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Phytochemical, Antimicrobial and Proximate Composition of *Nicotiana tabacum* Leaves Extract

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Abstract: - *Nicotiana tabacum* has been widely used as a therapeutic plant in Asian countries including China, India, Cambodia, Nepal, Malaysia and Iran. The leaves of *Nicotiana tabacum* have been reported to activate biological mechanisms such as antibacterial, antifungal, antimicrobial, anthelmintic and anti-Alzheimer's activities. This study was conducted to investigate the proximate, antimicrobial and phytochemical composition of tobacco leaves (*Nicotiana tabacum*) extract. *Nicotiana tabacum* leaves were subjected to proximate analysis and the alcoholic extract was investigated for antimicrobial inhibition against a wide range of bacteria using agar well diffusion method. The extract was also investigated for phytochemical composition. The alcohol extract subjected to analyses was prepared using 95% ethanol as extracting solvent. The proximate analysis showed that the leaves of *Nicotiana tabacum* contain significantly high protein, carbohydrate and fibre while the fat, ash and moisture content were significantly low. Phytochemical analysis of the alcohol extract of *Nicotiana tabacum* revealed the presence of alkaloids, phenolic compounds, tannins, flavonoids, steroids, terpenoids, resins and essential oil. The antimicrobial analysis results revealed that the alcohol extract of *Nicotiana tabacum* inhibited the growth of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus faecalis*, *Pseudomonas syringae* and *Xanthomonas axonopodis* by 2.1, 2.2, 2.0, 2.7, 2.5 and 2.3cm respectively. At a concentration of 0.2mg/ml of *Nicotiana tabacum* leaves extract, inhibition of 1.3, 1.2, 1.4, 1.5, 1.3 and 1.4cm was observed on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus faecalis*, *Pseudomonas syringae* and *Xanthomonas axonopodis* respectively which showed an increase progressively. At a concentration of 0.5mg/ml of *Nicotiana tabacum* leaf extract, an inhibition of 1.8, 1.9, 1.8, 2.1, 2.0 and 1.9cm was observed on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus faecalis*, *Pseudomonas syringae* and *Xanthomonas axonopodis* respectively. The results of the different analyses presented here may suggest that the extract of *Nicotiana tabacum* may be used as natural preservative ingredients in food and/or pharmaceuticals for combating infections caused by bacteria because of its nutritional, medicinal and pharmacological properties.

Keywords: *- Antimicrobial, Phytochemical, Proximate, Nicotiana tabacum, Extract, Inhibition*

I. INTRODUCTION

Wide varieties of plant materials are believed to possess different medicinal value. Tobacco particularly has been found to be one of the most commonly studied plants and a very important economic crop due to its potentials in healing of various ailments such as abdominal discomfort, constipation, urinary tract obstruction, dental pain, gastrointestinal disorders and dermatitis [1, 2]. Tobacco is an agricultural product processed from the leaves of plants in the genus Nicotiana. It is most commonly used as drugs and it is a valuable cash crop for countries such as India, China and the United States [3]. *Nicotiana tabacum* have been reported to activate biological mechanisms such as antibacterial, antinoniceptive, antifungal, antimicrobial, anthelmintic and anti-Alzheimer's activities [2]. Lately, there have been a drastic increase in antibiotic resistant strains of clinically important pathogens, which have resulted in the emergence of new bacterial strains that are multi-resistant [4]. This therefore poses a need to look for substances from other sources with proven antimicrobial activity and a 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 new synthesis of antimicrobial drugs [5]. *Nicotiana tabacum* possesses various pharmacological activities which have been reviewed [3]. However, it is imperative that more clinical and pharmacological studies be conducted to investigate the unexploited potentials of this plant. The specific aim of this work was therefore to investigate the proximate, antimicrobial and phytochemical composition of tobacco leaf (*Nicotiana tabacum*) extract.

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II. MATERIALS AND METHODS

➢ *Test Samples*

Fresh leaves of *Nicotiana tabacum* were purchased from Oja-Oba market, in Ondo state, Akure and authenticated by crop science and production department of the Federal University of Technology, Akure. The tobacco leaves obtained were thereafter washed under running water to remove the contaminants and other extraneous matter. Thereafter, they were chopped into small pieces and thereafter sun-dried for a week. To ensure complete drying, the sun-dried leaves were transferred into an oven at a 120^oC for 2 hours and then ground to fine particles with blending machine. The powdered leaves were kept in an air-tight container and kept in the refrigerator at 4^oC prior to laboratory analysis.

A. Extraction

The extraction process was carried out in the analytical research laboratory, Chemistry department of Federal University of Technology, Akure. 500g of the powder was weighed into the soxhlet extractor. The extraction was carried out under 72 hours using 900ml of 95% ethanol. The ethanol extract was concentrated into dryness by evaporation of the solvent in a steam bath and the weight was recorded. The sample was labelled and then stored in a sterile container which was thereafter stored in refrigerator at 4^oC prior to analyses.

B. Determination of Proximate Composition

The proximate composition of the plant material gives information on the level of acceptance of the material in general nutrition. The moisture content, protein, crude fat, crude fibre, carbohydrate and ash were determined using the method of AOAC [6]. All chemicals used were of analytical grade and as recommended by the manufacturer.

C. Phytochemical Analyses

The phytochemical evaluation of the ethanol extract of *Nicotiana tabacum* was performed to identify the plant chemicals of alkaloids, phenolic compounds, tannins, flavonoids, steroids and terpenoids.

➢ *Test for Alkaloids (Dragendorff's, Mayer's and Wagner's Tests)*

3 ml filtrate of the extract was loaded equally into three test tubes. Each test tube was treated with few drops of Drafendorff's, Mayer's or Wagner's reagents. Orange red precipitate (Dragendorff's) indicates the presence of alkaloids; creamy white precipitate (Mayer) points out the presence of alkaloids; and reddish brown precipitate (Wagner) demonstrates the presence of alkaloids [7].

➢ *Test for Phenolic Compounds (Ferric Chloride Test)*

1 ml filtrate of the extract was pipetted into the test tube and added with 2 or 3 drops of 5%-$FeCl_3$. The formation of greenish precipitate indicates the presence of phenolic compounds [8].

➢ *Test for Tannins (Ferric Chloride Test)*

10 ml filtrate of the extract was transferred into the test tube and added with 2 or 3 drops of 0.1% of $FeCl_3$. The brownish green or blue-black coloration interprets the presence of tannins [9].

➢ *Test for Flavonoids (Ammonium Test)*

10 ml filtrate of the extract was taken into the test tube and added with 1 ml of 1%-ammonium solution. The mixture was shaken vigorously. The formation of yellow color observed in the ammonia layer demonstrates the presence of flavonoids [10].

➢ *Test for Steroids (Liebermann Burchard Test)*

2 ml chloroform was added to the extract of 100 mg and filtered into the test tube. The mixture was added with 1 ml of glacial acetic acid, followed by carefully the addition of 1 ml of H_2SO_4 down the side of the test tube. Greenish color indicates the presence of steroids [11].

➢ *Test for Terpenoids (Salkowski Test)*

5 ml chloroform was added to the extract of 100 mg and filtered into the test tube. The mixture was added carefully with 3 ml of H_2SO_4 down the side of the test tube. Reddish brown color at the interface of the two liquids characterizes the presence of terpenoids [12].

➢ *Test for Cardiac Glycosides (Keller-Kiliani Test)*

2 ml of glacial acetic acid with 2 drops of 2%-$FeCl_3$ were added to 100mg of the extract in the test tube. The mixture was added with 1 ml of H_2SO_4 down the side of the test tube. Brown ring at the interface indicates the presence of cardiac glycosides [12]

➢ *Test for Essential Oils (NaOH-HCl Test)*

In the test tube, 2-ml filtrate of the extract was added with 100 µl of 1M-NaOH, followed by the addition of few drops of 1M-HCl. The mixture was shaken. White precipitate demonstrates the presence of essential oils [13].

➢ *Test for Saponins (Froth Test)*

10 ml of distilled water were added to 200 mg of the extract and filtered into the test tube. The mixture was shaken for 10 min until the formation of stable persistent froth. Formation of stable five-minute-persistent froth indicates the presence of saponins [14].

➢ *Test for Resins (Turbidity Test)*

10 ml of distilled water were added to 200 mg of the extract and filtered into the test tube, and the mixture was observed. Occurrence of turbidity shows the presence of resins [13].

➢ *Test for Quinones (H_2SO_4 Test)*

1 ml of H_2SO_4 was added to 1-ml filtrate of the extract. Red color indicates the presence of quinones [15].

➢ *Test for Polypeptides (Biuret Test)*

3 ml filtrate of the extract was added with 1 ml of 40%-NaOH, followed by the addition of 2 drops of 1%-$CuSO_4$. Violet color indicates the presence of polypeptides [16].

D. Antimicrobial Analysis of the Leaf Extract

Six different microorganisms were used which included gram (-) bacteria *Escherichia coli*, *Pseudomonas syringae*, *Xanthomonas axonopodis* and gram (+) bacteria *Staphylococcus aureus*, *Bacillus cereus* and *Streptococcus faecalis*. The clinical isolates were obtained from the department of microbiology, Federal University of Technology, Akure. The microorganisms were isolated on Nutrient Agar and sub-cultured 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. 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 using a digital vernier caliper.

➢ *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. 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 0.5, 0.3, 0.2mg/ml. The same procedure was carried out for the ethanol extract. 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.

➢ *Statistical Analysis*

The experiment results were expressed as mean ± SD of three replicates. Data obtained were statistically analyzed using one-way Analysis of Variance (ANOVA), a tool in Statistical Packages for Social Sciences (SPSS 17.0). The level of significance was set at $p \leq 0.05$. Means were separated with Duncan multiple range test.

III. RESULTS AND DISCUSSION

Proximate composition	*Nicotiana tabacum* leaves
Moisture content	4.10 ± 0.04
Crude protein	34.5 ± 0.06
Crude fibre	22.20 ± 0.08
Crude fat	6.35 ± 0.15
Ash	4.39 ± 0.17
Carbohydrate	23.46 ± 0.15

Table 1:- Proximate Composition of *Nicotiana tabacum* Leaves (%)

Values are mean ± standard deviation of triplicate determination (n=3).

Phytochemicals	Tests	Met. Ext
Alkaloids	Dragendorff Mayer Wagner	++ ++ ++
Phenolic compounds	Ferric chloride	+
Tannins	Ferric chloride	+
Flavonoids	Ammonium	+
Steroids	Burchard	+
Terpenoids	Salkowski	+
Resins	Turbidity	+
Essential oils	NaOH-HCl	+
Saponins	Froth	+
Quinones	H_2SO_4	+
Polypeptides	Biuret	-

Table 2:- Phytochemical Screening of the Leaf Extract of *Nicotiana tabacum*

Note: +ve; Positive (present); -ve; Negative (absent); Met Ext.: Methanol Extract

The proximate composition of *Nicotiana tabacum* leaf are shown in the table 1. The results showed that *Nicotiana tabacum* leaves contain significant high protein, carbohydrate and fibre. Carbohydrate are known to be good sources of high energy generation compounds. The appreciably low moisture content suggests a longer shelf life and reduced activity of micro-organisms.

The results of the phytochemical analysis of *Nicotiana tabacum* leaf extract are shown in the table 2. The phytochemical analysis of *Nicotiana tabacum* indicates the presence of high concentration of alkaloid and presence of phenolic compounds, flavonoid, tannins, quinones, saponins, steroids, terpenoids and resins also. This agrees with the results obtained by [17]. It was observed by [17] that nicotine, an alkaloid is the most biologically active component of tobacco. It was also found out by [18] that

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glycoside was positively tested in the leaves of *Nicotiana tabacum* which is in agreement with the result obtained in this research work. The presence of essential oil was also confirmed in tobacco leaf by [2] which is also in agreement with our study. Alkaloids, being one of the largest group of phytochemicals in plants have pronounced effect on humans which have led to development of pain killer medication [5].

Test organisms	Zone of inhibition
Escherichia coli	2.1cm
Staphylococcus aureus	2.2.cm
Bacillus cereus	2.0cm
Streptococcus faecalis	2.7cm
Pseudomonas syringae	2.5cm
Xanthomonas axonopodis	2.3cm

Table 3: Agar Well Diffusion (Zone of Inhibition)

Test tube no.	1	2	3
Concentrations	0.5 mg/ml	0.3 mg/ml	0.2 mg/ml
Test organism			
Escherichia coli	1.8	1.5	1.3
Staphylococcus aureus	1.9	1.6	1.2
Bacillus cereus	1.8	1.6	1.4
Streptococcus faecalis	2.1	1.8	1.5
Pseudomonas syringae	2.0	1.7	1.3
Xanthomonas axonopodis	1.9	1.7	1.4

Table 4:- Minimum Inhibitory Concentration of *Nicotiana tabacum* Extract on Test Organisms (cm)

The result of the antimicrobial test in table 3 revealed that the ethanol extract of *Nicotiana tabacum* exhibited antibacterial effect against gram positive and gram negative bacteria. It expresses inhibitions in agar well diffusion 2.1cm for *Escherichia coli.* 2.2cm for *Staphylococcus aureus*, 2.0cm for *Bacillus cereus*, 2.7cm for s*treptococcus faecalis*, 2.5cm for *Pseudomonas syringae* and 2.3cm for *Xanthomonas axonopodis*. The results showed the susceptibility of the microorganisms to the ethanol extract obtained from *Nicotiana tabacum* leaf extract. This probably indicates that there are bioactive ingredients that are inhibitory to the growth of these pathogens [5]. It has also been confirmed that alcohol extracts exhibited the highest inhibitory effect on these microorganisms [19]. The antibacterial activity exhibited by the extract may be attributed to the presence of alkaloids, flavonoids and other phytochemicals present in substantial amount as evident from the phytochemical analyses. Tannins have been reported to have pronounced antimicrobial activities. The result of this study justifies the use of ethanol extract of *Nicotiana tabacum* in medicine for treatment of infectious diseases by bacteria.

IV. CONCLUSION

The results obtained from the proximate analysis indicated that tobacco leaf is a good source of protein, carbohydrate and fibre. These properties suggest its potential as an excellent supplement for cereal diets and a source of dietary fibre. The results obtained from the phytochemical and antimicrobial analyses revealed that the leaves of *Nicotiana tabacum* has nutritional, pharmacological and medicinal properties which projects its potential as a source of medicine against a wide range of bacteria.

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