Journal of Medical Pharmaceutical And Allied Sciences
RESEARCH ARTICLE

www.jmpas.com
ISSN 2320-7418

TOBACCO PLANT *(Nicotiana tabacum L)* CRUDE EXTRACT AND ITS ANTIBACTERIAL ACTIVITY ON *ESCHERICHIA coli* AND *STAPHYLOCOCCUS AUREUS*

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Keywords
Phytochemical, crude extracts, Nicotiana tabacum, antibacterial and MIC

Received
12/02/2019
Reviewed
16/02/2019
Revised/ Accepted
22/02/2019

ABSTRACT

The Phytochemical constituents of aqueous and ethanolic crude extracts of dried and fresh *Nicotiana tabacum* leaves, stems, flowers and roots were examined and also investigated for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The Phytochemical constituents examined were flavonoids, alkaloids, cardiac glycosides, saponins, tannins, phenols, free anthracene, phytosterol, steroids and carbohydrates. Both the aqueous ethanolic extracts yielded phytochemical properties and also had antibacterial activity. The result also showed that all the four different parts of *Nicotiana tabacum* used had antibacterial activity with the leaves exhibiting highest activity. Generally, fresh *Nicotiana tabacum* extracts had higher antibacterial activity when compare to the dried parts of the plant. The Minimum Inhibitory Concentration (MIC) of both extracts against the test organisms was also determined.

INTRODUCTION

Antimicrobial agents are special kinds of chemotherapeutic substances usually obtained from microorganisms and plant which hinder the growth and activities of other microorganisms (Sofowora, 1984).The development of microbial resistance to available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants (Dash, *et al*., 2005; Naz, *et al*., 2010).

Despite the advances made in orthodox medicine, there has been an increasing interest on herbal medicines (Kollowoki, 1972). Globally, researchers are using extracts of plants for their antibacterial, antifungal and antiviral activities (Bakht *et al*., 2011a and b). It is reported that more than 400, 000 species of tropical flowering plant have medicinal properties (Yildirim *et al*., 2000).

Tobacco (*Nicotiana tabacum L.*) belongs to the family *Solanaceae* which also includes some other important crop species such as tomatoes, potatoes, peppers, etc. Tobacco nicotine inhibits the growth of pathogens which is dose dependent (Maria *et al*., 2007; Wang *et al*., 2008; Suresh *et al*., 2008).

Escherichia coli and *Staphylococcus aureus* are common pathogens known to resist some common available antibiotics. It is pertinent to note that great majority of orthodox drug product have some of the plants used as their source materials. With new diseases and causal strains of microbes emerging every day, research to determine medicinal value of plant species is not out of place. It is expected that extract from a common shrub like *Nicotiana tabacum L.* can be used to develop drugs for treatment of some infectious diseases cause by resistant strains of some microorganisms hence this study is aim at testing the antibacterial activity of aqueous and ethanolic extracts of *Nicotiana tabacum* against clinical isolates of *Escherichia coli* and *Staphylococcus aureus*.

MATERIALS AND METHOD

Sample collection

Nicotiana tabacum plants were collected from the villages near south core, University of Agriculture Makurdi, Benue state, Nigeria and taken to the Microbiology laboratory, University of Agriculture, Makurdi for analysis.

Qualitative Phytochemical Screening

The phytochemical screening of aqueous and ethanolic crude extract of both the dry and fresh leaves, stems, flowers and roots of *Nicotiana tabacum* was carried out using methods of Sofowora, (1984); Evans (1983); Obadoni and Ochuko (2001) respectively. The phytochemical properties screened were Carbohydrate, saponins, alkaloids, flavonoids, steroids, tannin, cardiac glycosides, phytosterol, free anthracene and phenol.

Preparation of the plant extract

Four (4) different parts of the *Nicotiana tabacum*: the leaves, stems, flowers, and roots of both fresh and dried were examined. These parts were allowed to dry at room temperature for one week and separately grounded to obtain powdery particles using wooden mortar and pestle. The resulting powdery substance of each part of plant was weighed to obtain two portions of each part of the plant. 40g from each part of the plant were soaked in 400ml of sterile distilled water and thoroughly mixed. The mixture was allowed to stay for 48hrs. Similarly, same 40g of the powder from each part of the plant was soaked in 400ml of 95% ethanol, thoroughly mixed and allowed to stay for 48hrs.

The same treatment was given to fresh parts of the plants that were properly grounded. Thereafter, all the mixtures were filtered first with muslin clothe filter followed by Whatman No 1 filter paper. The filtrates were concentrated using water bath at 45^0 to obtain dry extracts (Philip *et al*., 2009; Nagappan, 2012).

Test organisms

The test organisms (*Escherichia coli* and *Staphylococcus aureus)* were obtained from the Microbiology Laboratory of Federal Medical Centre Makurdi and confirmed in the Microbiology Laboratory of Federal University of Agriculture, Makurdi before using.

Determination of Antibacterial sensitivity

Agar diffusion techniques as described by Cheese rough (2000) were used to determine the antibacterial activity of the extracts. A stock solution of each plant extracts was prepared by dissolving 100 mg of extract with one ml of their respective solvents. Thereafter, Mueller Hinton agar was prepared and allowed to solidify.

The inoculum density was standardized to achieve a final concentration of 1.5 x 108 CFU/ml. Three single colonies of each of the test organisms from an agar plate culture were suspended in five ml of Mueller-

Hinton broth and incubated at 37 ºC for 18 hours (0.5 McFarland standard) (Jorgensen and Turnidge, 2015). A sterile cotton swab was dipped into the standardized bacterial inoculum suspension and was then streaked over the whole dried surface of the Mueller-Hinton agar plates earlier prepared. A sterile corn borer was used to bore four (4) holes in each of the seeded petri dishes after which 0.3mls each of the extract and each from the solvents used in the extraction as controlled was introduced into the holes. A broad spectrum commercial antibiotic disc (Gentamicin 10 µg) was placed at the centre of the plate as one of the controls. Each plate was allowed for 1 hour to diffuse before incubating for 24 hours at 37^0c. The diameter of the zones of inhibition was measured after 24 hours.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using methods described by (Owuama, 2015 and 2017; Gberikon *et al*., 2015).

RESULTS AND DISCUSSION

The result of phytochemical screening of the four different parts of the *Nicotiana tabacum* is presented in table 1 below.

The result of the phytochemical screening of four parts of the fresh and dried *Nicotiana tabacum* (the leaves, stem, roots and the flowers) showed the presence of the following compounds: flavonoids, alkaloids, cardiac glycosides, saponins, tannins, phenols, free anthracene, phytosterols, steroids and carbohydrates (table 1). This agreed with Okere *et al*, (2016) that reported the presence of tannins, phenols, alkaloids, steroids, glycosides, flavonoids, reducing sugars and starch from aqueous and methanolic extracts of *Nicotiana tabacum* leaves. In the same vein, Dua *et al*, (2017) reported the presence of saponin, tannin, flavonoid, terpenoid, nathoquinone, alkaloid, inulin and carbohydrate from aqueous, methanolic and ethanolic extracts of *Nicotiana tabacum* stem. The ethanolic extracts yielded more phytochemical compounds than the aqueous extracts. These result confirmed the evidence in previous studies that alcoholic solvents like ethanol and methanol are more suitable than other solvents such as water in extracting components of medicinal plants (Gberikon *et al*, 2015; Okere *et al*, 2016). The leaves, indeed fresh leaves yielded more phytochemicals than the other parts. This again support the reason while the leaves are mostly used in traditional medicine (Lopez *et al.*, 2001; David and Abuotor, 2000; Wennig and Robert, 2009; Zaidi and Gul, 2005).

Table 1: Result of phytochemical screening of the four different parts of *Nicotiana tabacum* (fresh and dried) using both solvents.

Class of compounds	Leaf		Flower		Stem		Root	
	*	#	*	#	*	#	*	#
Carbohydrates	+	+	+	+	+	+	+	+
Steroids	+	+	-	+	-	+	-	-
Phytosterol	-	+	+	+	+	+	+	+
(Free anthracene)	-	+	+	+	+	+	-	-
Phenols	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+
Saponins	+	+	-	-	+	+	-	+
(Cardiac glycosides)	+	+	+	+	-	+	-	-
Alkaloids	+	+	-	+	-	+	-	-
Flavonoids	+	+	-	-	-	+	+	+

KEY: + = Present, - = Absent, * = Aqueous extracts
= Ethanolic extract

Table 2: The result of zones of inhibition for aqueous extracts of fresh and dried leaves, stems, flowers and roots of *Nicotiana tabacum* (mm)

Extract	Test organisms	
	Escherichia coli	*Staphylococcus aureus*
AFL	14	15
AFS	12	13
AFF	11	10
AFR	08	05
ADL	10	13
ADS	10	11
ADF	08	10
ADR	06	NA
Distilled water	NA	NA
95% ethanol	NA	NA
Gentamicin 10µg	12	08

KEY: AFL = aqueous fresh leaves, AFS = aqueous fresh stem,
AFF = aqueous fresh flower, AFR = aqueous fresh root,
ADL = aqueous dried leaves, ADS = aqueous dried stem,
ADF = aqueous dried flower, ADR = aqueous dried root,
NA = no activity

Table 2 above shows the result of zones of inhibition for aqueous extracts of fresh and dried leaves, stems, flowers, and roots of *Nicotiana tabacum*.

Table 3: The result of zones of inhibition for ethanolic extracts of fresh and dried leaves, stems, flowers and roots of *Nicotiana tabacum* (mm)

Extract	Test organisms	
	Escherichia coli	*Staphylococcus aureus*
EFL	20	18
EFS	16	14
EFF	11	12
EFR	08	03
EDL	16	15
EDS	13	12
EDF	12	10
EDR	10	09
Distilled water	NA	NA
95% ethanol	NA	NA
Gentamicin 10µg	12	08

KEY: EFL = ethanolic fresh leaves, EFS = ethanolic fresh stem,
EFF = ethanolic fresh flower, EFR = ethanolic fresh root,
EDL = ethanolic dried leaves, EDS = ethaolic dried stem,
EDF = ethanolic dried flower, EDR = ethanolic dried root
NA = no activity

Table 3 above shows the result of zones of inhibition for ethanolic extract of fresh and dried leaves, stems, flowers and roost of *Nicotiana tabacum.*

Table 4: The result of minimum inhibitory concentration (MIC) of aqueous extract of fresh and dried leaves, stems, flowers and roots of *Nicotiana tabacum*

Test organisms	**concentration of extract (mg/ml)**							
	AFL	ADL	AFS	ADS	AFF	ADF	AFR	ADR
Escherichia coli	25.0	100	50.0	25.0	100	100	50	100
Staphylococcus aureus	50.0	25.0	100	25.0	100	100	50	NA

KEY: AFL = aqueous Fresh leaves, AFS = aqueous Fresh stem,
AFF =aqueous Fresh flower, AFR = aqueous Fresh root,
ADL = aqueous dried leaves, ADS = aqueous dried stem,
ADF = aqueous dried flower, ADR = aqueous dried root,
NA = no activity

Result of minimum inhibitory concentration (MIC) of aqueous extract of fresh and dried leaves, stems, flowers and roots of *Nicotiana tabacum.*

Table 5: The result of minimum inhibitory concentration (MIC) of ethanolic extract of fresh and dried leaves, stems, flowers and roots of *Nicotiana tabacum*

Test organisms	concentration of extract (mg/ml)							
	EFL	EDL	EFS	EDS	EFF	EDF	EFR	EDR
Escherichia coli	25.0	50.0	50.0	25.0	100	50.0	100	50.0
Staphylococcus aureus	50.0	25.0	25.0	25.0	100	100	50.0	50.0

KEY: EFL = ethanolic fresh leaves, EFS = ethanolic fresh stem,
EFF = ethanolic fresh flower, EFR = ethanolic fresh root,
EDL = ethanolic dried leaves, EDS = ethaolic dried stem,
EDF = ethanolic dried flower, EDR = ethanolic dried root

Result of minimum inhibitory concentration (MIC) of ethanolic extract of fresh and dried leaves, stems, flowers and roots of *Nicotiana tabacum.*

In the same vein, Dua *et al*, (2017) reported the presence of saponin, tannin, flavonoid, terpenoid, nathoquinone, alkaloid, inulin and carbohydrate from aqueous, Methanolic and ethanolic extracts of *Nicotiana tabacum* stem. The ethanolic extracts yielded more phytochemical compounds than the aqueous extracts. These result confirmed the evidence in previous studies that alcoholic solvents like ethanol and methanol are more suitable than other solvents such as water in extracting components of medicinal plants (Gberikon *et al,* 2015; Okere *et al*, 2016). The leaves, indeed fresh leaves yielded more phytochemicals than the other parts. This again support the reason while the leaves are mostly used in traditional medicine (Lopez *et al.,* 2001; David and Abuotor, 2000; Wennig and Robert, 2009; Zaidi and Gul, 2005).

The result of this work also indicates that both aqueous and ethanolic extracts of *Nicotiana tabacum* has antibacterial activity (table 2 and 3). This conformed to the result of Yildirim *et al*, (2001) that ether extracts of both the leaves and seeds and ethanol extract of leaves had shown to have antimicrobial activities on *Staphylococcus* species. Similarly, Wang *et al*, (2008) reported inhibition of the activities of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* by crude polyphenols extracted from

tobacco leaf by 80% ethanol solution. According to Russel *et al*, (1981), extracts from tobacco is equally effective against Gram–positive and Gram–negative bacteria, along with the acid–fast *Mycobacterium phlei* and opportunist fungi, *Candida albicans* and *Cryptococcus neotformans*. Table 2 and 3 also showed that the ethanolic extracts have higher antibacterial activity indicated by higher zones of inhibition. This was so because of its efficacy in the extraction of bioactive substances when compared to the aqueous extracts hence these substances are responsible for the inhibitory effects.

Furthermore, (table 2 and 3) showed the result of aqueous extract of fresh and dried parts of *Nitiana tabacum* on the test organisms. Generally, the aqueous extract of the fresh *Nicotina tabacum* has higher antibacterial activity when compare to the aqueous extract of the dried *Nicotiana tabacum. Staphylococcus aureus* is more susceptible with the range of 8-15mm zones of inhibition. In addition, aqueous extract of fresh leaves exhibit high antibacterial activity. This may be due to the high phenolic content of the leaves of *Nicotiana tabacum* as reported by Nasr *et al*, (2014).

The controls (ethanol and distilled water) exhibited no inhibition against the test organisms. This clearly indicated that the inhibitory effect is from the plant and not the solvent used in the extraction. In another development, gentamicin, a broad spectrum antibiotic that was used inhibited all the test bacteria.

Table 4-5 showed the result of ethanolic extract of fresh and dried *Nicotiana tabacum* on the test organisms. From this result too, fresh ethanolic extract has wider zones of inhibition when compare to the dried ethanolic extract. The highest zone of inhibition of 20mm was recorded from the fresh ethanolic extract of the leaves against *Escherichia coli.*

The minimum inhibitory concentration (MIC) range from 25mg/ml to 100mg/ml (table 6 and 7) which is high compared to the concentration of control (Gentamicin) of 10µg. This variation can be attributable to the fact that the extract is a crude extract and thus the active phytochemical constituents are in small quantity. The leaf and stem extracts showed lower minimum inhibitory concentration (MIC) than the flowers whose minimum inhibitory concentration (MIC) is usually very high around 100mg/ml.

CONCLUSION

It is clear from this study that, the leaves, stems, roots and flowers of *Nicotiana tabacum* (both fresh and dried) have useful phytochemical properties which are responsible for its antibacterial activity. The antibacterial activity of this plant can be described as broad spectrum having inhibited both Gram negative bacteria (*Escherichia coli*) and Gram positive bacteria (*Staphylococcus aureus*). Thus, the plant can be used as an alternative source for production of chemotherapeutic agent especially against *Escherichia coli* and *Staphylococcus aureus*. The minimum bactericidal concentration (MBC) can further be determined.

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