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Effect of Tobacco Snuff on *Staphylococcus aureus* and *Lactobacillus* species among Smokeless Tobacco Snuff users within Gusau Metropolis

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ABSTRACT
The *Nicotiana tabacum* L. (tobacco) is a popular economic plant in human history and it is a notable fumitory plant, which include cigarettes, cigars, chewing tobacco, snuff, and loose pipe tobacco. The analysis was aimed to determining the effect of tobacco snuff (*Nicotiana tabacum*) on *Staphylococcus aureus* and *Lactobacillus* spp isolated from the anterior nares and mouth of user of smokeless tobacco products (STPs) in Gusau. Ten (10) samples of smokeless tobacco product STPs were purchased in Gusau metropolis and analyzed using standard procedures for the presence of bioactive compounds. Extraction of tobacco snuff was determined using maceration method for both aqueous and methanol solvent. Phytochemical constituents of tobacco snuff were determined such as flavonoid, tannins, saponins, terpenoids, cardiac glycosides, phenols, steroids, polypeptides, resins. A stock isolates of *Staphylococcus aureus* and *Lactobacillus* spp were obtained from microbiology laboratory Federal University Gusau. Confirmatory test was done using Gram staining and Biochemical test to confirm the isolates. The activity of aqueous and methanol extract of tobacco snuff against the test isolates was determined in which the methanol extract shows a low activities against *S. aureus* and *Lactobacillus* spp with an average zone of inhibition of 17.00mm and 12.33mm, MIC of 100mg/ml and 50mg/ml respectively, while aqueous extract shows no activities against both S. aureus, and lactobacillus spp. This study showed that the tobacco snuff examined has very low or no effect on Staphylococcus aureus and lactobacillus spp,
Keywords: Tobacco, Phytocompounds, *Staphylococcus aureus, lactobacillus* species, snuff

1.1 INTRODUCTION

Tobacco snuff (*Nicotiana tabacum*) is a vital economic crop which belongs to the family of Solanaceae. In Commercial level of production tobacco can also attain from its other sister species such as *Nicotiana rustica,* which is smaller in height with fewer leaves than *Nicotiana tabacum.* On the dependence of dose, the nicotine in tobacco restrains the pathogen (Tong *et al.,* 2021). *Nicotina tobacum* L. is commonly known as Tobacco, and locally called "Utaba", "Anwuru" (Igbo), "Ewe taba", "Kataba", "Sobo" (Yoruba), and "Taba" (Hausa). It is an erect, perennial stout, glandular-pubescent, herbaceous plants; leaves large, pubescent, green, and oblong-lanceolate; flowers rosy or reddish, compact-shaped, in many –flowered inflorescence, usually panicle d racemes; fruits capsules, 1.5-1.8cm long, the plant is native to tropical and sub-tropical America and presently cultured worldwide as a cash crop (Burlill, 2015). The name tobacco snuff is the dried and processed leaves of *Nicotina tabacum* of the flowering plant family *Solanaceae* (Udoye and okeke) Tobacco Snuff is a smokeless tobacco made from finely ground or pulverized tobacco leaves. It is snorted or "sniffed" (alternatively sometimes written as "snuffed") into the nasal cavity, delivering a swift hit of nicotine and a lasting flavored scent (especially if flavoring has been

blended with the tobacco). Traditionally, it is sniffed or inhaled lightly after a pinch of snuff is either placed onto the back surface of the hand, held pinched between thumb and index finger, or held by a specially made "snuffing" device (Sharma *et al.,* 2016)

The mouth is home to an excess of 700 bacterial species, which are adapted to its inherently distinct ecological niche. (Petrova *et al.,* 2015) more than 50% of these species colonize the periodontal pocket, and the remnant are distributed across other sites of the oral cavity.

Some common oral flora belongs to the genera: *Enterococcus, Actinomyces, Peptococcus, Streptococcus, Staphylococcus, Corynebacterium, Eubacterium, Campylobacter* etc.

Even though the ecology of the oral cavity is diversified and has a hall mark of high equilibrium called microbial homeostasis. Resistance *Lactobacillus* is a part of the normal human flora of the bacteria usually reside in or on humans' body, but are not causing infection, it is called colonization. But if allow to enter the internal tissues or blood stream, theses bacteria may cause a variety of potential serious infection" Humans are often most colonized with *Lactobacillus*

Staphylococcus aureus is a member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. (Laura *et al.,* 2015). *Staphylococcus aureus* are opportunistic pathogens and are widespread in nature, their normal habitats being the skin and mucous membranes of man and birds. Most important pathogenic strains are *Staphylococcus aureus*, which can cause both superficial and deep pyogenic infections as well as a number of toxin-mediated illnesses (Stoneham *et al*,. 2021)

Statistically, within a society of 100% individual's population about 40% of the individuals present in same society are addicted to Tobacco snuff which result to nicotine addiction. Some of the individuals misuse or abuse it by taking this snuff orally while some inhale this tobacco snuff through the nose on daily bases without an appropriate knowledge about the adverse effect of tobacco snuff. We therefore are creating an eye open/ awareness to the society about the adverse effect of tobacco snuff against the normal flora of the mouth (Kurma *et al.,* 2016)

2.0 MATERIALS AND METHODS

2.1 Study area

Gusau is the Administrative capital of Zamfara State, Nigeria. It is located between Latitude 12˚13'N to 12˚18N and Longitude 6˚29E to 6˚45E with the total population of 383,162 people and an estimated 682,700 inhabitants in 2022 (NBS, 2022), from its annual growth rate of 2.7% (NPC, 2016). The climate of Gusau is a typical semiarid/arid which is influenced by the northerly wet and south moving dry continental air masses, resulting in two alternate rainy and dry seasons respectively (Kanoma and Abdulkadir 2022)

2.2 Sample Collection

Dried pounded Tobacco snuff was purchased from local retailers of herbs at Gusau old market Zamfara State and identified as *Nicotiana tabacum* by Herbarium from Biological science of Federal University Gusau, the samples were stored in air tight polythene for further analysis.

2.3 Extraction of Tobacco Snuff

The tobacco snuff was extracted with two different solvents (aqueous and methanol) using procedure described by Bashir *et al.* (2013). Fifty gram (50g) of the powdered tobacco snuff was weighed using a weighing balance and put into a clean dry beaker after which 500ml of aqueous and methanol was poured into it. The sample was stirred with a sterile rod, soaked for 168hrs and filtered using a musilin sieving cloth into a clean and dry stainless plate and then placed on the water bath for concentration and drying at 50^0C which took 96hours. The extract was then scrapped. measured and placed into a sterile sample bottle using a sterile spatula kept for further analysis (Anumudu *et al*., 2019).

2.4 Phytochemical Tests

Tobacco snuff extract was subjected to qualitative phytochemical screening according to standard procedures to test for the presence of the following secondary metabolites; alkaloids, flavonoids, saponins, tannins, glycosides and steroids as described by Odebiyi and Sofowora, (1978); Sofowora,

(1984); Ogukwe *et al*. (2004); Hassan *et al*. (2005); El-mahmood and Doughari, (2008); Adewuyi *et al*. (2021).

2.5 Confirmations of the isolates

The *Staphylococcus aureus* and *Lactobacillus* spp isolate obtained were confirmed using Gram staining and biochemical test as prescribed by Castro (2018).

2.6 Antibacterial Activity

2.6.1 Preparation of Concentrations

This was carried out according to the method described by Cheesbrough, (2006). Stock solution of tobacco snuff aqueous and methanolic extracts were prepared by dissolving 4g of each of the plant extracts in 10ml of dimethyl sulphoxide (**DMSO**). The stock solution was double-diluted to get three varied extracts concentrations in addition to it to make them four different concentrations of (200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml. (Mukhtar *et al.,* 2017)

2.6.2 Standardization of inoculum

The isolates were adjusted to 0.5 McFarland standard (1.5x 108 CFU/ml) turbidity for bacterial isolates by adding normal saline. McFarland standards were used as a reference to adjust the turbidity of microbial suspension so that the number of microorganisms was within a given range. For the preparation of the 0.5 McFarland standard, 0.05ml of barium chloride (BaCl2) (1.17% w/v BaCl2, 2H2O) was added to 9.95ml of 0.18M H2SO4 (1.0% w/v) with constant stirring. To aid comparison the standard was compared against a white background with contrasting black line (Assafiri *et al*., 2020).

2.6.3 Bioassay

The bioassay was carried out using the agar well diffusion method described by (Cheesbrough, 2006). About 0.1ml of the standardized inoculum (1.5 x 10^8 CFU/ml) of *Staphylococcus aureus* and *Lactobacillus* was inoculated onto sterile prepared Mueller Hinton Agar. six wells of 6mm wide, 4mm depth were made with a sterile cork borer into the agar plates containing the bacterial isolate and 0.1ml of the four different concentrations from the stock solution of the extracts at concentrations (200, 100, 50, and 25mg/ml) were introduced into their respective wells and 200mg/ml streptomycin was as positive control, test isolate and DMSO as negative control. The inoculated plates were left to stand for about 30minutes to allow diffusion of extract before incubating at 37^{o}C for 24 hours for the bacterial isolates The zones of clearance produced around the wells after incubation was observed and measured using a vemier caliper and recorded (in mm). The mean results were taken for the test organisms (Mohammed and Mohammed 2022).

2.6.4 Determination of the Minimum Inhibitory Concentration (MIC) of the Extracts

The minimum inhibitory concentration of the aqueous and methanol extracts of the *Nicotiana tabacum L*. (tobacco snuff) by serial doubling dilution and incorporated into test tubes containing 2ml nutrient broth.0.1 ml of standardized inoculum of the isolates were introduced and the test tube were incubated at 37^{0}C for 24hours (Wang *et al* 2016).

2.6.5 Determination of minimum Bactericidal concentration (MBC)

This was carried out with some modifications according to Lajuyigbe and Afolayan, (2012) by using fresh Mueller Hinton agar; isolates were inoculated with one loop full of culture taken from each of the first three broth cultures that showed no growth in the MIC tubes. Minimum bactericidal Concentration assay plates were incubated for 24hrs. After the incubation period, the lowest concentration of the extract that did not show any fungal growth on the solid medium was regarded as MBC results.

2.7 Statistical Analysis

The data obtained (recorded) after the laboratory tests will be subjected to statistical analysis of ANOVA using the Statistical Package for Social Sciences (SPSS 22).

3.0 RESULTS AND DISCUSSIONS

3.1 Physical characteristics of aqueous and methanol extract

The physical properties and the average weights observed from different extract revealed that aqueous extract appeared to be brown in colour with percentage yield of 9.1, while the methanol appeared dark brown in colour with percentage yield of 23.0 as shown in table 4.1

Table 3.1, Physical characteristics of *Nicotiana tabacum L.* (tobacco snuff) extract

Extract	Colour of extract	Texture of extract	Weight of tobacco extract(g)	Percentage yield (%)
Aqueous	Dark brown	Gummy	9.1	18.2
Methanol	Dark brown	Slightly gummy	23.0	46

3.2 Phytochemical Screening

The result of preliminary phytochemical analysis of tobacco snuff leaf extract, showed the presence of tannins, alkaloid, steroid renins, essential oil, flavonoid glycoside, in both aqueous and methanol while phenol, Saponin, terpenoids and quinones are presence in methanol. (Table 3.2).

Table 3.2: Phytochemical constituent of Tobacco snuff

Constituents	Solvent	
	Aqueous	Methanol
Alkaloid	+	+
Saponins	-	+
Glycoside	+	+
Renins	+	+
Flavonoid	+	+
Trepenoid	-	+
Essential oil	+	+
Steroid	+	+
Tanins	+	+
Phenolic compound	-	+
Quinones	-	+

Keys: + = Positive, - = Negative

3.3 Gram stain and Biochemical tests results

The result of confirmatory test of *Staphylococcus aureus* and *Lactobacillus* spp using gram staining and biochemical test was presented in table 3.3

Table 3.3; Gram staining and Biochemical test on *Staphylococcus aureus* and *Lactobacillus* spp

BIOCHEMICAL TEST	*S. aureus*	*Lactobacillus* Spp
Gram staining	+	+
Motility	+	-
Catalase	+	-
Coagulase	+	-
Triple sugar ion (T.S.I)		
Glucose (T.S.I)	-	+
Lactose (T.S.I)	+	+
Gas (T.S.I)	+	-
H_2S (T.S.I)	-	-
Citrate	+	-
Urease	+	-
Indole	-	-
Methyl red (MR)	+	-
Voge's proskauer (VP)	+	-
Cellobiose	-	+

Keys: + positive: - = negative

3.4 Result Antibacterial Activity of the Extract

Tables 3.4 and 3.5 present the evaluation of antibacterial activity by agar well diffusion method which indicated that all the isolates tested showed no zone of inhibition towards the aqueous extracts, Methanolic extracts showed low activity of 17mm and 16mm again both *S. aureus* and *Lactobacillus*. The standard antibacterial drug (Streptomycin) used as positive control showed the growth inhibition of 30mm respectively

Table 3.4 Activity of Aqueous extract against *S. aureus* and *Lactobacillus*

	Zone of inhibition (mm)					
Concentration	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.50 mg/ml	Control (30mg/ml)
Isolate						
S. aureus	0.00	0.00	0.00	0.00	0.00	30.00
Lactobacillus	0.00	0.00	0.00	0.00	0.00	30.00

Table 3.5: Activity of Methanol extract against *S. aureus* and *Lactobacillus*

	Zone of inhibition (mm)					
Concentration	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.50 mg/ml	Control (30mg/ml)
Isolate						
S. aureus	17.00	13.00	6.00	5.00	4.00	30.00
Lactobacillus	16.00	12.00	8.00	2.00	0.00	30.00

Results of minimum inhibitory concentration and bactericidal concentration of the plant extracts against the test organisms presented in table 3.6 shows MIC at 50mg/ml for *S. aureus* and 100mg/ml for *Lactobacillus Spp*.

Table 3.6: Minimum Inhibitory Concentration of Methanol extract of tobacco snuff against *S. aureus* and *Lactobacillus* spp

Isolate	Extract	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.50mg/ml	P-value
S. aureus	Methanol	-	MIC	+	+	+	0.040
Lactobacillus	Methanol	-	-	-	MIC	+	0.751

Keys: - = Absence of Turbidity, + = Presence of Turbidity, MIC = Minimum Inhibitory Concentration

Table 3.7: Minimum Bactericidal Concentration of tobacco snuff against *S.aureus* and *Lactobacillus* spp

Isolate	Extract	200mg/ml	100mg/ml	50mg/ml
S. aureus	Methanol	+	+	OX
Lactobacillus	Methanol	+	+	+

Keys: - = Absence of growth, + = Presence of growth, OX = No MBC test carried out

DISCUSSIONS

The solvents used for the extraction of tobacco snuff was selected based on their polarity and the average percentage yield was observed to be high (46%) in methanol extract while low average percentage yield (18.2%) was observed in aqueous extract this may be due to different in polarity of the solvents this finding is similar to the finding of Haris *et al*. 2017

The bioactive components such as tannins, alkaloid, steroid renins, essential oil, flavonoid glycoside, in both aqueous and methanol while saponins, terpenoids, phenol and quinines are only present in methanol extract This is in agreement to the finding of Oyekunle *et al.,* (2019). The presence of phytochemical constituents in plants extract has been reported to be responsible for antimicrobial activity, particularly Alkaloid, Saponins and Tannins are well documented for antimicrobial activity (Okere *et al.,* 2016). The antibacterial activities of aqueous and methanol extract of tobacco snuff against *S. aureus and Lactobacillus spp* indicate that, the aqueous extract shows no activity in both *S. aureus* and *Lactobacillus* this may be due to the absence of some secondary bioactive compounds and the development of resistant strain by the isolates as a result of the frequent used of the tobacco snuff , while Methanolic extract shows a very low activity with zone of inhibition of 17mm, 13mm, 6mm, 5mm, 4mm and 16mm, 12mm, 8mm, 2mm , 0mm for *S aureus* and *Lactobacillus* respectively this may result to solubility of almost all the bioactive constituents in methanol, this finding was similar to the work of Haris *et al*, (2017) .

CONCLUSIONS

The physical properties of Methanolic extract of tobacco snuff extract show a high percentage yield of 46% than aqueous extract with 18.2%. the bioactive constituents identified were more soluble to methanol than the aqueous solvent, conclusively, this result shows that *Nicotiana tabacum* (tobacco snuff) extract has no effect or shows very less activity against *S aureus* and *Lactobacillus spp* isolated from the nose and mouth respectively.

RECOMMENDATIONS

Further research on effect of tobacco snuff against some pathogenic microorganisms should be carry out both in vitro and in vivo

Research should be conducted on the possible antimicrobial the potentials of *Nicotiana tabacum* in pharmaceutical formulation of drugs and health benefit.

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