

Identification Phytochemicals and Antibacterial Activity of Coir Pith Part of Cassava Root

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Abstract: The purpose of the present study was to identify phytochemicals and anti-microbial activity of the coir pith part extract of cassava root. Preliminary Phytochemical screening was carried out for all the three extracts: n-hexane, methanol and ethanol as per the standard methods. Antibacterial activity of methanol crude extract of the coir pith part (*M. esculenta*) was performed and it was carried out by using agar well diffusion method. The preliminary phytochemical screening shows alkaloids and flavonoids present only on methanol crude extract and tannins, saponins, flavonoids, glycosides steroids, terpenoids and phenols are available on ethanol, methanol and n- hexane crude extracts. The zone of inhibition of methanolic extract of *M. esculenta* is 13.67 ± 0.58 mm, 16.67 ± 0.57 mm and 18.33 ± 0.58 mm at concentration of 25mg/ml, 50mg/ml and 100mg/ml in *P. aeruginosa*, respectively, and 12.33 ± 1.53 mm, 13 ± 1.00 mm and 15.33 ± 0.58 mm at concentration of 25mg/ml, 50mg/ml and 100mg/ml in *S. aureus*, respectively. Moreover, the extract also showed moderate antibacterial activity at all concentrations in the range between 12 mm to 18mm on both tested organisms. So, the present study concludes that the coir pith extract anti-nutrients because of the presence of tannins, saponins, steroids and phenols.

Keywords: Anti-Bacterial, Cassava Root, Phytochemical

1. Introduction

Phytochemicals are responsible for medicinal activities of the plants. Based on this fundamental knowledge several pharmaceutical industries are established. The phytochemical constituents that are playing a significant role in medicines can be identified using crude extracts/drugs of the plants [17]. These are non-nutritive chemicals that protect human beings from various diseases.

Phytochemical are basically divided into two major groups, there are primary and secondary metabolites that are categorized based on the function in plant metabolism. Primary metabolites comprise common carbohydrates, amino acids, proteins and chlorophylls, while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids and tannins [9]. The present study focusses on secondary metabolites of coir pith part of cassava root. The development of antimicrobial-resistant bacteria species stems from a number of factors which include the prevalent and sometimes

inappropriate use of antibiotics, extensive use of these agents as growth enhancers in animal feed, and increased transboundary passage of antibiotic-resistant bacteria [10]. Against this backdrop, the development of alternative drug classes to treat such infectious diseases is urgently required [3]. Some natural products are highly efficient in the treatment of bacterial infections [6].

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 [12].

Cassava root contains anti-nutrients such as phytate,

nitrate, polyphenols, oxalate, saponins, tannins and steroids. However, some of these compounds can also act as anti-carcinogens and anti-oxidants depending on the amount ingested [18]. From cassava root various functional groups were identified like alkyl, aliphatic aromatic, benzene stretching ring, phenol, aromatic and aromatic hydrogen were reported by [8]. Cassava supplements may stomach upset, bitter taste, nausea, thyroid disease and vomiting in some.

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 [1], [2]. Cassava root is a good source of carbohydrates, but a poor source of protein [19].

The Cassava (*Manihot esculenta*) plant contains various phytochemicals such as saponins, steroids, glycosides, alkaloids, phenols, terpenoids, tannins and flavonoids [14]. 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 focused on preliminary phytochemical screening and anti-bacterial activity in the coir pith part of cassava root.

2. Materials and Methods

This study was conducted in Wolaita Zone, SNNP region of Ethiopia. Considering the amount of cultivation of cassava, four Woredas: Kindo Koysha, Kindo Didaye, Ofa and Humbo Woredas of the Zone. Three sites were selected for cassava root collection based on high production in each of the purposively selected Woredas.

2.1. 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 agar 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.

2.2. 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. 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 agar plates. Micro pipette was used to inoculate (make spread) bacteria suspension into agar plate. Petri dishes and incubator were used to cultivate bacteria, beakers to prepare crude extract solution. Bunsen burner was used to sterilize Cork borer and inoculating loop was used to inoculate or to transfer bacteria into broth. Laminar air flow cabinet was used for aseptic conditions and tetracycline disc was used as positive control.

2.3. 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. 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. Extraction

Two hundred fifty gram 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 Whatman 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. 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 Whatman 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.

1L of methanol solvent was added in remaining residue

and stayed for 48hrs in vacuum filtration and again then filtrated with Whatman 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.

1L of ethanol was added to the residue and stayed for 48hrs in vacuum filtration and then filtrated with Whatman 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.

2.5. Mass of Crude Extract

Depending on the method indicated, 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 in 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

2.6. Phytochemical Screening

Photochemical examinations were carried out for all the extracts as per the standard methods reported in the literature.

2.7. Anti-Bacterial Activity

Depending on the aim of the study anti-bacterial activity were examined on crude extract of methanol. It was carried out by using agar well diffusion method as described by [15]. The activity of the plant extract was evaluated by using two different bacterial species; Gram positive bacteria (*S. aureus*) and a Gram-negative bacterium (*P. aeruginosa*). The investigate organisms were obtained from microbiology laboratory in Wolaita Sodo University Teaching and Referral Hospital. All the cultures were maintained at 4°C in nutrient agar slants until used. The microorganisms were activated by inoculating loopful of each investigate organisms in the separate test tube that contains nutrient broth and incubated at 37°C for 24 h. After 24h, 0.2 ml of inoculum was inoculated into the prepared Mueller Hinton agar plate. The inoculum was spread on the agar plate by using sterile cotton swab. Then, a well was made in the seeded agar plates with the help of a cork-borer (5 mm). Then after, the prepared plant extract was introduced into the well and all the plates

were incubated at 37 °C for 24 h. The experiment was performed in triplicate under aseptic conditions. After 24h, microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values were presented. In this experiment tetracycline was used as positive control.

2.8. Statistical Analysis

Antibacterial activity results were analyzed using descriptive statistics. One-way ANOVA was employed to test the statistical significance of the values. All statistical analysis was performed by SPSS statistical software version 20 (SPSS Inc. Chicago, USA).

3. Results and Discussions

3.1. Mass of Crude Extract

Depending on the protocol, 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.

3.2. Phytochemical Screening

The extracts from each solvent were analyzed quantitatively for selected phytochemicals, such as alkaloids, flavonoids, phenols, glycosides, Terpenoids, Tannins, saponins and steroids by previously described methods [7], [13], [16].

The qualitative investigation for phytochemicals in coir pith part of cassava root is presented in Table 2. All the 8 investigated phytochemicals were present in coir pith part of cassava root. N-hexane crude extract showed the presence of phenols, glycosides, saponins, steroids, terpenoids and tannins but absence of alkaloids and flavonoids. On the other hands, crude extract of methanol indicates the presence of alkaloids, flavonoids, phenols, glycosides, saponins, steroids, terpenoids and tannins. Crude extract of ethanol indicates the presence of phenols, glycosides, saponins, steroids, terpenoids and tannins but lacks alkaloids and flavonoid. Alkaloids and flavonoids compounds are obtained only in a methanol crude extract.

During the present screening investigation, diverse types of results observed in different solvents for coir pith part of cassava root. The presence or absence of phytochemicals in one or another solvent provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds.

In preliminary phytochemical evaluation of our study, methanol extract revealed a positive result in all of investigated phytochemicals. This showed that methanol extract is more effective than other solvent extracts in this study. Our results suggest that coir pith part of cassava root possess several known and unknown bioactive compounds, which are a potential source of useful drugs other than food source. Thus, by isolating and identifying these bioactive compounds, new drugs can be formulated to treat various diseases and disorders.

Table 2. The Phytochemical Analysis of coir pith part of cassava root in this work.

Bio-active components	Reagent used	Solvent used to extraction					
		n-hexane extract		Methanol extract		Ethanol extract	
		Result	Observed Color	Result	Observed color	Result	Observed color
Alkaloids	Dragendroff's reagent	-	Red color	++	Red color	-	Red color
Flavonoids	Alkaline reagent	-	Deep yellow color	++	Deep yellow color	-	Deep yellow color
phenols	Ferric chloride	++	Bluish black color	++	Bluish black color	-	Bluish black color
Glycosides	Keller-Killani test	++	Light reddish brown	++	Light reddish brown	++	Light reddish brown
Terpenoids	Salkowski test	++	Radish brown color	++	Radish brown color	++	Radish brown color
Tannins	Ferric chloride	++	Brownish green	++	Brownish green	++	Brownish green
Saponins	Distilled water	++	Strong foam formation	++	Strong foam formation	++	Strong foam formation
Steroids	Liebermann Buchardrxn	++	Light blue green	++	Light blue green	++	Light blue green

Key: Strong (++) Absent (--)

Table 3. Mean inhibition zone (millimeter) of the methanol extract of *M. esculenta* in this work.

Investigate organisms	Zone of inhibition			
	25mg/ml	50mg/ml	100mg/ml	Control (tetracycline) 30µg/disc
<i>P. aeruginosa</i>	13.67±0.58	16.67± 0.58	18.33±0.58	27±0.00
<i>S. aureus</i>	12.33±1.53	13 ±1.00	15.33±0.38	28±0.00

3.3. Antibacterial Analysis

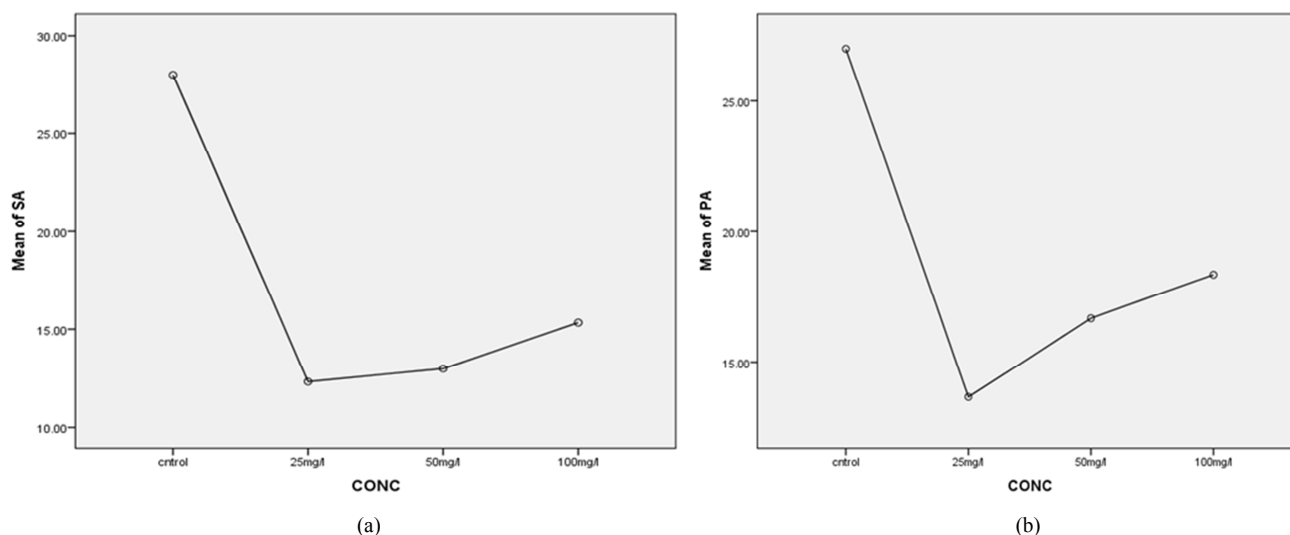
In the present study, anti-bacterial activity of the crude methanol extract of coir pith part of Cassava root was performed and the result of mean antimicrobial activity was presented in Table 3.

The mean inhibition zone observed for *P. aeruginosa* is 13.67± 0.58mm, 16.67 ± 0.58 mm and 18.33 ± 0.58 mm at concentration of 25mg/ml, 50mg/ml and 100mg/ml, respectively while the mean inhibition zone observed for *S. aureus* is 12.33 ± 1.53 mm, 13 ± 1.00 mm and 15.33 ± 0.38 mm at concentration of 25mg/ml, 50mg/ml and 100mg/ml, respectively. The zone of inhibition also observed on positive control (tetracycline) and it is 27±0.00 mm in *P. aeruginosa*

and 28±0.00 mm in *S. aureus*.

When comparing the mean inhibition zone of the extracts with positive control (tetracycline), the positive control showed better antibacterial activities on both test organisms at all a concentration which is 27±0 mm and 28±0 mm in *P. aeruginosa* and *S. aureus*, respectively.

Extracts have also showed moderate antibacterial activity at all concentrations in the range 12 mm to 18 mm on both tested organisms. While comparing the mean inhibition zone of the extracts, depending on the tested organisms, the extracts are more effective on Gram negative bacteria (*P. aeruginosa*) than Gram positive bacteria (*S. aureus*) at all concentration. The concentrations of the extracts increase when the inhibition zone increases in both bacterial species (Figure 1).

**Figure 1.** (a) The means verses concentration for *Staphylococcus aureus* (SA). (b) The means verses concentration for *Pseudomonas aeruginosa* (PA).

3.4. Discussion

Some anti-nutrient phytochemicals are observed from the

crude extract such as tannin, saponins, steroid and phenols. So, the presence of these phytochemicals in coir pith part of cassava root makes this part not to be suggested for nutrition. Methanol crude extract showed effective inhibits zone in two

investigated bacteria; these also indicates that the coir pith part of cassava root is anti-nutrient.

Previous researchers have reported that the extract of *M. esculenta* is more effective on *P. aeruginosa* than *S. aureus* [20, 21] also reported that the extract of *M. esculenta* is more effective on Gram negative bacteria than gram positive bacteria. The interesting aspects of the antibacterial investigation of coir pith part crude extract is that it shows positive response by developing significant clear zone of inhibition against wide range of bacterial which confirms its anti-bacterial potential. The biological activity of plant extract is used to heal cardiovascular problems, skin diseases, asthma, wound healing, protect from drug toxicity and UV radiations [11].

A correlation of the antimicrobial activity of the compounds investigated in this study with their chemical structure and functional groups suggests a number of observations. Alcohols are known to possess bactericidal rather than bacteriostatic activity against vegetative cells. The alcohol terpenoids in this study did exhibit activity against the investigate microorganisms, potentially acting as either protein denaturing agents' solvents [5] or dehydrating agents. The presence of an oxygen function in the framework increases the antimicrobial properties of terpenoids [4]. 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. *P. aeruginosa* and *S. aureus* being the most significantly affected (Table 3).

Tannins bind to adhesions, inhibits enzyme, and may also complex with cell wall. It also makes intestinal mucosa more resistant and reduces secretion. Glycosides inhibit release of autacoids steroids and enhance intestinal absorption of Na^+ and water. Saponins aids vacuolization and disintegration of teguments and also inhibit histamine release in vitro. The presences of these phytochemical components and their mechanism of action confirmed their antibacterial activity on crude extract of coir pith part of cassava root.

4. Conclusion and Recommendations

4.1. Conclusion

In this study, identification of phytochemicals screening and antibacterial activity of coir pith part of cassava roots were carried out. The results of the present study identified the qualitative phytochemicals of coir pith part of cassava root. Alkaloid and flavonoid are only found in methanol crude extract and tannins, steroids, saponins, phenols, terpenoid and glycosides are found in ethanol, methanol and n-hexane. The presence of tannin, saponins and phenols in the coir part of cassava root suggests that the studied samples for medicinal benefit rather than nutritional value.

The zone of inhibition of methanolic extract of *M. esculenta* coir pith part of the root was observed to be 13.67 ± 0.58 mm, 16.67 ± 0.57 mm and 18.33 ± 0.58 mm at concentration of 25mg/ml, 50mg/ml and 100 mg/ml, respectively in *P. aeruginosa*. The inhibition zone was seen to

be 12.33 ± 1.53 mm, 13 ± 1.00 mm and 15.33 ± 0.58 mm at concentration of 25mg/ml, 50mg/ml and 100 mg/ml, respectively in *S. aureus*. Moreover, the extract also showed moderate antibacterial activity at all concentrations ranging from 12mm to 18mm on both tested organisms.

4.2. Recommendations

In present study showed that the coir pith part of cassava root crude extract possesses the result showed that because the presence of tannins, saponins and steroids which are responsible for the moderate anti-bacterial activity. 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, phytochemicals and anti-bacterial activity in coir pith part of cassava root will be crucial for awareness campaigns to its users. advanced spectroscopic investigations will be needed for the identification and structural elucidation of compounds present in the coir pith part of cassava root.

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