
Identification of Functional Groups of Coir Pith Part of Cassava Root Using FTIR Spectroscopy

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Abstract: Cassava plant has various medicinal properties to remedy for various inflammatory, analgesic and carcinogenic conditions. Cassava is also significantly rich in calcium, manganese, beta carotene, vitamin C and vitamin A. Having toxic cyanide, cassava is a remedy for number of ailments if prepared properly; such as digestive disorders, liver disease, celiac disease and diabetes. Cassava have a broad spectrum of biological activates, anti-oxidant, oxygen radical scavenging activity which are mainly due to presence of phenols and flavonoids. The purpose of present study was to identify the functional groups of the coir pith part extract of cassava root. FTIR identification was carried out by Shimadzu FTIR spectrometer 4000 series with the scan range between 4,000–400 cm^{-1} . FTIR spectroscopic investigation of methanol crude extract showed the presence of characteristic peak values of different functional groups such as carboxylic acid, amide, nitro compounds, alkanes and alkyls, aromatic-nitro containing compound, ether, alkanes, alcohols and alkenes and alkyls. An intense peak of 3388.17 cm^{-1} in coir pith part of cassava root was observed in the FTIR spectra which correspond to the carboxylic acid. So, the present study concludes that the coir pith extract possesses strong functional groups, anti-nutrients because of the presence of tannins, saponins, steroids and phenols.

Keywords: Cassava Root, FTIR, Functional Group

1. Introduction

An atomic and molecular spectrum provides detailed information about the structure and chemical properties of atoms and molecules. Spectroscopy has been an essential tool in the development of models for atomic and molecular structure, prompting scientists to refine existing models to accurately reproduce the experimentally observed spacing between energy states [4].

Medicinal plants are the important bio-active resources of drugs for conventional system of medicine [13]. FTIR Spectroscopy has been recognized as an extensively used method for finding functional groups present in the plant extracts and they were determined with the aid of IR region in the range of 4000- 400 cm^{-1} [6]. It is used to categorize chemical constituents and has been used as a necessary method to identify the medicines for pharmacopeia in several countries [14]. The wavelength of light fascinated is a characteristic of the chemical bond which might be seen in

the annotated spectrum. The chemical bonds in the molecules have been predicted using FTIR [12].

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi and viruses. Antimicrobial drugs either kill microbes or prevent the growth of microbes. Anti-microbial drugs play an important role in the treatment of many infectious diseases. Anti-microbial is given to weaken or kill some of the invading pathogens. Hopefully, the body tissues can then destroy the rest. Antimicrobial drugs are used in relatively low concentrations in or upon the bodies of organisms to prevent or treat specific infectious diseases without harming the host organism. So, plants can be a major source for search of new anti-infective agents [10].

Cassava (*Manihot esculenta*) is an extensively cultivated tuber crop and a staple food for millions of people in the tropical regions of Africa, Latin America and Asia [3]. Cassava root is a good source of carbohydrates, but a poor source of protein [15].

Cassava plant has various medicinal properties to remedy for various inflammatory, analgesic and carcinogenic

conditions. Cassava is also significantly rich in calcium, manganese, beta carotene, vitamin C and vitamin A. In spite having toxic cyanide, cassava is a remedy for number of ailments if prepared properly; such as digestive disorders (Gastritis, gastro duodenal ulcer, constipation and colitis), liver disease, celiac disease and diabetes. Cassava has been reported to have a broad spectrum of biological activities, anti-oxidant, oxygen radical scavenging activity which are mainly due to presence of phenols and flavonoids. The beneficial effects of cassava in diabetes have been confirmed by a number of studies in experimental animals [1]. So, the aim of the present study is to identify functional groups in the coir pith part of cassava root.

2. Materials and Methods

2.1. Study Area

This study was conducted in Wolaita Zone, SNNP region of Ethiopia. Considering the amount of cultivation of cassava, four Woredas: Kindo Koysa, Kindo Didaye, Ofa and Humbo were selected from the 16 Woredas of the Zone. Three sites were selected for cassava root collection based on high production in each of the purposively selected Woredas. Anaze, Sorto and Bele from Kindo Koysa; Zaro, Bereda and Pateta from Kindo Didaye; Busha, Galda and Sere Esho from Ofa and Ampo koysh, Gututo larna and Bossa wanche from Humbo were selected.

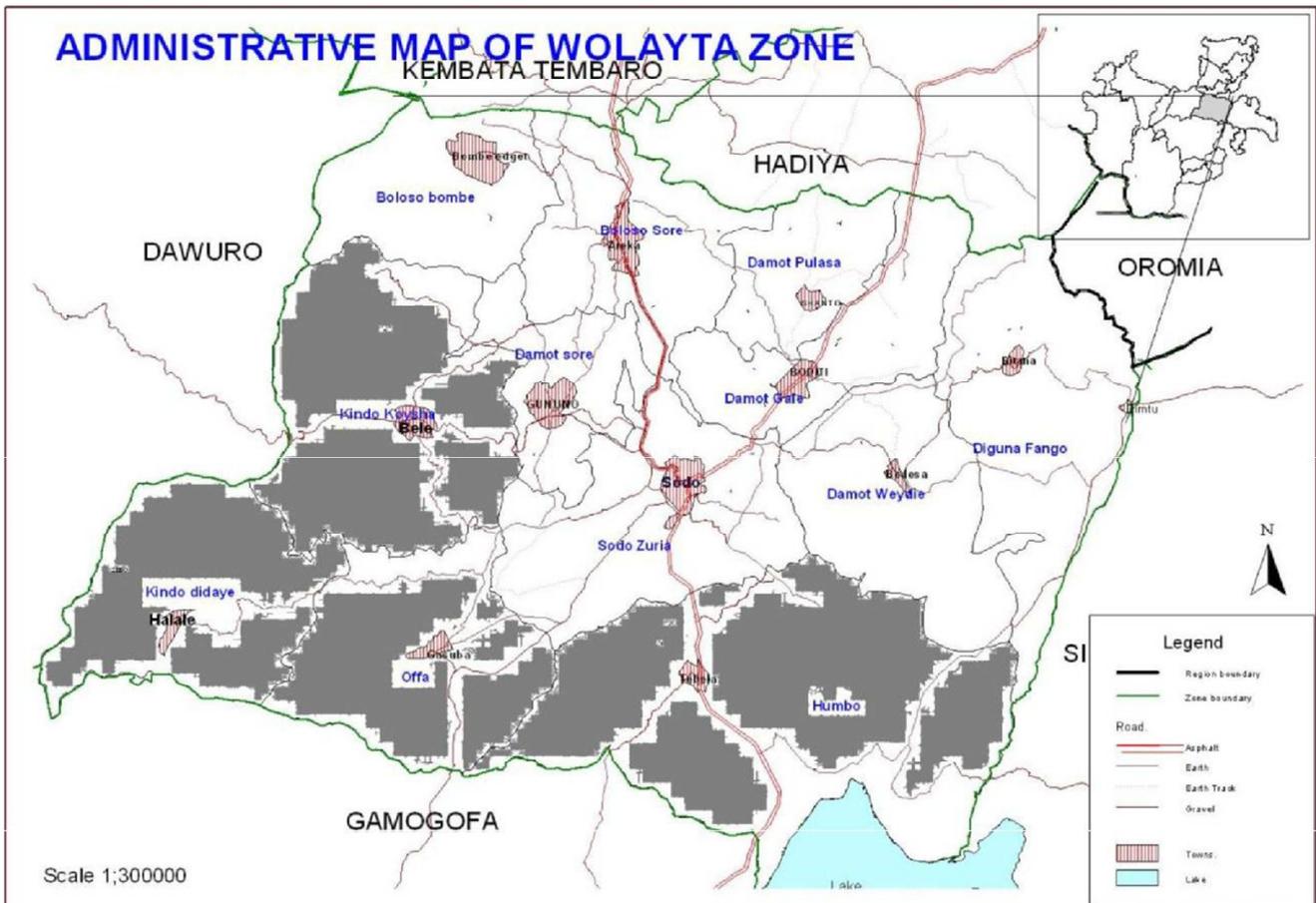


Figure 1. Administrative map of Wolaita Zone, with shaded study area (Source: Wolaita Zone Finance Economy and Development Bureau).

2.2. Chemicals

N-hexane (> 95%), Methanol ($\geq 99.9\%$) and ethanol (96%) were used for gradient extraction for plant material while Dragendroff's, Mayer's and Hager's reagents were used to test alkaloids. Alkaline reagent was used to test flavonoid and ferric chloride reagents were used to test Phenols while distilled water used to test glycosides. Chloroform reagent was used to test saponins and conc. sulphuric acid were used to test tannin and Liebermann Burchard reaction reagents were used to test steroid. Salkowski reagent was used to test Terpenoids. Dilute

hydrochloric acid (37%) was used to dissolve the extracts, nutrient broth and Mueller Hinton ager to cultivate bacteria. Dimethyl sulfoxide was used to dissolve crude extract for bacterial activity. All the reagents used in this experiment are of analytic grade and were purchased from Ran-chem. PLC Addis Ababa, Ethiopia.

2.3. Materials

Beaker was used for maceration of plant materials and Hood was used to dry the crude extract and test tubes were used for reaction handling. Filter paper was used for filtration and measuring cylinders were used to measure the solvent while

analytical beam balance (England Adam (AFP-110L)) was used to measure mass of the crude extracts. Rotary evaporation (Heidolph) was used to concentrate the solution to crude extract while Grant (GLS 400) thermostatic bath shaker was used for maceration of plant materials. Perkin Elmer BX infrared spectrometer ($4000\text{--}400\text{ cm}^{-1}$) in IR region was used to record spectra of the sample. Knife (Kiwi) was used to separate coir pith part from cassava root and fork was used to dig a cassava root. Autoclave was used for sterilization and Cork borer was used to make a well (hole) on the prepared ager plates, beakers to prepare crude extract solution.

2.4. Methods

2.4.1. Sample Collection and Preparation

Cassava roots were collected from four selected sites in Wolaita Zone. Fresh cassava roots were collected from the farm fields by digging it carefully. Healthy matured cassava plant about 1kg from each site was chosen and collected from the farm plot. Cassava tubers were washed and peeled (to remove the skin) by water to remove any impurities. From

peeled cassava tuber, coir pith part was extracted by knife after one day. Site samples were mixed together. The extracted part of cassava tuber was dried under room temperature for 14 days and milled to suitable size locally by human labor. Finally, the milled coir pith part of cassava root of 250g powder was prepared, and stored under refrigerator below 4°C until it was used for extraction.

2.4.2. Extraction

250g of powdered coir pith part of Cassava root was sequentially extracted with n-hexane, methanol and ethanol. One liter of each solvent was added by using maceration techniques for 48hrs with continuous shaking. The extracted matter was filtered using Whatmann no 1 filter paper, and the residual solvent in each gradient extract was removed using rotary evaporator under reduced pressure (1atm). The mass of the crude extracts of each solvent was determined using analytical balance (England Adam (AFP-110L)) and stored in hood for further analysis. General schematic flow chart indicates general extraction techniques according to as follow.

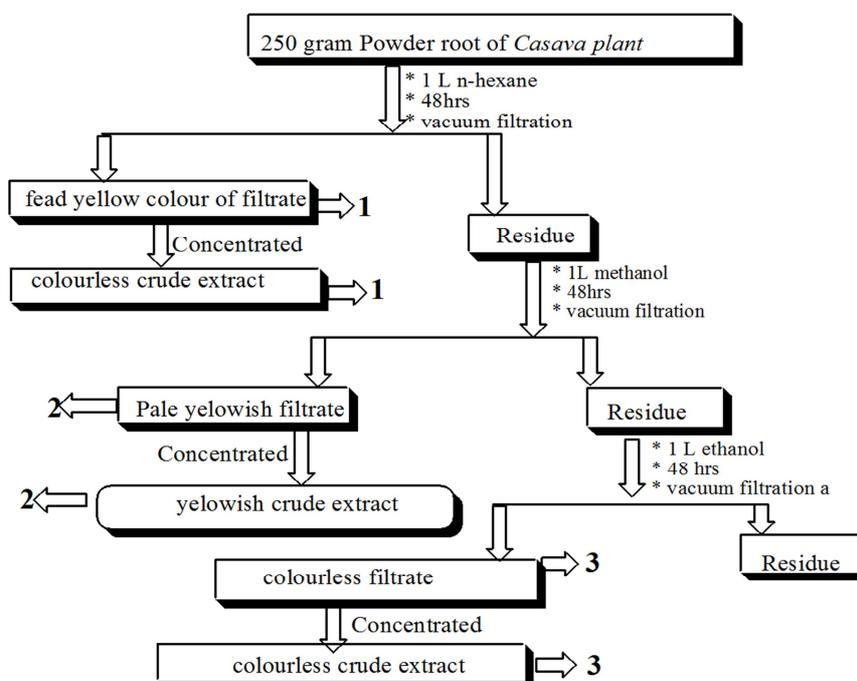


Figure 2. General outline for the extraction of coir pith part of Cassava root [5].

Firstly, powdered form of 250g of coir pith part of cassava root was added in 1L of n-hexane and stayed for 48hrs in vacuum filtration then filtrated with Whatmann. no 1 filter paper and then a fead yellow color (pure n-hexane and important compounds) and residue was obtained. A fead yellow color was concentrated by rotary evaporation at 1atm and n-hexane was removed and only 0.8g colorless crude extract was obtained. Secondly, 1L of methanol solvent was added in remaining residue and stayed for 48hrs in vacuum filtration and again then filtrated with Whatmann no 1 filter paper. Gave pale yellowish extract (pure methanol and important compounds) and residue was obtained. Pale

yellowish extract was concentrated again by rotary evaporation at 1atm and then methanol was removed and only 2.5g of yellowish crude extract was obtained. Thirdly, 1L of ethanol was added to the residue and stayed for 48hrs in vacuum filtration and then filtrated with Whatmann no. 1 filter paper that gave rise to colorless filtrate and residue. Colorless filtrate was concentrated by rotary evaporation at 1atm and ethanol was removed and 0.99g colorless crude extract was obtained. Finally, from three solvent system 4.29g crude extract was obtained and remaining residue was let over. Each crude obtained from extraction stored under fuming hood until becomes dry.

2.4.3. Mass of Crude Extract

Depending on the method indicated (in figure 2), the crude extracts was carried out and allowed to dry completely. The percentage yield of successive extracts of coir pith part of

Cassava was determined (Table 1). The mass in each crude extract and percentage yield was calculated as follows:

$$\text{Percentage yield} = \frac{\text{mass of extract (g)}}{\text{mass of used for extraction (g)}} \times 100\%$$

Table 1. The Percentage yield of solvent system used for extraction in this work.

No.	Solvent	Mass of Crude extract (gram)	Crude Yield (%)
1	n-hexane	0.8	0.32
2	Methanol	2.5	1
3	Ethanol	0.99	0.396

2.4.4. FTIR Spectroscopic Method

Dried powder of methanol solvent extracts of coir pith part cassava root (*M. esculenta*) was used for FTIR analysis. One milligram of the dried extract powder was encapsulated in 100mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each extract was loaded in FTIR spectroscope (Shimadzu, Japan), with a scan range from 4000 to 400 cm⁻¹ with a resolution of 8 cm⁻¹. The analysis was repeated twice for the spectrum confirmation.

2.5. Statistical Analysis

All statistical analysis was performed by SPSS statistical software version 20 (SPSS Inc. Chicago, USA).

3. Results and Discussions

Depending on the protocol indicated in Figure 2, the crude extracts was carried and allowed to dry completely. The percentage yield of successive extracts of coir pith part of Cassava root is displayed in Table 1. The gradient extraction of the roots of the targeted plant using different solvents produced different percentage yields as in table 1. The percentage of crude extract of methanol is found to be higher than the ethanol and n-hexane. Due to the polarity range of solvents the gradient extraction of methanol became higher than that of other solvent.

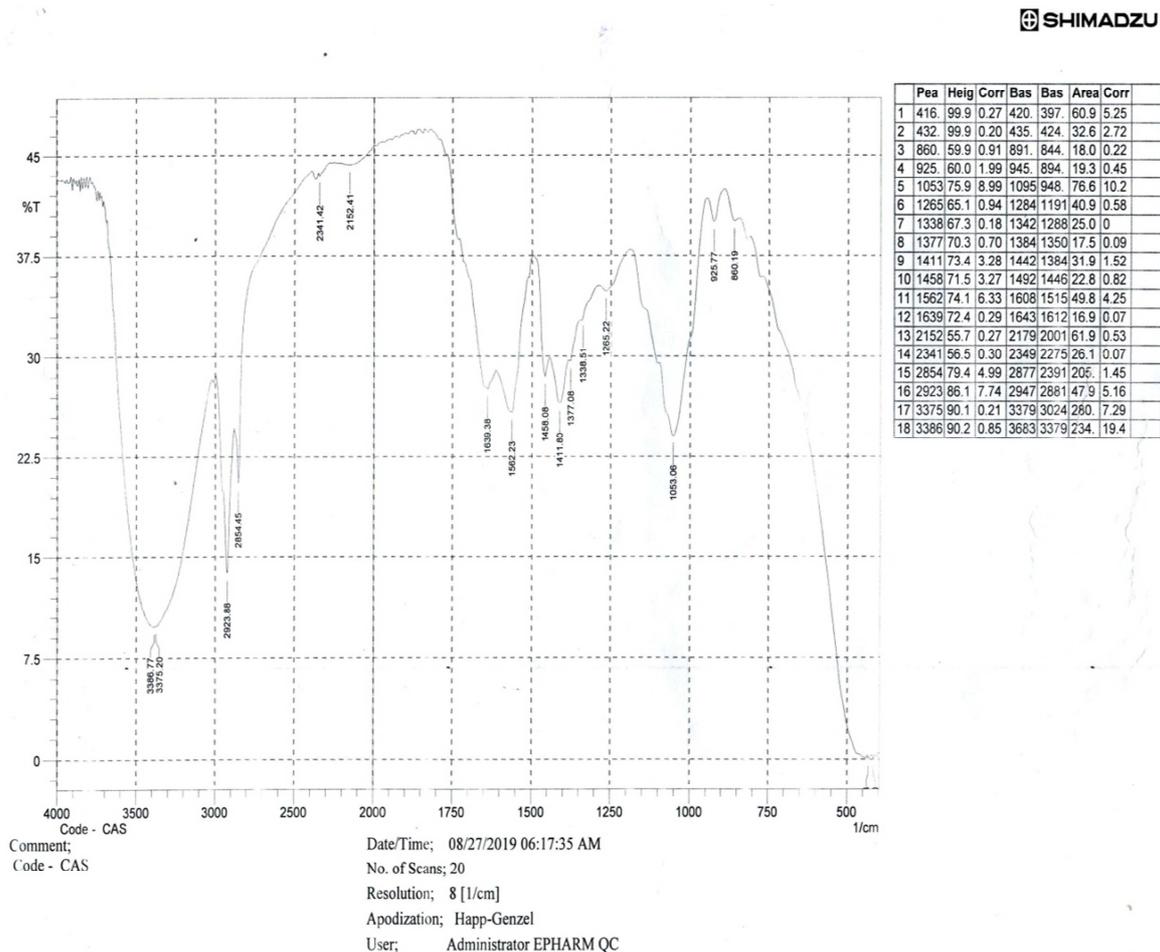


Figure 3. FTIR results.

Table 2. Summary of FTIR spectrum (cm^{-1}) on crude extract of methanol on coir pith part of cassava root recorded in this work.

Class of compound	Absorption	Range wave number	Intensity	Functional group	References
Carboxylic acid	3388.17	3570–3200	Broad peak	RCOOH	[2]
	2923	2915-2940	Sharp peak	C-H ₃	[11]
Alkanes	2854.45	2835-2895	Sharp peak	-CH ₂	[11]
Amides	1639.38	1580 -1660	Sharp peak	R-C(O)-NR'R' C=O stretch	[7, 8, 11]
Nitro Compound	1562.23	1580 -1660	Sharp peak	R-NO ₂ and (N-O symmetric)	[8, 11]
	1458.08	1485–1445	Sharp peak	(C-H) bending	[2, 11]
Alkanes and alkyls	1411.80	1470–1430	Sharp peak	α CH ₂ bending	[2]
	1377.08	1395-1365	Sharp peak	CH ₃ -CH bending	[2]
Aromatic-nitro containing compound	1338.51	1355–13204	Sharp peak	(Ar-NO ₂) asymmetric stretching both two bands	[2]
Ether	1265.22	1270–1230	Sharp peak	(Ar-O-R) (=C-O-C)	[7]
Alcohols	1053.06	~1050	Sharp peak	R-CH ₂ -OH (1°) or C=C-CH(R)-OH in the presence of C-O stretching	[2]
	925.77	1055–1000	Sharp peak	RCH=CH ₂	[7]
Alkenes and alkyls		895–885	Sharp peak	R=C-H bend and R	[2]

3.1. Spectroscopy Analysis

Methanol extract of coir pith part of cassava root was obtained as light yellowish gummy appearance. This crude was selected to determine the functional group of crude extract of coir pith part of cassava root.

Functional groups were identified in coir pith part of cassava root extract by comparing corresponding functional group peak absorption value at a particular wave number with FTIR Standards [2, 7].

Analysis of FTIR (KBr) spectrum (Appendix) shows that the crude extract of methanol has no a doublet band at/near 2850 and 2750 cm^{-1} that indicates the crude has no aldehyde functional group. The presence of weak bands in the range of 1650 and 2000 cm^{-1} indicates that the crude has aromatic functional group. On the other hands, the observed stretching band at 3388.17 cm^{-1} indicates the presence of carboxylic acid (RCOOH). The strong band at 2923 cm^{-1} represents C-H stretch of alkanes whereas the bands at 2854.45 cm^{-1} indicates the C-H stretching of methyl groups (R-CH₃).

The stretching band at 1639.38 cm^{-1} indicates the presence of amide R-C(O)-NR'R' C=O stretch and the observed stretching band at 1562.23 cm^{-1} indicates the presence of nitro- compounds R-NO₂ and (N-O symmetric). The observed stretching band at 1458.08 cm^{-1} indicates the presence of alkanes and alkyls (C-H) bending. The observed stretching band at 1411.80 cm^{-1} indicates the presence of alkanes and alkyls α CH₂ bending.

The observed stretching band at 1377.08 cm^{-1} indicates the presence of alkanes and alkyls (CH₃- CH) bending while the observed stretching band at 1338.51 cm^{-1} indicates the presence of aromatic compound with the presence of nitro group (Ar-NO₂) asymmetric stretching. The observed stretching band at 1265.22 cm^{-1} indicates the presence of aromatic ether (Ar-O-R) (=C-O-C) asymmetric and a symmetric arrangement while the observed stretching band at 1053.06 cm^{-1} indicates the presence of alcohols R-CH₂-OH (1°) or C=C-CH(R)-OH in the presence of C-O stretching. The observed stretching bands at 925.77 cm^{-1} and 860.19 cm^{-1} indicate the presence of alkenes and alkyls (RCH=CH₂, R=C-H bend and R).

3.2. Discussion

As can be seen from Table 2, some hazard functional groups were observed based on the FTIR spectral data. The presence of carboxylic acid, alcohol and amide may lead to vomiting, loss of consciousness and cause of stomach problem when taken in excess. Carboxylic acids that are present in plant-based foods such as cassava have a negative impact on the bioavailability of magnesium and calcium. This anti-nutritional agent binds calcium and leads to formation or extraction through urine. The crystals from calcium oxalate majorly contribute to kidney stones. It is highly advisable to reduce oxalate intake and promote the intake of calcium among individuals who are risk of kidney stones [9].

From this study, aromatic-nitro containing compounds: ether, amides, nitro compounds and carboxylic acid are oxygen containing functional groups and their presence makes the coir pith part of cassava root a modest antimicrobial activity.

4. Conclusion and Recommendations

4.1. Conclusion

In this study, identification of functional groups of coir pith part of cassava roots were carried out. FTIR spectroscopic investigation of methanol crude extract showed the presence of characteristic peak values of different functional groups such as carboxylic acid, amide, nitro compounds, alkanes and alkyls, aromatic-nitro containing compound, ether, alkanes, alcohols and alkenes and alkyls. An intense peak of 3388.17 cm^{-1} coir pith part of cassava root is observed in the FTIR spectra and it corresponded to the carboxylic acid. These shows coir pith part of cassava root could be suggested as anti-nutrient.

4.2. Recommendations

In present study showed that the coir pith part of cassava root crude extract possesses strong functional groups like carboxylic acid, alcohol and amides which are anti-nutrients

candidates. So, the society should be carefully and extract coir pith part of cassava root when they cook tuber at home. The society should also take care when mixing cassava product with other grains like teff and maize for commercial purpose. Before mixing with another grain, first it is advisable to extract coir pith part from cassava root. The information obtained in this study on, functional group in coir pith part of cassava root will be crucial for awareness campaigns to its users. In future advanced spectroscopic investigations will be needed for the identification and structural elucidation of compounds, phytochemistry and anti-bacterial activities present in the coir pith part of cassava root.

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