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Characterization and antibacterial activity of tannin from selected food and agricultural wastes

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Abstract
Tannins provide several advantages being as good biomaterial, antimicrobial, antioxidant, pharmaceutical, biopesticide, and nutraceutical agents. This study aimed to characterize tannin from three (3) agrowastes (Corn cob, cocoa pod, and coco peat) and compare their antimicrobial activity with that of synthetic tannin. The wastes were air-dried and blended. The tannin content was estimated using UV-Visible Spectrophotometric method. FTIR analysis of the samples was also carried out. Antimicrobial activity of different extracts of samples tannin and synthetic tannin on four (4) pathogenic bacteria (*Salmonella Typhi*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Klebsiella pnuemonae*) was determined. Results obtained revealed the tannin content of samples in the order: coco peat (5.500 mg/g) >cocoa pod (4.740 mg/g) >corn cob (3.212 mg/g). The FTIR analysis showed a broad band at 3285-3216 cm^{-1} for the three samples representing bonding –OH groups. The samples displayed peaks around 2928-2917cm^{-1} representing aliphatic chains, -CH2- and –CH3. The peak around 1616-1576 cm^{-1} corresponds to C-O vibrations. The peak around 1363-1256 cm^{-1} corresponds to C (O)-O stretching vibrations and –OH in plane vibrations. These peaks correspond to peak values obtained from synthetic tannic acid. The four organisms were susceptible to aqueous extracts of tannin from all samples except *Bacillus subtilis* which was not susceptible to cocoa pod tannin. *Salmonella typhi* was not susceptible to synthetic tannin at all concentrations. Results from this study indicate that some agrowastes may be utilised as a source of cheap, readily available tannin and may also serve as a more effective antibacterial agent.

Keywords: Tannin, Agrowastes, FTIR, Antimicrobial, Characterization, Pathogenic bacteria

1. Introduction
Plants' secondary metabolites have been well-documented to be of great benefit to human health. One such is the phenolics which exhibit significant pharmacological properties such as antioxidant, anticancer, and ability to inhibit and mortify certain pathogenic bacteria. An example of such phenolic secondary metabolites is tannin. Synthetic tannins have been shown to possess unhealthy

side effects. Thus, the use of natural products as a source of tannin has become a focus. This study is therefore, aimed at extraction and evaluation of the antibacterial effect of tannin from three (3) selected agriculture wastes- cocoa pod, coco peat, and corn cob.

2. Literature review

2.1. Tannins

Tannins are a group of water-soluble polyphenolic compounds (with molecular weight ranging from 0.5 – 20 kD) naturally found in plants and have been reported to be the fourth most abundant plant constituents after cellulose, hemicellulose and lignin (Vuolo et al., 2018; Ire & Nwanguma, 2020). They are amorphous, astringent substances occurring widely in the bark, wood, leaves, and resinous exudations of plants (Pizzi, 2008; Zivkovic et al., 2009; Tomak & Gonultas, 2018; Giovando et al., 2019). Biochemically, tannins are sort of secondary metabolites predominantly available in plant-based foodstuffs and beverages grapes, blackberries, strawberries, walnuts, cashew nuts, hazelnuts, mangoes, and tea (Clifford & Scalbert, 2000). Tannins are considered defensive molecules to protect plant tissues from herbivorous attacks because of their astringent taste (Chang et al., 2019; Sharma, 2019; Kwan et al., 2022). Tannins possess antioxidant activity. This property is related to their chemical structure as they possess phenolic rings able to bind to a wide range of molecules and act as electron scavengers to trap ions and radicals (Shirmohammadli et al., 2018; de Hoyos-Martínez et al., 2019).

Extensive research work is currently being carried out by researchers on the extraction and application of tannins (Arinaa & Harisuna, 2019; Chen et al., 2020a, 2020b; Liao et al., 2020; Martins et al., 2020). One such is the application of tannin as an antimicrobial agent.

2.2. Natural source of Tannin

In recent decades, natural products and ingredients have gained an increasing demand instead of the use of synthetic additives. Consumers opt for this option as they are safer and eco-friendly and they show plenty of health benefits while avoiding side effects associated with synthetic antimicrobials (Rana & Paul, 2017; Sharma et al., 2020). Several studies have suggested that the food production system could impact the circular economy model by adapting its manufacturing model and adding value to the by-products of the agri-food industry (Jimenez-Lopez et al., 2020). Hence, considering that tannins are widely distributed among the vegetation of the terrestrial and aquatic environments and the disposal of by-products of the agri-food industry such as leaves, peels, or seeds that can be used as tannin-rich sources (Aires et al., 2016; Grenda et al., 2018). The presence of tannins in agricultural wastes opens the possibility to obtain them from sustainable and affordable sources.

3. Research methodology

Samples: Three (3) agricultural wastes (cocoa pod, coco peat and corn cob) were used. Cocoa pod was sourced from Ekiti state, South West Nigeria, while coco peat and corn cob were sourced in Sheda village and its environs, Kwali Area Council of FCT, Nigeria.

Chemicals and Reagents: All chemicals and reagents used in this study were of analytical grade.

3.1. Preparation of samples

The samples were air-dried and then pulverized to smaller particles using pulverizing machine. The pulverized samples were sieved, then packaged into air tight polythene bag and kept until when needed.

3.2. Tannin Assay

The quantity of tannins was determined by using the spectrophotometric method. 0.5g of the waste samples was weighed into plastic bottle; 50mL of distilled water is added and stirred for 1 hr. The sample was filtered into a 50 mL volumetric flask and made up to mark. 5 mL of the filtered sample was then pipetted out into test tube and mixed with 2 mL of 0.1M HCl and 0.008M $K_4Fe(CN)_6.3H_2O$. The absorbance was measured with a spectrophotometer at 395nm wavelength within 10 min.

3.3. FT-IR Spectroscopy

Fourier transform infrared (FTIR) spectroscopic analysis was used to study the surface chemistry of the waste samples. Dried waste samples were characterized using a Nicolet IS 5 Thermo Fisher Scientific, USA FTIR spectrophotometer. Each sample was scanned between the wavelength of 400 and 4000cm^{-1}.

3.4. Antibacterial activity

Four (4) pathogenic bacteria such as *Staphylococcus auerus*, *Salmonella typhi, Bacillus subtilis*, and *Klebsiella pneumonia* were collected from the Biotechnology Advanced Research Centre, Sheda Science and Technology Complex, Abuja and were confirmed using appropriate standard media. The antibacterial activity was investigated using Agar Well diffusion methods as described by CLSI (2020). The bacteria were grown on tryptic soy agar for 24h. Three to four colonies were inoculated in to muller hinton broth and incubated for 4h and the turbidity was compared with 0.5 MacFarland. A sterile swab was used to spread each of the inoculum on the Muller Hinton Agar. Five wells were bored on the media using cork borer of 6mm, the wells were sealed using MHA, and the plates were allowed to stay for 15 min. 0.1 mL of different concentration of the extracts was dispensed into different well, allow to stay for 30 min. On the control plates 0.1 mL of 10% DMSO (Negative) and 30 μg/mL (Positive) was used. The plates were incubated for 18-24 h the zone of inhibition was measured in mm.

4. Data analysis

Descriptive statistics was employed in analyzing all generated data.

5. Results and discussions

5.1. Tannin determination and characterization

The quantity of tannins is determined by the preparation of tannins solutions and absorption of the tannins read at 395nm. Table 1 shows the absorbance and subsequent amount of tannin in the agrowaste samples. The result reveals the samples contain tannin in the order: coco peat (5.5 mg/g) >cocoa pod (4.740 mg/g) >corn cob (3.212 mg/g).

Table 1: Estimation of Tannin Content of Selected Agrowastes

S/N	Sample	Sample qty (mg)	Abs @ 395nm	Tannin mg/g
1	Cocoa pod	500	2.370	4.740
2	Corn cob	500	1.606	3.212
3	Cocopeat	500	2.75	5.500

Figure 1-3, presents the individual spectra of selected agro-wastes tannin while the spectral attributes are described in Table 2. The following spectra are noticeable: a strong absorption around 3,795 - 3,216 cm^{-1} for the agrowastes with a wide and strong band centered at 3273. 85 cm^{-1} for synthetic tannin. This band is assigned to the hydroxyl groups (O-H) H-bonded broad and strong and C-H (aromatic medium) (Table 2). The obtained spectrum for OH bands is in agreement with the OH band spectrum reported by Wahyono et al. (2019) who applied FTIR spectroscopy to identify tannin compounds in the panicle of sorghum mutant lines and Marques *et al*. (2021) who carried out the extraction, quantification, and FTIR characterization of bark tannins of four forest species grown in Northeast Brazil. Preliminary information on the occurrence of a polymerization process is provided by the shape of the OH-stretching band (Kassim et al., 2011). At range of 2925.43-2917.16 cm^{-1}, a sharp peak due to the alkane medium (C-H) was observed for the agrowastes. Such sharp peak was not obtained in the synthetic tannin spectrum (Table 2). These sharp peaks are associated with the symmetric and antisymmetric -C-H- stretching vibrations of CH_2 and CH_3 groups respectively (Steel & Torrie, 1960; Wahyono et al., 2019; Marques et al., 2021). The stretching related to aliphatic groups in tannins is diagnostical because it can give information on the methylation in the chemical structure of the compounds (Ricci et al., 2015; Mageshkumar & Karthikeyan, 2016). This type of stretching appears in the spectra as narrow peaks in the region around 2,970 - 2,929 cm^{-1} for methyl groups and around 2,920 - 2,780 cm^{-1} for methylene substituents, which was observed in the agrowastes in the present work.

According to Socrates (2004), the spectral region between 1,620 - 1,400 cm^{-1} is related mainly to vibrational movements of C=C bonds in the aromatic rings, with several weak and strong characteristic peaks. In the spectra of the agrowastes samples from this study, these events were observed at region 1616.42 – 1567.99 cm^{-1} while that of the synthetic tannin is 1694.33 cm^{-1}

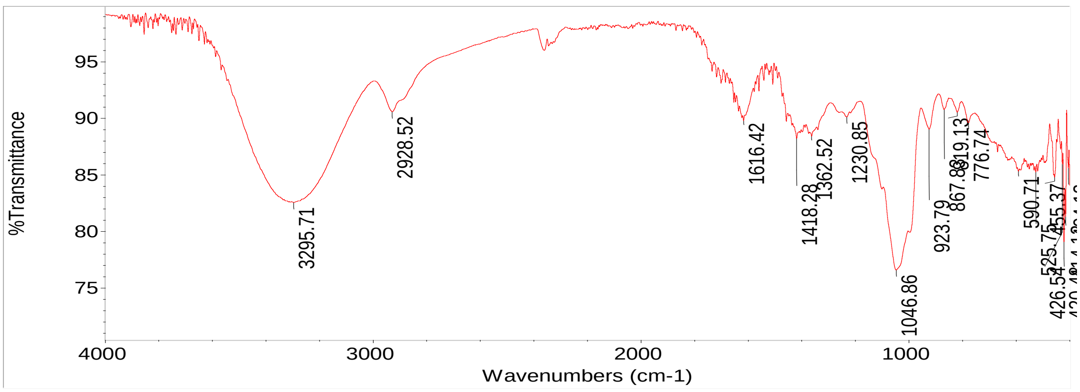

Figure 1: FT-IR Spectrum for Cocoa pod Tannin

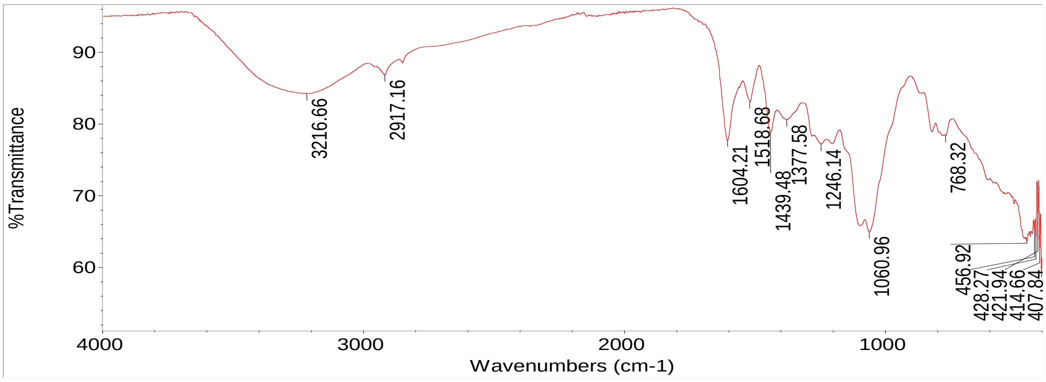

Figure 2: FT-IR Spectrum for Corn cob Tannin

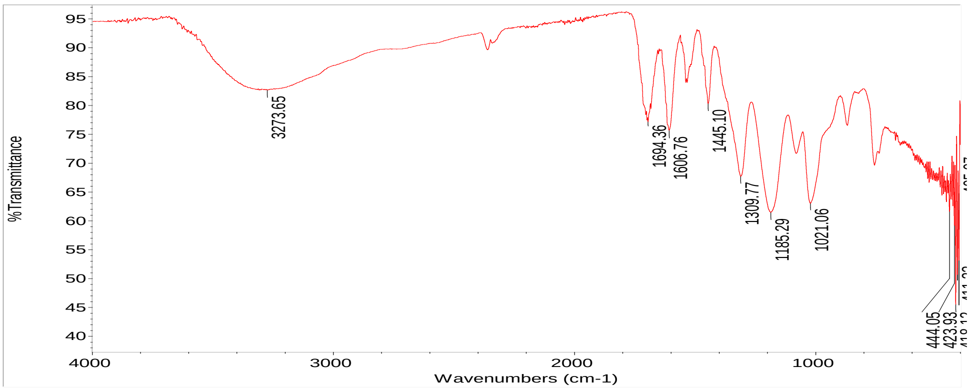

Figure 3: FT-IR Spectrum for Synthetic Tannin

Table 2: FT-IR Spectroscopy Identification of Tannins in Selected Agricultural Wastes

	Absorption bands (cm⁻¹)				
S/No	**Cocoa pod**	**Coco peat**	**Corn cob**	**Synthetic Tannin**	**Functional groups/ Event**
1	3295.71	3216.66	3250.59	3273.65	O-H stretching vibrations of hydrogen bonds
2	2928.52	2917.16	2925.06, 2341.78, 2359.77	2361.29	C-H asymmetrical stretching vibrations of aromatic sharp
3	1616.42, 1418.28	1604.21, 1439.48, 1518.8	1583.12	1694.33 1445.10, 1608.78,	C=C Aromatic weak
4	1362.52, 1230.85	1377.58, 1246.14	1393.40, 1256.06	1309.77, 1185.29	C-O
5	1046.86	1060.96	1029.48	1021.06 961.74,	C-O extension in the pyran ring of tannins and C-OH stretching vibration
6	921.47	-	-	961.74	C-O extension in the pyran ring of tannins and C-OH stretching vibration and C-OH stretching vibrations

7	817.26		816.83	865.81	Deformation of C-H from aromatic rings out-of-plane vibrations

5.2. Antibacterial Activity of Agricultural Wastes

The antibacterial activity of corn cob extracts from aqueous, methanol and ethanol extracts are as shown in Table 3. All the isolates (*Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Klebsiella pneumonia*) used were susceptible to the aqueous and ethanol extracts. This is in accordance with the studies of Boakye *et al*, 2016 and Bhalodia and Shukla, 2011. The isolates displayed resistance to methanol extract from corn cob. The highest zone of inhibition (35 mm each) was observed at 50 mg/mL aqueous and ethanol extracts in *Staphylococcus auerus* and *Klebsiella pneumonia*. The antimicrobial activity of tannin might be due its ability to penetrate the cell wall easily, inhibit metabolic activities of the cell and also reduce the amount of iron for bacteria uptake (Farha *et al.*, 2020).

Table 3: Antibacterial Activity of Corn cob Tannin

BACTERIA	AQUEOUS EXTRACTS					METHANOLIC		EXTRACTS			ETHANOLIC EXTRACTS				
	50.0	25.0	12.5	6.2	3.1	50.0	25.0	12.5	6.2	3.1	50.0	25.0	12.5	6.2	3.1
	ZONE OF INHIBITION in mm														
Staphylococc	35	30	28	26	15	0	0	0	0	0	33	20	12	10	0
Salmonella	30	20	0	0	0	0	0	0	0	0	25	0	0	0	0
Bacillus	25	0	12	0	0	0	0	0	0	0	30	0	0	0	0
Klebsiella	30	25	0	0	0	0	0	0	0	0	35	17	15	9	0

The antibacterial activity of aqueous, methanol and ethanol extracts of tannin from coco peat is as shown in Table 4. All the microorganisms used were resistance to the methanol and ethanol extracts with no zone of inhibition, the maximum zone of inhibition was seen in aqueous extract which inhibited the growth of *Staphylococcus aureus* with 40 mm at 50 mg/mL. *Bacillus subtilis* was more resistant to the aqueous extracts with only a 19 mm zone of inhibition at 25 mg/mL while *Salmonella typhi* and *Klebsiella pneumonia* had no zone of inhibition across all concentrations in both ethanolic and methanolic extracts.

Table 4: Antibacterial Activity of Coco peat Tannin

BACTERIA5	AQUEOUS EXTRACTS					METHANOLIC EXTRACTS					ETHANOLIC EXTRACTS				
	50.0	25.0	12.5	6.2	3.1	50.0	25.0	12.5	6.2	3.1	50.0	25.0	12.5	6.2	3.1
	ZONE OF INHIBITION (mm)														
Staphylococcus aureus	36	30	25	22	10	28	25	12	0	10	0	0	0	0	0
Salmonella Typhi	24	15	13	0	0	25	11	0	0	0	0	0	0	0	0
Bacillus subtilis	0	0	0	0	0	25	0	0	0	0	0	0	0	0	0
Klebsiella pnuemonae	25	0	0	0	0	30	20	10	0	0	0	0	0	0	0

The antibacterial activity of cocoa pods is as shown in Table 5. All the microorganisms used were resistance to ethanol extracts without zone of inhibition seen across all concentrations. *Bacillus subtilis* was resistant to aqueous extracts, but with 25 mm zone of inhibition seen in methanolic extracts. The highest zone of inhibition was seen in *Staphylococus aureus* with 36 mm at 50 mg/mL in aqueous extracts. This may be due to the peptidoglycan layer that allows easy penetration of tannin in to the cell and the lower activity seen in *Salmonella typhi* and *Klebsiella pneumonia* might be due to the presence of bilayer membrane (Dabbaghi, 2009; Theisen *et al*., 2014; Hull *et al.*, 2011, Belhaoues *et al.*, 2020). *Baccilus subtilis* was more resistant to most of the extract this could be due to its ability to degrade tannin (Unban *et al*., 2020; Hafiz *et al.,* 2022).

Table 5: Antimicrobial Activity of Cocoa pod tannin

BACTERIA 4	AQUEOUS				EXTRACTS	METHANOLIC					ETHANOLIC				EXTRACTS
	50.	25.	12.	6.2	3.13	5	25.	12.	6.2	3.13	5	25.	12.	6.2	3.13
	ZONE OF INHIBITION in mm														
Staphylococcus	40	35	35	30	20	0	0	0	0	0	0	0	0	0	0
Salmonella Typhi	0	27	11	0	0	0	0	0	0	0	0	0	0	0	0
Bacillus subtilis	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0
Klebsiella pnuemonae	0	30	20	10	0	0	0	0	0	0	0	0	0	0	0

The antibacterial activity of standard tannic acid is as shown in Table 6, *Salmonella* typhi was resistant to tannic acid at all concentrations while *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella puemoniae* were all susceptible to tannic acid.

Table 6: Antimicrobial Activity of Synthetic Tannic Acid

BACTERIA 6	TANNIC ACID mg/mL]					Positive Control (Chloramphenicol) (30mg/mL)	Negative Control 10% DMSO
	50.00	25.00	12.50	6.25	3.13		
ZONE OF INHIBITION (mm)							
Staphylococcus aureus	25	22	20	15	11	40	0
Salmonella typhi	0	0	0	0	0	35	0
Bacillus subtilis	25	23	20	20	10	35	0
Klebsiella pnuemonae	22	25	19	16	10	30	0

From all the extracts and tannic acid used the maximum zone of inhibition (40 mm) was seen in the aqueous tannin extract of cocoa peat in *Staphylococcus auerus*. This shows that the aqueous extract may have higher efficacy in the treatment of *Staphylococcus auerus* related infection than the synthetic tannin.

In this study, the activity of aqueous extracts was more effective with a broad spectrum of activity, this could be due to the ability of water to extract more tannin due to the presence of a hydroxyl group (Ky et al., 2016). The broad spectrum of activity observed in the synthetic tannin might be due to the high purification and concentration. The resistance of *Bacillus subtilis* is not in agreement with the findings of other researchers (Farha et al., 2020; Ekambaram et al., 2016; Dong et al., 2018).

6. Conclusion

The presence of tannin in plants reduces its nutritional value, this can be channeled to use in the treatment of infection. The disposal of waste to the environment leads to hazards, conversion of agricultural waste to wealth through the extraction of their tannin content and subsequent application in bacterial infection treatment will not only reduce such environmental hazards but provide an alternate cheap and readily available source of the antibacterial agent.

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