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Phytochemistry, Antimicrobial Potential and Proximate Evaluation of *Marantochloa Cuspidata* (Marantaceae)

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Abstract:
The leaves of Marantochloa cuspidata are widely used in wrapping food in African communities. It is believed to enhance the taste of such food items as well as preserve them. A 50% ethanol extract of the leaves was subjected to phytochemical screening, proximate analysis and evaluation of its antimicrobial potentials using standard methods. Phytochemical screening revealed the presence of alkaloids, cardiac glycosides, tannins and saponins. Proximate analysis showed moisture content 8.81%, ash content 7.22%, fibre content 65.55% and lipid content of 7.48%. The Median Lethal Dose (LD_{50}) was 2214.58mg/kg. The extract showed antibacterial and antifungal properties, thereby justifying its ethno-botanical uses.

Keywords: *Marantochloa cuspidata, antibacterial, antifungal, proximate analysis.*

1. Introduction

The leaves of *Marantochloa cuspidata* are widely used in wrapping food in African communities (Iwu *et al*, 1993). It is believed to enhance the taste of such food items as well as preserve them. The leaves are also used as receptacles for honey collection and rubber tapping (Miyaukaki and Sasaka, 2000; Betti, 2004). The edible and medicinal uses are unknown (Fern *et al*, 2014). This study is aimed at evaluating the benefits of its folkloric use.

2. Materials and Methods

2.1. Sample Collection, Authentication, Preparation and Extraction

The fresh leaves of *Marantochloa cuspidata* were harvested from Abak L.G.A of Akwa Ibom State. They were identified and authenticated by Dr. (Mrs) M.E. Bassey, a plant taxonomist in the department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State. The leaves were washed and air-dried under room condition until thoroughly dried. They were ground into powder manually using a mortar and pestle and further pulverized using a blender. The powdered sample was stored in an airtight bottle prior to evaluations. 200g of the sample was weighed into an extraction jar and extracted with 50% ethanol at room temperature (27 ± 2^0C) for 72 hours with frequent stirring. The crude extract was then filtered, concentrated to dryness at 40^oC and stored in a refrigerator at 4^0C.

2.2. Phytochemical Screenings

The dried ethanol extract was examined for secondary plant metabolites such as alkaloids, anthraquinones, cardiac glycosides, phlobatannins, saponins and tannins according to phytochemical methods as described by Sofowora (2008); Evans (2009).

2.3. Proximate Analysis

Crude protein content (as Nitrogen) was determined by micro-Kjeldahl method (Isengard, 2001), lipid content by petroleum ether extract analysis, crude fibre, ash content and moisture content by standard methods. Carbohydrate content was determined by differential of total contents (Edwards, 1999).

2.4. Antimicrobial Analysis

2.4.1. Test Organisms, Standardization, Inoculation & Incubation

The test microbial organisms were Gram positive bacteria (*Bacillus subtilis* NCTC 8853 & *Staphylococcus aureus* NCTC 6571), Gram negative bacteria (*Escherichia coli* NCTC 1048 and *Pseudomonas aeruginosa* NCTC 2785) and the yeast was a clinical isolate of *Candida albicans*. The test organisms were typed cultures provided by the department of Pharmaceutics and Pharmaceutical Technology incorporating Pharmaceutical Microbiological, Faculty of Pharmacy, University of Uyo.

The bacteria strains were sustained on nutrient agar slant at 4^0C while the *Candida albicans* was grown and maintained on Sabouraud's dextrose agar which was prepared and treated according to the manufacturer's (Biotec UK) guidelines. The cultures were standardized to 1×10^6 cfu/ml before use by dispensing 0.1ml of overnight culture of each organism into 20ml of sterile nutrient broth (for bacteria strains), incubated for 24 hours and Sabouraud's dextrose broth (for fungi) and incubated for 72 hours.

2.4.2. Determination of Antimicrobial Activity

The hole-in-plate bioassay method (Hugo and Rusell,1984) was used. About 0.1ml of the inoculums of each microorganism previously standardized to 1×10^6 cfu/ml was transferred into labelled sterile Petri-dishes with the aid of sterilized Pasteur pipettes (using different pipette for different organisms). 25ml of sterilized molten cooled agar but cooled to 45^oC was measured into each Petri dish, swirled gently for proper mixing and seeding of the microorganisms in the agar media and allowed to solidify in an aseptic environment.

A sterile cork borer of 4mm diameter was used to bore equidistant holes in the solidified agar (Reeves *et al*, 1979). Various concentrations of the plant extract were aseptically and carefully filled into separate wells avoiding overfilling and flooding. 0.04mg/ml solution of streptomycin (in cases of bacteria strains) and 0.01mg/ml Nystatin solution (for fungi strain) were filled into separate holes in each plate as positive controls respectively. 50% ethanol was equally filled into separate holes in each plate as negative control. The plates were covered and allowed to stand undisturbed at room temperature for about 1 hour to allow proper diffusion to occur. The bacteria strains-seeded plates where then incubated at 37^oC for 24 hours and fungi seeded plates at 28^oC for 72 hours. Antimicrobial activity was evaluated by measuring the zones of inhibition of microorganisms around each hole.

3. Results

Results of Phytochemical screening of *Marantochloa cuspidata* leaf extract could be seen in table 1, while tables 2 and 3 show the results of antimicrobial screening and proximate analysis
respectively.

TESTS FOR SECONDARY METABOLITES	CONCLUSIONS
Alkaloid	
a. Drangendorff Reagent	+ + +
b. Mayer's Reagent	+ + +
c. Picric acid test	+ + +
Anthraquinones	
a. Borntrager's combined anthraquinone test	+ + +
Cardiac glycosides	
a. Salkowski's test	+ +
b. Keller Killiani test	+ +
Phlobatannins	
a. Hydrochloride test	—
Saponins	
a. Frothing test	+ + +
b. Sodium bicarbonate test	+ + +
Tannins	
a. Ferric Chloride test	+ +

Table 1: Phytochemical screening of Marantochloa cuspidata leaf extract

Key

(+++) = Abundantly present
(++) = Moderately present
(+) = Scantily present
(—) = Absent

*Diameter of Zone of inhibition (mm)						
Organism	**Plant Extract (mg/ml)**				**Control drugs (mg/ml)**	
					Streptomycin	Nystatin
	25	50	100	200	0.04	0.01
Staphylococcus aureus	1.2	1.7	2.0	3.0	4.8	—
Pseudomonas aeruginosa	1.3	1.5	2.0	2.3	4.1	—
Escherichia coli	1.5	2.0	2.8	3.4	4.0	—
Salmonella typhi	1.2	2.0	2.3	2.9	6.7	—
Bacillus subtilis	1.3	1.5	2.0	3.0	4.1	—
Candida albicans	1.5	2.3	2.5	3.3	—	6.8
*Diameter excludes the diameter of the cork borer (4mm)						

Table 2: Antimicrobial activity of Marantochloa cuspidata leaves extract

Proximate Composition	**Content (%)**	**Caloric Value (J)**
Moisture content	8.81	—
Ash content	7.22	—
Fibre content	5.05	—
Protein	14.70	58.80
Lipids	7.48	67.32
Carbohydrate	65.55	262.20

Table 3: Proximate analysis

4. Discussion

The phytochemical screening of *Marantochloa cuspidata leaves* revealed the presence of abundant alkaloids and saponins in addition to moderate content of tannins and cardiac glycosides. The presence of these secondary metabolites suggest that the leaves of *Marantochloa cuspidata* could be of medicinal and industrial value. Many alkaloids have been shown to function as protective agents in plants that synthesize them (Isengard, 2001). Many alkaloids are still used in medicine (Wikipedia, 2015). Many synthetic and semi-synthetic alkaloids are structural modifications of alkaloids targeted to enhancing or changing the effect of the drug with reduced unwanted side-effects (Wikipedia, 2015). Saponins act as emulsifying agents and have been widely used in pharmaceutical preparations. In addition, their amphoteric nature confers on them surfactant property that can enhance penetration of macro-molecules through cell membranes (Horne, 1993). Eating saponins may help lower cholesterol levels reduce risk of heart disease and provide benefit to the immune function (Kunle, 2009; Kannall, 2015; Yacoub, 2015). The presence of tannins suggests that the plant could have antiviral and antibacterial activities and also could facilitate wound healing and burns (Haslem, 1989). *In vitro*, tannins showed antiviral (Lu *et al*,2004), antibacterial (Akiyama *et al*, 2001) and antiparasitic (Kolodziej and Kiderlen, 2005) properties. Tannins have astringent property and binds proteins and various organic compounds as well as function as metal ion chelators. Cardiac glycosides are cardio-active and useful in treatment of heart conditions (Oloyede, 2005). Digoxin, a cardiac glycoside, is used clinically to treat congestive heart failure and the associated symptoms of shortness of breath. The properties of phytochemicals could enhance nutritive agents in the leaves to seep into food wrapped with the leaves, thereby enhancing their tastes and nutritive values.

The Median Lethal Dose (LD_{50}) was 2214.58 mg/kg, a high value that suggests that the leaves could be safely used medicinally.

All test organisms showed sensitivity to the leaves extract in an increasing concentration-versus-effect-gradient manner. *E. coli* was most sensitive, *Staphylococcus aureus* effectively sensitive\

while *Pseudomonas aeruginosa* was least sensitive. The extract exhibited a broad spectrum of activity as both Gram positive, Gram negative bacteria, as well as fungi showed sensitivity.

Naturally retained moisture content of leaf was also within acceptable values, eliminating the possibility of chemical and microbial spoilages. The ethno-botanical use of the leaves is justified.

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