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DETERMINATION OF HEAVY METALS, EXTRACTION AND CHARACTERIZATION (PHYTOCHEMICAL SCREENING) OF ANTIMICROBIAL COMPOUNDS FROM CIGARETTE

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ABSTRACT

The study deals with the determination of heavy metals, extraction,and characterization (phytochemical screening) of antimicrobial compounds from a cigarette. Two packs each of Pall Mall and Marlboro cigarettes were purchased from a mini-market at Obakekere off campus, FUTA. The cigarette packs where taken to the Department of Chemistry laboratory, Obakekere for analysis. The extracts in organic solvents (ethanol) were prepared for the study. Ethanol extracts of cigarette showed the presence of tannin, oxalate, phenol, alkaloid and phytic acid. Antimicrobial activity of the cigarette extracts was tested against bacteria (*Streptococcus saluvarius, Lactobacillus paracasei, L. plantarum, L salvarius*) and fungi (*Pichia* spp.1, *Saccharomyces cereveseae, and Pichia* spp. 2)by agar well diffusion method. The ethanol extract of both samples showed a significant range of inhibitory effect against the microorganisms at minimal inhibitory concentration. The mineral analysis reveals the level of Cu, Cr, and Zn present in the two samples with Zinc having the highest in Pall Mall brand PMC (0.87mg/kg) followed by Copper (0.73mg/kg) in MBA brand. Thus, these results confirm the presence of antibacterial and antifungal compounds in the cigarette while compounds in cigarette showa higher zone of inhibition hence both the samples can be used as a source of antibiotics in the pharmaceutical industries.

KEYWORDS: Tobacco, Phytochemical, Cigarette, Antimicrobial, Heavy Metals.

INTRODUCTION

Nicotianatabacum (Tobacco) belongs to a family of Solanaceae. It's a perennial herbaceous plant that is found only in cultivation. It is native to tropical and subtropical America but today it is cultivated throughout the world. All the parts are sticky and are covered by shorts viscid-glandular hairs which exude a yellow secretion containing nicotine. Its synonyms are tobacco, tamak, andsiah (marma). 20% of tobacco resources are discarded as processing waste, which pollutes the environment and causes a big waste (Rawat and Mali, 2013). The antinociceptive activities of methanolic leaf extract of tobacco by tail immersion, hot plate, and acetic acid have revealed the abdominal constrictions in albino Wistar mice.

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It has also been known for its antifungal activity against *Fusarium solani* (Ponstein*et al.,* 1994). As a traditional medicine, for the treatment of tuberculosis and coughs, it was also screened for activity against *Mycobacterium tuberculosis* (Adeleye*et al.,* 2008).

Tobacco leaf contains several pyridine alkaloids, the principal one being a liquid alkaloid, nicotine. Other alkaloids present include nicoteine, nicotimine, anabaineanatalline and nornicotine. In topical times, there have been upsurges in antibiotic resistant strains of clinically imperative pathogens, which have led to the arrival of new bacterial strains that are multi-resistant (WHO, 2003). It is therefore very essential to stare for ingredients from other sources with recognized antimicrobial activity.

For years, plants have been a valuable source of natural products for maintaining human health. Researchers have already used the extracts of plants for various anti-bacterial, anti-fungal and anti-viral activities (Bakht, 2011a). The waste product of plants has also shown the presence of bioactive compounds such as stem or bark. Phytochemicals have revealed the active component of medicinal Plant that has shown an effect against on all types of microorganism and also their sensitive test against Gram positive bacteria & gram negative bacteria (Nascimento*et al.,* 2000). As far as the free radicals are concerned, antioxidant activity provides a medical revolution for health and disease management (Aruma, 2003; Sharm*et al.,* 2016). This study was carried out to investigate the concentrations of heavy metals in tobacco seed and study the phytochemical properties as well as investigate the antimicrobial activity of the brands against an active microorganism.

MATERIALS AND METHODS

Four brands of cigarettes coded as MP, MA, ML, and MB were purchased from the south gate of the Federal University of Technology, Akure Ondo State Nigeria. Four brands of cigarette. Concentrate trioxonitrate (v) acid (HNO_3) and concentrated perchloric acid ($HClO_4$), tannic acid, used are analytical grades

METHODS

DETERMINATION OF HEAVY METALS

For the determination of the metal content of the cigarette unwrapped and separated to into three different portions; wrap, filter,and the plant sample and oven dry at 50 ^{0}C to constant weight. The plant sample was utilized for the quantitative phytochemical analysis.

Extraction of metals from sample was by mixed acid digestion (Lacatusu, 2000 and Chale, 2002). Into a 250 mL digestion flask was added 1 g of blended sample and 25 mL concentrated nitric acid (HNO_3) with 5 mL concentrated perchloric acid ($HClO_4$).

The mixture was shaken thoroughly, digested on a hot plate. There was an evolution of red fume and the digestion was stopped when fume color changes to light yellow. The obtained solution was allowed to cool and then filter into a 50 mL volumetric flask and make to make with deionized water. Blank solution was also prepared. The sample was stored in a sample bottle and analyzed using using Atomic Absorption Spectrophotometer (AAS) BUCK SCIENTIFIC 210 VGP model. All the analysis was done in triplicates. They Include: (Pall Mall (MP), Marlboro (MA), London menthol (ML) and Benson & Hedge switch (MB); London menthol (plant, filter, and cloth), Benson and hedge (plant, filter, and cloth).

PHYTOCHEMICAL ANALYSIS

Quantitative analysis was carried out on the plant material in the cigarettes sample and the alkaloids, tannins, phytates, oxalates and total phenols were determined.

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DETERMINATION OF TANNIN CONTENT

For tannin determination, 10 mL of 70 % aqueous acetone was added to 200 mg of finely ground sample in a bottle and properly covered. The bottle was put in an ice bath shaker for 2 hr at 30°C. The solution was then centrifuged and the supernatant stored in ice. From the supernatant, 0.2 mL was pipetted into 0.8 mL distilled water. The standard tannic acid solution was also prepared in order to plot the graph. Folin reagent (0.5mL) was added to both sample (standard tannic acid and cigarette solution) followed by 2.5 mL 20 % Na_2CO_3. The solutions were vortexed and allowed to incubate for 40min at room temperature after which the absorbance was read at 725nm. The concentration of tannin in the sample was estimated from the standard tannic acid curve (Makkar and Goodchild, 1996).

DETERMINATION OF OXALATE

The titration method described by Day and Underwood, (1986) was used to determine the oxalate content of the tobacco plant. One gram of the sample was weighed into 100mL conical flask where 75mL of 3M H_2SO_4 was added and stirred with a magnetic stirrer for 1 hr, it was then filtered using no 1 What man filter paper and 25ml was taken from the filtrate and titrated while hot against 0.5 molar $KMnO_4$solutions until a faint pink colour persisted for at least 30sec.

Oxalate (mg/g) = Titre Value × 0.9004

0.1N $KMnO_4$ = 0.9004 calcium oxalate

DETERMINATION OF ALKALOIDS

The gravimetric harborne method described by Onwuka, (2006) was adopted. 5g of the oven dried sample was dispersed in 50 mL of 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stand for 4 hr before it was filtered. The filtrate was evaporated to one quarter of its original volume follow by addition ofconcentrated NH_4OH drop wise to precipitate the alkaloids. The precipitate was obtained by filtration using already weighed filter paper. The precipitate was washed with 1% NH_4OH solution and dried in the oven at 60ºC for 30 mins. Alkaloids are expressed in percentage.

Alkaloid (%) = $\left(\frac{weightof\ precipitate}{weightofsample\ used} \times 100\right)$

PHYTATE DETERMINATION

2g of the sample was weighed into a 250mL conical flask and 40mL 2.4% hydrochloric acid was added followed by shaking for 3 hr at room temperature (27 ± 2ºC), and the supernatant obtained by filtration. To 50 mL of the filtrate in a 250 mL beaker was water added to give a proper acidity and 10 mL of 0.3 % ammonium thiocyanate solution was added as an indicator. This was titrated against standard iron (iii) chloride solution, which contained 5.66g $FeCl_3$/L and the equivalent point was slightly brownish-yellow which persist for 5 min (Wheeler and Ferrel, 1971).

DETERMINATION OF TOTAL POLYPHENOLIC COMPONENT

The total phenolic content was determined with the Folinciocalteu method (Singleton 1965). Exactly, 0.5 mL of folin reagent was added to 7.5mL of the sample (1mg dissolve in 10mL of water), 2mL of 20% sodium carbonate was then added. The mixture was allowed to stand for 60 min in the dark before absorbance was read at 750nm. A calibration curve was done with a solution of gallic acid (80mg/L) the result was obtained in milligram of gallic acid equivalent (GAF) per gram of dark bark.

0MICROBIAL ANALYSIS

Exactly 60 g of the oven dried plant material of the cigarette was extracted with 95% ethanol (300 mL) using Soxhlet extractor. The obtained extract was then concentrated to dryness at room temperature. The sample was labeled and

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stored in a sterile container in the refrigerator at 4^{o}C until used.

TEST ORGANISMS

Clinical isolates of microorganisms of choice were obtained from the Microbiology Laboratory of the Federal University of Technology, Akure. The microorganisms were gotten from the healthy saliva of a human being. All bacterium isolates were cultured aerobically on Nutrient agar (NA) plates while fungi isolates were also cultured aerobically on protein dextrose agar (PDA) plates containing 8mm wells. These were sub-cultured on different agar slants and incubated at 37^{o}C overnight.

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY TEST (AGAR WELL DIFFUSION METHOD)

The microorganisms of choice used for this experiment are *Streptococcussalvurius*, *Lactobacillusparacasei*, *L. plantarum*, and *L. salvarius* respectively and *Pichia* spp. 1, *Saccharomyces cereveseae, Pichia* spp. Two different concentrations of each of the samples were prepared by serial dilution (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}). The nutrient agar plates for bacterium were incubated at 37^{o}C for 24 hr while protein dextrose agar plates for fungi were incubated at 27^{o}C for 48 hr. Control plates used was also set up using streptomycin sulphite on standard antibiotics while ketoconazole was used as a standard antifungal agent.

DETERMINATION OF MINIMUM INHIBITION CONCENTRATION (MIC)

For the determination of MIC, Serial dilutions of the extract were carried out and an aliquot of the extract (0.2g) as dissolved in 100 mL of distilled water to obtain 2.0 mg/mL.Exactly, 2.0 mg/mL was then double diluted in sterile distilled water to obtain concentrations of 1.0, 0.50, 0.025, 0.0125, 0.0625, 0.0325 mg/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.

STATISTICAL ANALYSIS

All analyses were performed in triplicates. Results were expressed by means of ± Standard deviation of three determinations. Statistical significance was established using Analysis of Variance (ANOVA) models to estimate the differences on mineral composition of the two brands of a cigarette with the help of the statistical software SPSS 20.

RESULTS AND DISCUSSIONS

RESULTS

The results of the mineral analysis are presented in Table 3.1., results of phytochemical screening analysis are presented in Table 3.2 and the results of the antimicrobial analysis are presented in Table 3.3.

Table 3.1.Result of Mineral Analysis of the different parts of the two brands of Cigarette (Pall Mall and Marlboro)

Sample	Zn (ppm)	Cu (ppm)	Cr (ppm)
PMA	0.51 ± 0.26^{ac}	0.18 ± 0.08^{ab}	0.02 ± 0.00^{b}
PMB	0.19 ± 0.25^{b}	0.06 ± 0.06^{b}	0.002 ± 0.00^{a}
PMC	0.87 ± 0.23^{bc}	0.23 ± 0.00^{a}	0.08 ± 0.03^{ac}
MBA	0.12 ± 0.02^{c}	0.73 ± 0.02^{c}	0.30 ± 0.03^{abc}
MBB	0.25 ± 0.03^{b}	0.21 ± 0.09^{ac}	0.43 ± 0.19^{b}
MBC	0.67 ± 0.02^{abc}	0.15 ± 0.01^{abc}	0.03 ± 0.03^{c}

*Data represented as the mean± Standard deviation of three determinations; The same superscript down the group show no significant difference at p<0.05; PM (Pall Mall); MB (Marlboro); A (Wrap); B (Filter); C (Plant)

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Table 3.2.Result of Phytochemical Screening of the two Brands of Cigarette Plant (Pall Mall and Marlboro)

Sample	Oxalate (mg/g)	Tannin (mg/g)	Alkaloid (mg/g)	Total phenol (mg/g)	Phytate (mg/g)
PM	2.39±0.32	48.50±0.71	84.06±1.51	82.33±1.04	0.354±0.01
MB	2.03±0.32	43.75±1.48	69.74±0.58	81.00±3.12	0.354±0.01

Data represented as the mean± Standard deviation of three determinations

Table 3.3.Result of Antimicrobial Screening and Minimal Inhibitory Concentration (MIC) of the two brands of Cigarette (Pall Mall and Marlboro)

	Antibacterial				Antifungal			
Sample	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
MB^{1}	10.0	10.0	9.0	10.0	5.0	5.0	5.0	15.0
MB^{2}	5.0	6.0	5.0	8.0	2.0	3.0	2.0	4.0
MB^{3}	4.0	5.8	4.5	3.5	1.0	2.0	2.0	-
MB^{4}	4.0	-	-	5.0	-	-	-	-
PM^{1}	7.0	11.0	11.0	11.0	6.0	5.0	4.0	8.0
PM^{2}	5.0	10.0	9.0	5.5	5.0	3.0	3.0	7.0
PM^{3}	4.5	5.5	3.0	4.5	2.0	2.0	2.0	-
PM^{4}	-	-	-	-	-	-	-	-

Key: PM (Pall Mall); MB (Marlboro)

The superscript on the sample represents the concentration of the sample (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}); Antibacterial (A, B, C, D represents *Streptococcussalvurius*, *Lactobacillusparacasei*, *L. plantarum*, and *L. salvarius* respectively); Antifungal (A, B, C, D represents *Pichia* spp. 1, *Saccharomyces cereveseae, Pichia* spp. 2).

DISCUSSION

HEAVY METALS

The result of the heavy metal analysis for the two cigarette brands is shown in Table 3.1 above, with Zinc having the highest in Pall Mall brand PMC (0.87mg/kg) and Copper (0.73mg/kg) MBA in Marlboro brand. It was also observed in Table 3.1 that heavy metal contents in the wrapping paper is much higher than the filter this may result from the additional metals in the wood components used for the production of the paper but does not have a significant effect with the level of metals in the cigarette. The metal content was higher in the plant of both PM and MB samples respectively and could be deleterious to health when such products are consumed in excess as tobacco smoking influences the concentrations of many elements in some organs. Smoking of tobacco leaves is one of the main routes of exposure to heavy metals. Metals contained in tobacco leaves originate from root uptake and transfer to the shoots and also from the deposition of aerosol particles on the leaves.

PHYTOCHEMICAL SCREENING TEST

Phytochemical screening was done in order to reveal the presence of secondary metabolites that were present in the methanolic extract of the two cigarette brands of *Nicotianatabacum*. Alkaloid, tannin, oxalate, phytate,andphenolics were determined and found to have moderate values (Table 3.2). In the Pall Mall brand, Alkaloid had the highest mean value (84.06 mg/g) and phytate with the lowest (0.354 mg/g). Also in the Marlboro brand, total phenols recorded the highest value (81.00 mg/g) and phytate with the least (0.35 mg/g). Tannins have been reported to form complexes with proteins and reduce their digestibility and palatability (Eka, 1985). However, their contents in tobaccos are known to reduce through heating when the cigarette is lit (Lewu*et al.,* 2010) therefore the amount of the

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tannin consumed during smoking is reduced as a result of the heat. Tannins concentration in the cigarette samples studied are in moderately acceptable limits. Tannins have traditionally been considered anti nutritional but it is now known that their beneficial or ant- nutritional properties depend upon their chemical structure and dosage. Condensed tannins inhibit herbivore digestion by binding to consumed plant proteins and making them more difficult for animals to digest, and by interfering with protein absorption and digestive enzymes (Muller-Harvey and McAllan, 1992), this is the reason why plant containing tannin compound such as tobacco leaf is not consumed by animals because of their indigestibility.

Alkaloids (nicotine) which are one of the largest groups of phytochemicals in plants have an amazing effect on humans and this has led to the developments of powerful pain killer medications (Kam and Liew, 2002). The result obtained for alkaloid and tannin correlated well with the result obtained for *Nicotianatobacum* by Okorondu*et al.,* 2015. It was also observed that the Pall Mall brand had the highest values for all the phytochemicals present in the cigarette sample with exception to phytate having the same value with the Marlboro brand (0.35mg/g). The nicotine content (alkaloid) in Pall Mall brand is slightly higher than that in Marlboro thus it could be said that the latter will be more useful in pain killers and not as effective in the proper functioning of the brain because it will have high intoxicating power compared to the former.

ANTIMICROBIAL SCREENING TEST

The purpose of the present study was to investigate the antibacterial activity of tobacco extracts from two different brands of cigarette by agar well diffusion method. Inhibition length was calculated in order to reveal its inhibitory effect against a gram positive (*Streptococcus saluvarius*) and three gram negative bacteria *(Lactobacillus paracasei, L. plantarum,and L. salvarius*) as shown in Table 3.3and its antibacterial activity compared with standard antibiotics such as streptomycin. Ethanol extracts of the samplehave shown an inhibitory effect against *Lactobacillus paracasei and L. plantarum*with a minimal inhibition concentration of MB^4 (10^{-4}) (Table 3.3) and against all the bacteria in question at PM^4 (10^{-4}) but showed no inhibitory effect at higher concentrations. Ethanolic extract of the same sample has also shown its inhibitory effect against antifungal such as *Pichia* spp. 1, *Saccharomyces cereveseae and Pichia* spp. 2 at MB^3D (10^{-3}) and MB^4 (10^{-4}) as well as at PM^3D (10^{-3}) and PM^4. Theantifungal activity compared well with standard antibiotics such as ketoconazole.

It is possible that the antibacterial and antifungal 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 (Okorondu*et al.,* 2015). This probably indicates that these bioactive ingredients 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 *Staphylococcusaereus* 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).

CONCLUSION

The present study concludes that cigarettes are the rich source of various phytochemicals having investigated and found to contain a high level of phytochemical components. These both shown antimicrobial (both antibacterial and antifungal) properties but comparatively the ethanolic extract of Pall Mall and Marlboro cigarette

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samples are rich in phytochemicals by the means of quantity and shows higher inhibition zone which can be attributed to the metabolites which are responsible for the activity. Hence the plant can be used as an antibacterial and antifungal agent and may serve as leads for the pharmaceuticals industries in developing countries like Nigeria.Greater work should still be done on this plant so as to fully utilize its medicinal and nutritional characteristics.

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