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Research Article

Phytochemical, Antimicrobial and Antioxidant Screening of Leaf and Tuber Extracts of Three Varieties of *Plectranthus Esculentus* Grown by Berom People of Nigeria

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ABSTRACT
Three varieties of *Plectranthus esculentus* (Livingstone potato) are grown for their edible tubers by Berom people of Nigeria. Phytochemical, antimicrobial and antioxidant screening; with quantification of total phenols and flavonoids were carried out on hexane, ethyl acetate, methanol and aqueous extracts of leaves and tubers of each variety. Results revealed substantial antimicrobial activity in hexane and ethyl acetate extracts of tubers; methanol and aqueous extracts of leaves. Antimicrobial activity was ascribed to phytochemicals present *viz* steroids, anthraquinones saponins, tannins, and flavonoids. Bebot variety has the highest antioxidant activity and phenolic content, followed by Riyom, while Long'at had the least.

Keywords: Livingstone potato, agar well diffusion, radical scavenging assay, reducing power assay, total phenols, total flavonoid.

INTRODUCTION

The war against microbial infections seemed to have been won with the introduction of penicillins about half a century ago, when they were referred to as miracle drugs. However, over the last decade, they started losing their effectiveness due to development of resistance by microbes, largely arising from misuse and mutation[1]. Resistance to antimicrobial agents is an important and pressing problem globally; many cases of antibacterial infection now involve strains that are resistant to at least one drug[2]. In time past, structural modification has been able to take care of the problem of resistance, but now an alternative solution is needed as parent structure alterations have failed to solve the problem[2]. This has necessitated the search for new antimicrobial agents. Natural products, especially plants, have been used by humans from time immemorial for the treatment of a wide spectrum of diseases, including those attributable to microbial infections[3]. Natural products, especially plants, have been noted to possess antimicrobial activity because of the unique secondary metabolites they contain[1,4].

On the other hand, plants have proven to be very rich sources of antioxidants, and this property has been linked mostly to their phenolic content. Phenolic compounds have been shown by many studies to have higher antioxidant capacities than other natural antioxidants, e.g., vitamins and carotenoids[5,6]. The acidic nature and presence of delocalised π electrons in phenolic compounds, i.e., their redox potential allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers and also confers on them their antioxidant property[7]. Flavonoids, an important class of phenols are very notable for their antioxidant activity; they are polyphenols structurally derived from benzo-γ-pyrone, and having a basic structure of diphenylpropane[8].

Plectranthus esculentus N.E. Br., a dicotyledonous perennial shrub that grows up to 2 m tall has very good prospects in phytomedicine, including treatment of microbial infections and use as antioxidant. It belongs to the family Lamiaceae, subfamily Nepetoideae[9,10]. It is pleasant smelling and has soft hairy leaves and hairy four-angled (square) stems. The Berom people of Plateau State observed that scorpions are usually attracted to places where the tubers are stored; this they attributed to its pleasant smell[11]. The long, cylindrical roots form clusters of short hairy edible tubers 5-10 cm long and up to 2cm in diameter[10]. It is widespread throughout Africa. In Nigeria, it is common in Plateau and Kaduna States, where it is grown mainly because of its tubers which are eaten fresh, cooked or roasted. In Plateau State, Nigeria, *Plectranthus esculentus* is grown by the Berom people and also the Taroh people of Langtang area. It is known locally as rizga or vat while the common English name is Livingstone potato. Three different varieties grown by the Berom people are named vat-long'at, vat-riyom and vat-bebot. The different varieties differ in the shape and size of the tubers and also in the height of the plant, with long'at having the tallest stem and longest tubers while riyom and

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Table 1: Antibacterial Activity of Tuber Extracts of the different varieties of *P. esculentus* (Diameter of zones of Inhibition in mm).

Organisms	*S. aureus*			*S. pneumonae*			*E. coli*			*P. aeruginosa*		
Concentration (mg/ml) Extracts	100	50	25	100	50	25	100	50	25	100	50	25
HBT	16.50	15.50	14.00	16.25	14.00	13.50	14.75	13.50	12.00	6.00	6.00	6.00
HRT	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
HLT	19.50	15.00	13.10	17.00	14.50	12.25	16.75	15.75	14.25	6.00	6.00	6.00
EBT	16.50	14.50	13.50	15.50	12.50	12.00	18.25	6.00	6.00	16.50	11.50	6.00
ERT	11.00	6.00	6.00	13.30	6.00	6.00	11.25	10.00	10.00	13.30	10.00	6.00
ELT	15.50	12.75	12.25	13.25	11.25	9.00	18.00	15.25	6.00	14.75	12.75	10.25
MBT	15.00	12.00	11.00	13.00	11.50	10.00	6.00	6.00	6.00	6.00	6.00	6.00
MRT	11.00	6.00	6.00	14.75	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
MLT	13.75	11.75	6.00	10.75	6.00	6.00	11.50	6.00	6.00	12.50	6.00	6.00
ABT	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
ART	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
ALT	6.00	6.00	6.00	12.50	10.50	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Gentamicin	40µg/ml		20µg/ml	40µg/ml		20µg/ml	40µg/ml		20µg/ml	40µg/ml		20µg/ml
	25.00		23.00	34.00		32.50	20.00		18.00	26.00		24.00
50% methanol	6.00			6.00			6.00			6.00		

Note: (1) Cup size diameter=6mm (2)HLT-Hexane extract of Long'at Tubers, ERT-Ethyl acetate extract of riyom Tubers, MBT- Methanol extract of Bebot Tubers, ALT-Aqueous extract of Long'at Tubers, etc.

bebot varieties have shorter and branched stems. The tubers of vat-riyom are similar to vat-long'at tubers and can sometimes be confused for each other, but on a closer look, they are shorter, with wider diameter. Vat-bebot tubers are very distinct, in that they are very short and wider in diameter than even the riyom tubers.

Although *Plectranthus esculentus* has not been evaluated for antimicrobial activity, co-generic species such as, *P. glaucocalyx, P. glandulosis* and *P. tenuiflorus* have been shown to have antimicrobial activity[12-14]. This suggests that *P. esculentus* may also possess antimicrobial activity. Furthermore, *Plectranthus esculentus* tubers and some other members of the genus has been shown to possess antioxidant properties[15,16]; however, there is no reported activity for the leaves.

This work therefore describes the antimicrobial, antioxidant and phytochemical screening of hexane, ethyl acetate, methanol and aqueous extracts of tubers and leaves of the three varieties of *P. esculentus* described above and also attempt to correlate the antioxidant activity to their phenolic content.

MATERIALS AND METHODS

Chemicals and Reagents

Organic solvents used for extraction (hexane, ethyl acetate and methanol) were obtained from Loba-chemie Pvt Ltd., Mumbai, India. Nutrient and Sabouraud dextrose agar were from Titan Biotech Ltd., Rajasthan, India. Folin Ciocalteu reagent, Trichloroacetic acid (TCA), Ferric chloride were also from Loba-chemie. 1,1-diphenyl-2-picryl hydrazyl (DPPH) radicals, standard gallic acid, rutin and methanol used for radical scavenging assay were from Sigma chemical company, St. Louis, USA.

Plant Collection and Processing

Fresh tubers and leaves of vat-long'at, vat-riyom, and vat-bebot varieties of *P. esculentus* were obtained from farms in Heipang, Plateau State, Nigeria in August. They were authenticated by Mrs. C. D. Gadu, of the National Root Crop Research Institute, Vom, Plateau State, Nigeria. Voucher specimens of the three varieties have been deposited in the herbarium at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Jos. Voucher numbers: UJ/PCG/HSP/17L01a (vat-long'at); UJ/PCG/HSP/17L01b (vat-riyom); UJ/PCG/HSP/17L01c (vat-bebot). The leaves and tubers were properly washed, sliced and then air-dried. The dried tubers and leaves were further chopped, air-dried and powdered.

Extract Preparation

The powdered tubers and leaves were individually macerated successively with solvents in an increasing order of polarity using, hexane, ethyl acetate, methanol and water. Aqueous extracts were lyophilized while other extracts were concentrated *in vacuo*. The extracts were stored in a desiccator until they were used.

Microorganisms

Table 2: Antibacterial Activity of Leave Extracts of the different varieties of *P.esculentus* (Diameters of Zones of Inhibition in mm).

Organisms	*S.aureus*			*S.pneuomonea*			*E.coli*			*P.aeruginosa*		
concentration (mg/ml) Extracts	100	50	25	100	50	25	100	50	25	100	50	25
HBL	12.25	6.00	6.00	13.00	6.00	6.00	11.75	6.00	6.00	11.00	6.00	6.00
HRL	11.75	11.25	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
HLL	9.25	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
EBL	12.25	10.25	6.00	6.00	6.00	6.00	11.25	6.00	6.00	10.25	6.00	6.00
ERL	11.00	9.00	6.00	12.75	10.00	8.75	11.75	9.00	6.00	6.00	6.00	6.00
ELL	11.25	13.00	11.50	11.00	6.00	6.00	6.00	6.00	6.00	11.75	9.50	6.00
MBL	20.00	17.75	14.85	25.00	19.60	14.50	10.00	6.00	6.00	11.75	6.00	6.00
MRL	19.50	16.25	14.50	17.25	14.75	13.50	17.25	15.25	13.50	20.75	18.00	13.50
MLL	18.50	10.25	6.00	15.25	10.25	6.00	10.75	10.00	9.50	13.75	9.75	6.00
ABL	21.75	15.75	11.50	13.75	6.00	6.00	17.00	13.00	11.50	11.25	10.50	6.00
ARL	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
ALL	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
40µg/ml gentamicin	40µg/ml 25.00		20µg/ml 23.00	40µg/ml 34.00		20µg/ml 32.50	40µg/ml 20.00		20µg/ml 18.00	40µg/ml 26.00		20µg/ml 24.00
50%methanol	6.00			6.00			6.00			6.00		

Note: (1) Cup size diameter=6mm (2) HLL-Hexane extract of Long'at Leaves, ERL-Ethyl acetate extract of riyom Leaves, MBL- Methanol extract of Bebot Leaves, ALL-Aqueous extract of Long'at Leaves, etc.

Table 3: Antifungal Activity of Tuber Extracts of the different varieties of *P.esculentus.* (Diameters of Zones of Inhibition in mm).

Organisms	*A.niger*			*P.notatum*			*C.albicans*		
Concentration (mg/ml) Extracts	100	50	25	100	50	25	100	50	25
HBT	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
HRT	17.00	13.50	11.50	20.00	20.00	21.00	15.00	17.00	19.00
HLT	6.00	6.00	6.00	25.00	24.00	20.00	6.00	6.00	6.00
EBT	6.00	6.00	6.00	15.00	6.00	6.00	23.00	16.00	14.00
ERT	6.00	6.00	6.00	6.00	6.00	6.00	21.00	18.00	14.00
ELT	6.00	6.00	6.00	20.00	18.00	16.00	11.5	10.00	6.00
MBT	6.00	6.00	6.00	12.00	10.00	6.00	6.00	6.00	6.00
MRT	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
MLT	11.00	6.00	6.00	14.50	12.00	6.00	11.50	10.50	6.00
ABT	17.50	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
ART	25.00	20.00	18.50	13.00	12.00	6.00	6.00	6.00	6.00
ALT	20.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Fluconazole(µg/ml)	40	20		40	20		40	20	
	30.00	26.00		28.75	24.75		19.50	14.25	
50%methanol	6.00			6.00			6.00		

Note: (1) Cup size diameter=6mm

Two Gram-positive bacteria: *Staphylococcus aureus* and *Streptococcus pneumonae*; two Gram-negative bacteria; *Escherichia coli* and *Pseudomonas aeruginosa* and three fungi; *Aspergillus niger*, *Penicillium notatum* and the yeast *Candida albicans* were used in this experiment. These were obtained from stock cultures of the Microbiology Unit, Department of Pharmaceutics and Pharmaceutical Technology, University of Jos, Nigeria. The organisms were maintained on nutrient agar and Sabouraud dextrose agar slants respectively and stored at temperature of 4˚C.

Inoculum Size Determination

Fresh cultures of each organism were prepared in nutrient broth for bacteria and peptone water for fungi to be used for the experiment. One ml of each culture was taken and made up to 10ml with sterile distilled water; these were further diluted serially to obtain five other concentrations. 0.1ml of each concentration was added to 20ml of sterile nutrient agar (for bacteria) and Sabouraud dextrose agar (for fungi) and mixed gently, after which it was poured into sterile Petri dishes and left to solidify. The plates were incubated at 37˚C for 24hours (bacteria) and 25˚C for 72 hours (fungi). At the end of the incubation period, the

Table 4: Antifungal Activity of Leaf Extracts of the different varieties of *P.esculentus.*

	A.niger			*P.notatum*			*C.albicans*		
Concentration(mg/ml	100	50	25	100	50	25	100	50	25
Extracts									
HBL	6.00	6.00	6.00	11.00	6.00	6.00	6.00	6.00	6.00
HRL	6.00	6.00	6.00	6.00	6.00	6.00	22.00	20.00	16.00
HLL	6.00	6.00	6.00	13.00	6.00	6.00	12.00	6.00	6.00
EBL	6.00	6.00	6.00	18.00	15.00	14.00	6.00	6.00	6.00
ERL	6.00	6.00	6.00	6.00	6.00	6.00	15.00	6.00	6.00
ELL	6.00	6.00	6.00	25.00	18.00	20.00	16.50	11.00	11.00
MBL	6.00	6.00	6.00	18.00	16.00	13.00	12.50	6.00	6.00
MRL	6.00	6.00	6.00	18.00	12.00	11.00	20.00	16.00	13.00
MLL	6.00	6.00	6.00	15.00	14.00	12.00	14.50	13.00	11.00
ABL	17.50	6.00	6.00	11.50	9.00	6.00	6.00	6.00	6.00
ARL	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
ALL	6.00	6.00	6.00	19.50	19.50	18.50	6.00	6.00	6.00
fluconazole	40µg/ml		20µg/ml	40µg/ml		20µg/ml	40µg/ml		20µg/ml
	30.00		26.00	28.75		24.75	19.50		14.25
50%methanol	6.00			6.00			6.00		

Note: (1) Cup size diameter=6mm

Table 5: Phytochemistry of Extracts of the different varieties of *P.esculentus* Tubers

Metabolite Extracts	Alkaloid	Saponin	Tannin	Flavonoid	Steroid/terpenes	Anthraquinone	Cardiac glycosides
HBT	-	-	-	-	+	+	+
HRT	-	-	-	-	+	-	-
HLT	-	-	-	-	+	+	+
EBT	-	-	-	-	+	-	+
ERT	-	-	Trace	-	+	+	Trace
ELT	-	-	-	-	+	+	-
MBT	+	-	+	+	-	-	-
MRT	+	-	+	+	-	-	-
MLT	+	-	+	+	-	-	+
ABT	+	+	+	+	-	-	-
ART	+	-	-	-	-	-	-
ALT	+	-	-	+	-	-	-

Note: + stands for present, – stands for absent and trace stands for minimal colour change.

numbers of colonies were counted[17].

Antimicrobial Assay

Agar well diffusion method[18] was employed with nutrient agar for bacteria and Sabouraud dextrose agar for fungi. Concentrations of 100, 50 and 25 mg/ml of the different extracts were prepared using 50% methanol in sterile distilled water. 0.1ml of each extract was filled into the wells. Plates containing bacteria were incubated at 37˚C for 24 hours while plates containing fungi were incubated at 25˚C for 72 hours after which diameter of the zones of inhibition were measured in millimetres (mm). Forty (40) and twenty (20) micrograms per millilitre (µg/ml) gentamicin and fluconazole were used as reference antibiotics respectively, while 50% methanol in distilled water was used to demonstrate lack of activity of the diluents (solvents).

Phytochemical Screening

The phytochemical screening of all extracts from the three varieties of *P. esculentus* studied was carried out according to standard procedures[19,20].

DPPH Radical Scavenging Assay

The standard test[21] was carried out with slight modifications as follows. Different concentrations of extracts were prepared in methanol: 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.905, 1.95 and 0.98 µg/ml. 0.02 mg/ml of DPPH was also prepared in methanol, 4 ml of this was added to 2 ml of each concentration of extract, after which the mixture was vortexed for 10 seconds and incubated for 30 minutes in the dark. Absorbance was read at 515 nm. Standard ascorbic acid and Gallic acid were analysed following the same method and using concentrations ranging from 100 to 0.195 µM. Percent inhibitory activity of each extract was determined from the following expression:

Table 6: Phytochemistry of Leave Extracts of the different varieties of *P.esculentus.*

phytochemical Extracts	Alkaloid	Saponin	Tannin	Flavonoid	Steroid/terpenes	Anthraquinone	Cardiac glycosides
HBL	-	-	-	-	+	-	-
HRL	-	-	-	-	+	-	-
HLL	-	-	-	-	+	-	-
EBL	-	+	+	+	-	+	trace
ERL	-	-	-	-	+	-	+
ELL	-	-	-	-	-	-	+
MBL	+	+	+	+	trace	-	+
MRL	-	+	+	+	+	+	+
MLL	-	-	-	trace	+	-	+
ABL	+	-	+	+	-	-	-
ARL	-	-	+	+	-	-	-
ALL	-	-	+	+	-	-	-

Table 7: IC_{50} and EC_{50} values for Radical Scavenging and Reducing Power Assay

Extract	DPPH IC_{50}(µg/ml)	RPA EC_{50} (mg/ml)	Extract	DPPH IC_{50}(µg/ml)	RPA EC_{50} (mg/ml)
HLL	>502	30	HLT	446.68	9.50
HRL	>502	32.75	HRT	501.19	10.30
HBL	>502	>35.00	HBT	>502	5.75
ELL	89.12	2.40	ELT	100.00	2.80
ERL	50.12	2.30	ERT	50.12	1.75
EBL	19.95	2.30	EBT	39.81	2.70
MLL	112.20	2.40	MLT	89.12	3.80
MRL	10.00	0.50	MRT	100.00	1.80
MBL	3.98	0.60	MBT	501.00	8.30
ALL	>502	2.30	ALT	>502	3.00
ARL	63.09	1.40	ART	>502	6.00
ABL	10.59	0.55	ABT	281.84	5.00
GA	0.56	0.06	GA	0.56	0.06
AA	1.78	0.13	AA	1.78	0.13

Note: HLL-Hexane extract of Long'at leaves, ERL-Ethyl acetate extract of riyom leaves, MBL- Methanol extract of Bebot leaves, ALL-Aqueous extract of Long'at leaves, HLT- Hexane extract of long'at tubers, etc. GA-Gallic Acid, AA-Ascorbic Acid

[(Absorbance of blank - Absorbance of sample) ÷ Absorbance blank] x 100

where blank represents the DPPH solution without the extract.

IC_{50} value for each extract was determined from the curve of % inhibitory activity versus concentration of sample. A low IC_{50} value is indicative of high antioxidant activity.

Reducing Power assay

The reducing power assay (RPA) was carried out according to the method of [22], with some modifications. Various concentrations of the samples and standards (ascorbic and gallic acid) in distilled water (250 µl) were mixed with 250 µl of phosphate buffer (0.6 M, pH 6.6), followed by 250 µl of 1% potassium ferricyanide. The mixtures were incubated at 50˚C for 20 minutes. 250 µl of 10% TCA was then added, followed by 1 ml of distilled water and finally, 200 µl of 0.1% ferric chloride. Each experiment was carried out in triplicate. Absorbance was measured at 700 nm. EC_{50} value was obtained from a plot of absorbance against concentrations of samples and standard. EC_{50} is the concentration of sample and standard having absorbance of 0.5.

Total Phenolic Content Determination

The total phenolic content (TPC) was determined spectrophotometrically, using Folin-Ciocalteu reagent (FCR), according to previous studies[23,24], with modifications. A standard calibration curve was first of all obtained as follows; concentrations from 100-10 µg/ml of Gallic acid were prepared using distilled water. 0.4 ml of each of these concentrations was added to 2 ml of FCR that has been pre-diluted 10-fold with distilled water. The mixture was allowed to stand for 5 minutes at room temperature after which 1.6 ml of 7.5% sodium carbonate (Na_2CO_3) solution was added. The mixture was vortexed for 10 seconds and incubated in the dark for 2 hours. Absorbance was then read at 765 nm. Standard calibration curve was obtained by plotting absorbance values against the corresponding concentrations.

Table 8: Total Phenolic and Flavonoid content.

Extract	TPC(mg/g)GAE	TFC(mg/g)RE	Extract	TPC(mg/g)GAE	TFC(mg/g)RE
HLL	-5.96±1.57	13.94±0.26	HLT	-1.99±0.26	26.54±0.08
HRL	-5.19±0.97	26.20±0.29	HRT	-6.86±1.05	24.13±0.8
HBL	-4.62±2.04	47.60±0.19	HBT	-6.47±0.46	102.80±0.58
ELL	8.65±0.22	102.00±0.39	ELT	16.70±0.44	95.54±1.12
ERL	10.10±0.26	89.71±0.38	ERT	3.40±0.13	131.00±1.6
EBL	26.50±0.68	166.30±1.35	EBT	0.96±0.8	66.54±1.25
MLL	7.37±1.05	88.56±0	MLT	1.47±0.26	45.96±0.1
MRL	31.50±0.46	188.20±0.77	MRT	3.01±0.34	83.37±1.15
MBL	54.00±0.22	264.20±1.17	MBT	-6.86±0.51	20.77±1.63
ALL	4.94±0.51	28.69±0.46	ALT	-4.94±0.43	16.19±0.06
ARL	17.00±0.78	65.87±0.58	ART	0.83±0.46	17.31±0.1
ABL	81.90±0.68	216.70±0.67	ABT	-3.40±0.26	15.87±0

Table 9: Correlation coefficients.

Assay	Correlationnnon
DPPH vs. TPC (Leaves)	-0.742
DPPH vs. TPC (Tubers)	-0.063
DPPH vs. TFC (Leaves)	-0.825
DPPH vs. TFC (Tubers)	-0.772
RPA vs. TPC (Leaves)	-0.582
RPA vs. TPC (Tubers)	-0.572
RPA vs. TFC (Leaves)	-0.603
RPA vs. TFC (Tubers)	-0.581
DPPH vs. RPA (Leaves)	0.736
DPPH vs. RPA (Tubers)	0.962

To determine the total phenolic content of the extracts; 100 µg/ml of each extract was first prepared in distilled water. The experiment was then carried out as described above. The total phenolic content of each extract in mg/g gallic acid equivalent (GAE) was obtained using the formula; TPC = c.v/m, where, TPC = Total phenolic content in gallic acid equivalent, c = concentration of gallic acid from the calibration curve corresponding to the absorbance value of the extract, v = volume of extract used, and m = weight of extract used in mg.

Total Flavonoid Content Determination

A standard calibration curve was first obtained as follows; concentrations from 100-1000µl of rutin were prepared. To 1ml of each concentration (in triplicate), 4ml of distilled water was added, followed by 300µl of 5% sodium nitrite solution, after 5 minutes, 300µl of 10% aluminium chloride was added. The mixture was allowed to stand for 6 minutes, after which 2ml of 4% sodium hydroxide was added, this was immediately followed by 2.4 ml of distilled water to make up the final volume to 10 ml. The mixture was vortexed for 10 seconds after which absorbance was read at 510 nm. Standard calibration curve was obtained by plotting absorbance values against the corresponding concentrations.

To determine the total flavonoid content of the samples, 4 mg/ml of each sample was treated as above. Total flavonoid content in mg/g rutin equivalent (RE) was calculated using the formula described above for TPC[25].

Qualitative Analysis by Thin Layer Chromatography (TLC)

Analytical normal phase silica gel coated plates were used. Different solvent systems were tried in order to obtain the solvent system that worked best on all members of each category of extracts. Hexane extracts were developed in toluene: chloroform: methanol, in the ratio 2.9:7:0.1 respectively. Ethyl acetate extracts were developed in methanol:dichloromethane, ratio: 1:9; methanol extracts were developed in ethyl acetate:acetic acid:methanol, in the ratio:8.5:1:0.5, while the aqueous extracts were developed in acetone:butanol:acetic acid:water, in the ratio: 2:6:1.5:0.5. Standard ascorbic acid was developed in ethyl acetate:acetic acid:toluene, in the ratio:5.5:1:1.5, standard gallic acid on the other hand was developed in ethyl acetate:toluene:formic acid, in the ratio: 3.5:5:0.5. After development, the plates were air-dried and viewed under the ultra-violet light at 365 nm.

The plates were then either dipped in 0.01% DPPH for qualitative antioxidant assay or 0.5% ferric chloride for qualitative assay of phenolic content.

Statistical Analysis

Each experiment was performed in triplicate and means were calculated. Results for TPC and TFC were expressed as mean±SEM. Correlation coefficient was obtained on Microsoft excel.

RESULTS

The inoculum size of all bacteria used fell within the range of 10^{6-7} colony forming units per millilitre (cfu/ml) while that of fungi was the within the range of 10^{5-6} spore forming units per milimetre (sfu/ml).

Results of antibacterial screening of tuber and leaf extracts of the three varieties are presented in Tables 1 and 2, while antifungal activity test results are shown in Tables 3 and 4. The results of phytochemical screening of tuber and leaf extracts of the three varieties are shown in Tables 5 and 6. Also, results of radical scavenging and reducing power assay are presented on Table 7, those of total phenol and flavonoid are presented on Table 8. Correlation coefficients describing the correlation between antioxidant assays and phenolic content are presented on Table 9. Figures 1 and 2 show the relationship between IC_{50} (DPPH), EC_{50} (RPA), TPC and TFC values for the leaves and tuber extracts.

DISCUSSION

Plectranthus esculentus tuber is an important delicacy among the Berom people of Plateau state, Nigeria. The

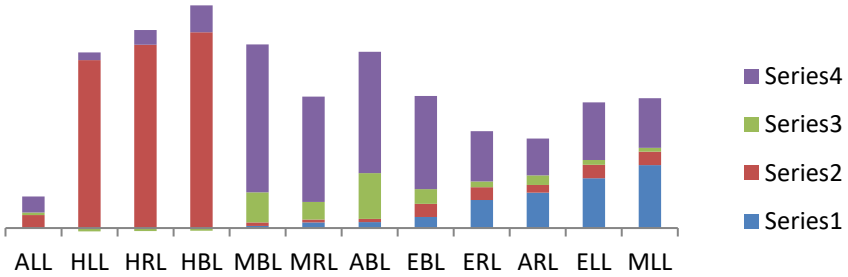

Figure 1: Chart showing Relationship between IC_{50} (DPPH), EC_{50} (RPA), TPC and TFC values of Leaf extracts
Series 1: IC_{50} values (DPPH)
Series 2:EC_{50} values (RPA)
Series 3: TPC values
Series 4: TFC values

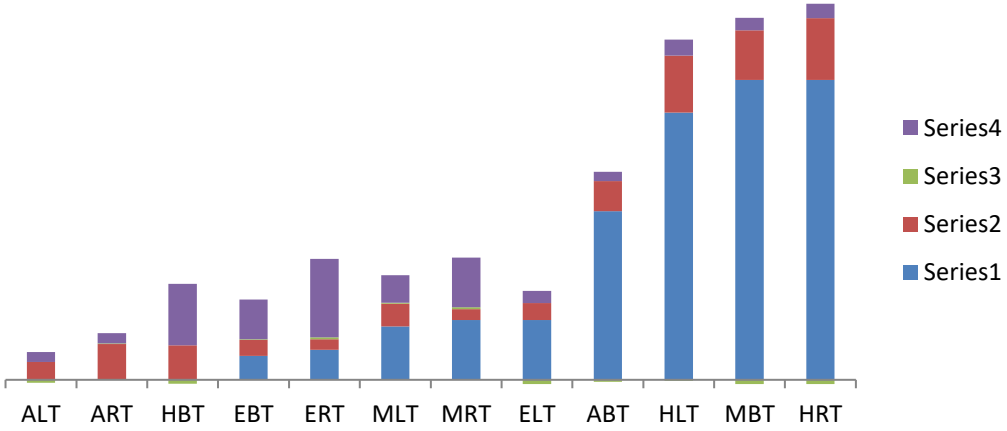

Figure 2: Chart showing Relationship between IC_{50} (DPPH), EC_{50} (RPA), TPC and TFC values of Tuber Extracts
Series 1: IC_{50} values (DPPH)
Series 2: EC_{50} values (RPA)
Series 3: TPC values
Series 4: TFC values

Tarok people in Plateau state are also not left out in the consumption of this delicacy, although among them, the different varieties are known by different names, such as Aku and Ananjoh. As important as this plant is to the people of Plateau state, it was gathered that the leaves are of no value at all to the locals as they are usually allowed to waste away on the farm after the tubers are harvested. The collection of the leaves for analysis was actually questioned as it is of no known economical, health or food value. However, considering the fact that leaves of other members of the *Plectranthus* family have proven to be useful in disease management, leaves of *P. esculentus* were investigated in this work alongside it's tubers and this has led to interesting discoveries, as some of the leaf extracts showed very good antimicrobial and antioxidant activities with corresponding high phenolic content much greater than in the tubers.

Information gathered in the course of sample collection also revealed that the bebot variety is gradually becoming extinct, such that it took a lot of effort before it could be obtained. The farmers complained that the variety has a low yield compared to other varieties, and as a result, they concentrate on the cultivation of the other varieties. As a matter of fact, the long'at variety is the most popular of all the varieties, as that is what is seen on the trays and farm of most of the local sellers and farmers. However, it is interesting to note that from the result of this work, bebot and riyom varieties showed meaningful antimicrobial activity, however, long'at variety also proved to be less useful as an antimicrobial. Furthermore, leaves of the bebot variety is the richest in antioxidant, phenolic and flavonoid content, closely followed by those of riyom variety, while leaf extracts of the long'at variety has the lowest antioxidant and phenolic content. Among the tuber extracts, the bebot variety is also the richest in antioxidant activity, however, highest phenolic content is found in the long'at tubers, i.e., in its ethyl acetate extract. Flavonoid content on the other hand is generally higher in the riyom followed by the long'at tubers.

From the results of antibacterial screening of tubers (Table 1), certain trends are discernible. For instance, bebot and long'at varieties have similar level of activity and are clearly more active than riyom variety. Hexane and ethyl acetate extracts have comparable activities but were slightly more active than methanol extract; aqueous extracts were mostly inactive against the test organisms. Ethyl acetate extracts of the three varieties showed some activity against *Pseudomonas aeruginosa*, a notably difficult Gram-negative bacterium. The Gram-positive bacteria. Gram-positive bacteria (*Staph. aureus* and *Strep. pneumonae*) were more susceptible than Gram-negative (*E. coli* and *Ps. aeruginosa*); *E. coli* was more susceptible than *Ps. aeruginosa*. Results of the antibacterial screening of leaf extracts (Table 2) revealed that leaves were generally less active than tubers. Among aqueous extracts of the three varieties, only bebot was active. Methanol extract of the three varieties were most active, followed by ethyl acetate and hexane extract, aqueous being the least active. Also, *Staph. aureus* was the most susceptible test organism.

In terms of antifungal activity of tubers (Table 3), ethyl acetate extracts of the three varieties showed activity against *Candida albicans*. In addition, the ethyl acetate extract of long'at showed activity against *Penicillium notatum*. Hexane extract of riyom was active against all three fungal organisms. Aqueous extracts of all three varieties were active against *Aspergillus niger*. In addition, aqueous extract of riyom was active against *Penicillium notatum*. Methanol extract of bebot and long'at were active against *P. notatum* and that of long'at was also active against *Candida albicans.* The results of antifungal screening of leaf extracts (Table 4) showed that no leaf extract had activity against *Aspergillus niger*. Methanol extract of the three varieties showed activity against *P. notatum* and *C. albicans*. Hexane and ethyl acetate extract of long'at and riyom also showed activity against *C. albicans*. Aqueous, ethyl acetate and hexane extracts of bebot and long'at varieties had activity against *P. notatum*.

Phytochemical screening of tuber extracts (Table 5) revealed a positive test for alkaloids in the methanol and aqueous extracts of all three varieties. In addition, the methanol extract of the three varieties tested positive for flavonoids and tannins. Hexane and ethyl acetate extracts also tested positive for anthraquinones, steroids/ terpenes and cardiac glycosides. In the phytochemical screening of leaf extracts (Table 6) methanol and aqueous extracts also tested positive for flavonoids and tannins. In addition, the methanol extract tested positive for saponins. Methanol and aqueous extract of bebot tested positive for alkaloids. Hexane extracts of all varieties contained steroids and terpenes. Also, ethyl acetate and methanol extracts of the three varieties tested positive for cardiac glycosides.

IC_{50} and EC_{50} values obtained during the antioxidant assay, TPC and TFC of each extract are presented on tables 1 and 2. From table 1, it can be seen that some of the extracts have IC_{50} values greater than 501µg/ml, this is because, their highest percent inhibition is less than 50%, hence, the exact IC_{50} value (concentration of sample leading to 50% reduction in the initial concentration of DPPH) could not be measured.

The qualitative assay using TLC, as presented on figure1 suggests that extract HBT and MBT contains some compounds with antioxidant activity; also, the IC_{50} values of these extracts obtained from the quantitative test is very high. This suggests that the antioxidant compounds in this extracts are very low in concentration. The qualitative assay also suggests that extracts HLT, HRT, HBT and MBT contain low concentrations of phenols.

Although phenolics are known with other biological activities, they are most popular for their antioxidant capacity. The role of these natural compounds in counteracting the negative effects of free radicals, and hence, in the prevention of human diseases have been documented by many authors[26]. The ability of phenolic compounds to scavenge free radicals is as a result of their acidity, i.e. ability to donate protons and also, their delocalized π-electrons (ability to transfer electrons while remaining relatively stable).

The result of the DPPH scavenging properties and total phenolic content of the leaves is in line with reports[27] that there is a correlation between antioxidant activity and phenolic content. Thus extracts with low IC_{50} values, signifying high antioxidant ability also gave very high phenolic content. The study therefore further proves that the phenolic content of plants is largely responsible for their antioxidant properties; for instance, MBL with IC_{50} value of 3.98 µg/ml also has TPC value of 54±0.22 mg/g GAE; ABL with IC_{50} value of 10.59 µg/ml also has TPC value of 81.9±0.68 mg/g GAE; and MRL with IC_{50} value of 10.00 µg/ml also has TPC value of 31.5±0.46 mg/g GAE. On the other hand, extracts having very high IC_{50} values, such as MLL (112.20 µg/ml) have very low TPC value 7.37±1.05 mg/g GAE.

Leaf Extracts HLL, HRL, HBL and ALL whose exact IC_{50} values could not be determined are very peculiar, however, their TPC values provided a clear explanation for their behaviour; HLL, HRL and HBL have negative TPC values, indicating absence of phenolics, while ALL have a very low TPC value (4.94±0.51 mg/g GAE). The correlation coefficient of -0.742 obtained is also an indication of a good correlation between the antioxidant properties of the leaf extracts and their corresponding TPC. The correlation coefficient show that as IC_{50} values increases, TPC values decreases, and *vice versa*. Gallic acid, a simple phenol, gave IC_{50} value of 0.56 µg/ml which is much lower than that of ascorbic acid (1.78 µg/ml). This goes further to show the relevance of phenolic compounds in the management of oxidative stress. A similar correlation was observed between DPPH scavenging activity and TFC of the leaf and tuber extracts, with correlation coefficients of -0.825 and -0.772 respectively

DPPH scavenging antioxidant activity of the tuber extracts, on the other hand does not correlate well with its TPC (correlation coefficient =-0.063). The antioxidant activity reported in some of this extracts may not be totally as a result of phenolic compounds. Other classes of phytochemicals other than phenols have also been reported by some researchers to be good antioxidants[28,29].

Furthermore, RPA for both leaves and tuber extracts correlates partially with the corresponding TPC and TFC values. A closer look at Tables 7 and 8 reveal that only the extracts with very high TPC and TFC values have consistent high RPA. The various correlation coefficients are presented on table 9, while relationships between the various parameters are presented on figure 1 and 2.

In DPPH radical scavenging activity, antioxidants present in the samples react with DPPH by donating hydrogen to it, thereby scavenging it. The DPPH (deep violet colour) is converted to a yellow coloured α,α-diphenyl-β-picryl hydrazine[30,31]. The radical scavenging potential is indicated by the degree of colour change from violet to yellow[30,31]. In reducing power assay, antioxidants in the sample bring about a reduction of Fe3+ to Fe2+. This reduction is usually monitored by measuring the formation of Perl's Prussian blue at 700 nm[30,31].

Lastly, it is evident from the results above that antioxidant activity and phenolic compounds are high in concentration in the polar extracts; for instance, The IC_{50} value of MBL is just slightly higher than that of the standard Ascorbic acid with IC_{50} value of 1.78µg/ml. This supports the claim that hydrophilic extracts have much higher antioxidant capacity than hydrophobic ones[32]. The report[33] that phenolic content increases with polarity of the solvent of extraction is also supported by this study.

In conclusion, the tubers and leaves of the three varieties of *P. esculentus* showed moderate antibacterial and antifungal activities. Phytochemical screening indicates that all three varieties possess compounds with good potential as antimicrobial agents. A very good antioxidant activity was also observed in the leaves of bebot and riyom variety, while the leaves of long'at variety and tubers of the three varieties showed moderate to poor antioxidant activity. This activity correlated well with the phenolic content in the leaves, however, a poor correlation was observed with the tubers.

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