okigbo and Omaolamiro, 2007).

Materials and Methods

Test samples

Fresh leaves of *Nicotiana tabacum* (Tabacco) were purchased from old market, in Owerri, Imo State. These leaves were taken for proper botanical identification at the crop science department of the Federal University of Technology Owerri.

Extraction

The leaves were cut into small pieces and sundried for 4 days and ground using a blending machine unit until a fine powdery form was obtained. 30g of the powder was weighed into the soxlet extractor. The extraction process was done in Chemistry Department of Federal University of Technology Owerri. It was done under 3-4 hours using 150mls of ethanol. The ethanol extract was concentrated into dryness by evaporation of the solvent in a steam bath and the weight was noted.The sample was labeled and was stored in sterile container and refrigerated at 4°C until used. According to the method of El-fallal and El-kattan (1997) 10 g of the powder plant material were mixed with 100 ml of boiled distilled water, put glass beaker in the incubator vibrators in temperature of 28 °C for 30 min., was nominated mix the use of medical gauze, distributed filtrate in glass tubes and have renounced at 3000 r/min. for 10 min., collecting the filtrate in glass dishes (diameter 20 cm) of water and dry it in the oven at a temperature 40 °C until the water evaporates completely, to get a hot water extract powder, The dried residues were collected in a labeled sterile McCartney bottles.

Test Organisms

Clinical isolates of Gram positive *Staphylococcus aureus* and gram negative *Escherichia coli* were obtained from Microbiology Laboratory, Federal Medical Center Owerri, Imo State. The organisms were isolated on Nutrient agar and subcultured onto nutrient agar slants. The slants were incubated at 37°C overnight.

Antimicrobial Susceptibility Test (Agar well diffusion test)

The level of susceptibility of each of the test organism was determined using agar well diffusion method (Pelczer and Chan, 1977). Nutrient agar was prepared according to the manufacturer's instructions, autoclaved and dispensed into sterile Petri dishes and allowed to set before use. The plates were inoculated with the test isolates. Afterwards, a sterile cork borer of 5mm diameter was used to

make holes on the nutrient agar plates. 0.2ml of the extract was filled into each appropriately labeled well. The inoculated plates were kept at room temperature for 30 minutes to allow the extract to diffuse into the agar and were incubated at 37°C for 18-24 hours. Antimicrobial activity was determined by zones of inhibition and this is quantified by measuring the diameter of zone of inhibition in (cm) using a meter rule after incubation.

Determination of Minimum Inhibition Concentration (MIC)

The minimum inhibitory concentration was defined as the lowest concentration of the assayed extract that inhibited any visible growth of the test organism (Prescott, *et al.*, 1999; ShahideBonjar, 2004). To determine the MIC, Serial dilutions of the extract were carried out and an aliquot of the extract (0.2g) as dissolved in 100mls of distilled water to obtain 2.0mg/ml. This 2.0mg/ml was then double diluted in sterile distilled water to obtain concentrations of 1.0, 0.50, 0.025, 0.0125, 0.0625, 0.0325mg/ml. The same procedure was carried out for the ethanolic and water extracts. Overnight cultures of the nutrient broths were standardized using 0.5 McFarland's standards. Then cultured to Nutrient agar plates before for Kirby Bauer method for MIC test.

Phytochemical Tests

Freshly prepared ground samples were chemically tested for the presence of chemical constituents using standard procedures (Trease and Evans, 1983). Water and ethanol are commonly used in the extraction of phytochemicals such as alkaloids, Tannins, saponins, flavonoids and Cyanogenic Glycosides.

Test for Alkaloids

In line with methods from AOAC (2005), 1.0ml of extract of the sample is shaken with 5.0ml of 2% HCl on a steam bath and filtered. The filtrate was evaporated to dryness on a steam bath, the impure crystalline substances dissolved in 5ml of pore chloroform, and 3mls of sulfuric acid and the mixture was carefully shaken. A layer appears and was allowed to separate. The lower chloroform layer was removed and the upper layer retained. These steps were repeated until the upper layer became colourless. Concentrated ammonia was added to make it alkaline. 3mls of chloroform was added to the extract and evaporated to dryness, and the pore crystals produced are alkaloids.

Test for Flavonoids

In line with methods from AOAC (2005), 1.0ml of extract was placed in a test-tube and 1.0ml of 10% lead acetate is added. The formation of yellow precipitate is taken as positive for flavonoids.

Test for Tannins

A mixture of 1ml of the plant extract is added to an equal volume of bromine water, the formation of a greenish to red precipitate is taken as evidence for presence of condensed tannins.

Test for Saponins

A mixture of 1.0ml of extract is boiled with 5.0ml of water for 5 minutes and decanted while still hot. The filtrate is used for the frothing test. 1.0ml of the filtrate is diluted with 4.0ml of distilled water, shaken vigorously and observed on standing for stable froth.

Test for Cyanogenic glycosides (Qualitative)

In line with methods from AOAC (2005), 1.0g of the plant extract is covered with sufficient water in a stoppered flask into which sodium pierate paper is suspended by trapping it with a cork. The flask is placed in a water bath for 1hour. A change from the yellow colour of the paper to brick red colour is a positive result for cyanogenic glycosides.

Test for Cyanogenic Glycoside (Quantitative)

In line with methods from AOAC (2005), 1.0g dry ground sample is weighed into a 250mlround bottom flask.200ml of distilled water is added and allowed to stand for 2 hours. An antifoaming agent (silicon oil) is added before distillation. Full distillation is then carried out and 150-170ml of distillate is collected in a 250ml conical flask containing 20ml of 2.5% NaOH. To 100ml of the distillate containing cyanogenic glycoside, 8ml of 6N NH_4OH and 2ml of 5% KI is added, mixed and titrated with 0.02N silver Nitrate ($AgNO_3$) using a micro burette. Permanent turbidity indicates end point.

Cyanogenic glycoside content of sample is calculated thus;

$$Cyanogenic\ glycoside\ mg/100g = \frac{Titre\ value\ (ml) \times 1.08(g) \times extract\ vol\ (ml) \times 100}{\text{Aliquot vol (ml)} \times \text{sample Weight (g)}}$$

Metal Analysis of *Nicotiana tabacum*

Metal Analysis was carried out using Atomic Absorption Spectrophometer (NETAL CAPHA D3110). Sample preparation was by acid digestion, followed by filtration through a 0.45 micro membrane filter then aliquots of the filtrate were used for analyses of the various metals AOAC, 2005.

Sample Digestion

Ash was obtained from the extracts and 3g each of the ash sample was digested with 5ml nitric acid to a minimum value of about 5ml. The digest was filtered through a whatman No.44 filter paper directly into acid. A properly washed and well rinsed plastic container was

made up to 50ml mark in a volumetric flask. A reagent blank using 5ml nitric acid was also incorporated.

Instrumental Analysis

The heavy metals were determined using a flame atomic absorption spectrophotometer SOLAAR 32 AA and using the appropriate hollow cathode lamp and resonance wavelength of the metals.

$$The\ concentration\ (mg/kg) = \frac{(x - y)\,V_1}{V_2}$$

where x = concentration of the metals obtained from atomic absorption spectrophotometer for sample (mg/l)

y = Concentration of the metal obtained from atomic absorption spectrophotometer instrument for blank.

V_1 = Volume of digest sent for analysis (ml).

RESULTS

The results shows the zone of inhibition of the *Nicotiana tabacum* extract with *Staphylococcus aureus* and *Escherichia coli* using the agar diffusion method. It was deduced that the sample, extracted with ethanol proved to be more effective against the test organism by expressing a wider diameter of inhibition around the bored wells on the surface of the culture medium. *Staphylococcus aureus* and *Escherichia coli* exhibited diameters of 2.8cm and 3.6cm respectively, while the sample extracted with water on the other hand showed a narrower diameter of inhibition in the antimicrobial assay with the above organism showing diameter of 1.2cm and 1.4cm respectively as seen in table 1.

The antimicrobial assay of the plant shows that the growth inhibition of *Staphylococcus aureus* under the highest concentration of 1.00mg/ml is 2.3cm, 0.5 = 1.8cm, 0.025 = 1.4cm, 0.0125 = 1.2cm, *Escherichia coli* at concentration of 1.00mg/ml is 2.8cm, 0.50 = 2.2cm, 0.025 = 1.8cm, 0.125 = 1.4cm, 0.0625 = 1.0cm. The minimum inhibitory concentration was recorded at concentration of 0.0625mg/ml for *Escherichia coli* and 0.125mg/ml for *Salmonella typhi respectively* as shown in table 2.

It was deduced from the qualitative phytochemical screening of *Nicotiana tabacum* (tobacco leaf) that tannin, alkaloid, saponnin, flavonoid and cyanogenic Glycosides were all present in the test sample.

The mineral estimate in *Nicotiana tabacum* (Tabacco leaf) using atomic absorption Spectrophotometers (AAS) shows that it contains iron (Fe) 648.53mg/kg, magnesium (mg) 640.33mg/kg, sodium (Na) 7021.30mg/kg potassium (k) 3128.63mg/kg, calcium (Ca) 17.551.33mg.kg, and zinc (Zn) 46.30mg/kg as shown in table 4.

Table 1: Agar well diffusion (Zone of Inhibition)

Solvent	Test organisms	Agar diffusion method (zone of inhibition)
Ethanol	*Staphylococcus aureus*	2.8cm
	Escherichia coli	3.6cm
Water	*Staphylococcus aureus*	1.2cm
	Escherichia coli	1.4cm

Table 2: Minimum inhibitory concentration of *Nicotiana tabacum* (tobacco leaf) extract on test organisms (cm)

Test tubes number	1	2	3	4	5	6
Concentrations	1.00 mg/ml	0.50 mg/ml	0.025 mg/ml	0.125 mg/ml	0.0625 mg/ml	0.0325 mg/ml
Test organism						
Staphylococcus aureus	2.3	1.8	1.4	1.2	-	-
Escherichia coli	2.8	2.2	1.8	1.4	1.0	-

Table 3: Phytochemical screening of *Nicotiana tabacum* (Tobacco leaf) sample

Alkaloid	flavonoids	Tannin	Saponnin	Hydrogen cyanide
+	+	+	+	106.38ppm

(+) present

Table 4: Mineral estimate in *Nicotiana tabacum*(Tobacco leaf)

(Fe)	Mg	Na	K	Ca	Zn
648.53 mg/kg	640.33 mg/kg	7021.30 mg/kg	3128.63 mg/kg	17.551.33 mg/kg	46.30 mg/kg

Iron (Fe),) Magnesium (mg), Sodium (Na) Potassium (K) Calcium (Ca), Zinc (Zn)

Discussion and Conclusion

The ethanol extract of *Nicotiana tabacum* exhibited antibacterial effect against gram positive and gram-negative bacteria. It expressed inhibitions in agar well diffusion 2.8cm for *staphylococcus aureus* and 3.6cm for *Escherichia coli,* while the extract water extract expressed inhibitions of 1.2cm for *staphylococcus aureus* and 1.4cm for *Escherichia coli* water extract showed appreciable activity on the tested organism *staphylococcus aureus* and *Escherichia coli.* The results obtained from ethanol extract of *Nicotiana tabacum* (Tabacco leaf) showed the susceptibility on *Escherichia coli* and *staphylococcus aureus.* This probably indicates that there are bioactive ingredients that are inhibitory to the growth of these common pathogens. (Etani *et al.,* 1998). Previous studies have also shown that Ethanol

extracts exhibited the highest inhibitory effect on *Staphylococcus aereus* and *Escherichia coli,* compared to hot water (Nwankwo and Amaechi, 2013; Nwankwo *et al.,* 2014). This effect is as a result of the degree of polarity and the nature of the solute as it has been reported by some workers that organic solvent is better than aqueous extracts due to its ability to dissolve organic components in the plant (Okigbo *et al.,* 2003). This study tried to show that the conservation of this plant should be a priority to many plant scientists.

As shown in table 2, at a concentraton of 1.00mg/ml of *Nicotiana tabacum* leaf extract *Staphylococcus aureus,* an inhibition of 2.3cm was observed while on *Escherichia coli,* the same concentration of the extract inhibited 2.8cm. The minimum inhibitory concentration of ethanolic extrats of *Nicotiana tabacum* was recorded at concentration of 0.0625mg/ml for *Escherichia coli* and 0.125mg/ml for *Salmonella typhi* respectively as shown in table 2. The least zone of inhibition was observed with *Staphylococcus aureus,* which expressed the lowest inhibition for both ethanol and water extract sample of tobacco with metric value of 2.8cm and 1.2cm respectively. It is possible that the antibacterial activity exhibited by the extracts of this tobacco leaf may be attributed to the presence of Alkaloids, flavonoids and other phytochemicals in substantial amounts as observed in the phytochemical screening. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effect on humans and this has led to the developments of powerful pain killer medications (Kam and Liew, 2002). The result of this study justifies the use of ethanol extract of *Nicotiana tabacum* (Tabacco leaf) in medicine for the treatment of infectious disease caused by bacteria. The heavy metals were determined using flame atomic absorption Spectrophotometer (AAS) showed the presence of iron Fe(648.53mg/kg) Magnesium (Mg 640.33mg/kg), Sodium (Na 7021.30mg/kg) Potassium (K 3128.63mg/kg) Calcium (Ca 17,551.33mg/kg) zinc (zn 46.30mg/kg).

Conclusion

In this study, the ethanol extract from *Nicotiana tabacum* (Tabacco leaf) have the highest antimicrobial property. It has a wide spectrum of activity as it was able to inhibit gram positive *Staphylococcus aureus* and gram negative bacteria *Escherichia coli* whereas the other solvent water had little effect on the

organisms. Furthermore, the phytochemical screening of *Nicotiana tabacum* (Tabacco leaf) extract shows the presence of alkaloid, tannin, saponnin, flavonoids and cyanogenic glycosides and the mineral estimate in *Nicotiana tabacum* (Tabacco leaf) using atomic absorption spectrophotometer (AAS) shows the presence of Fe=648.53mg/kg, Mg=640.33mg/kg, Na=7021.30mg/kg, K=3128.63mg/kg, Ca=17,551.33mg/kg, and Zn=46.30mg/kg. Greater work should be done on this plant so as to utilize its medicinal and nutritional characteristics.

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