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PROXIMATE AND PHYTOCHEMICAL COMPOSITIONS, AND TOXICITY STUDIES ON *Zapoteca portoriscensis* ROOT METHANOL EXTRACT AND ITS FRACTIONS

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Abstract

In this study, the proximate and phytochemical compositions, toxicity profile and characterization of the crude methanol extract (CME), methanol (MF) and ethyl acetate (EAF) fractions of *Zapoteca portoricensis* roots were carried out using standard methods. Results of proximate analysis showed a high percentage of carbohydrate, relatively low percentage of protein, moisture and ash, and very low crude fiber and fats contents. The presence of tannins, flavonoids, alkaloids, saponins, HCN, terpenoids, steroids, phenols, glycosides, reducing sugars and soluble carbohydrates were detected in the CME, MF and EAF while reducing sugars and soluble carbohydrates were not detected in EAF. No mortality and behavioral changes were observed in the test animals up to 5,000 mg/kg body weight in the toxicity studies. The FTIR studies revealed the presence of OH, NH, C-OH, C-C, C-N, C-H and C=C in the CME, MF and EAF. The presence of these functional groups indicates the presence of biologically active compounds. The results from this study may explain the potential medicinal and therapeutic activities of the plant roots and a possible indication of the safety of the plant root to its users.

Keywords: *Zapoteca portoricensis, proximate, phytochemical, toxicity, FTIR*

Introduction

In recent years, there has been a gradual revival of interest in the use of medicinal plants especially in developing countries because herbal medicines have been reported safe and without any adverse side effects especially when compared with synthetic drugs. Thus, new drugs with better and cheaper substitutes of plant origin are a natural choice. The medicinal values of these plants lie in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al*., 2005). *Zapoteca portoricensis* (jacq) H.M Hernandez, commonly called white stick and popularly known as "*Elugelu*" in eastern Nigeria is a perennial seasonal plant with unarmed branches (Jyothi *et al*., 2012). It belongs to the family Fabaceae. It is a native of West Africa (eastern and southern Nigeria), West Indies and Atlantic coast of America. The peoples of eastern and southern Nigeria have found the extract from different parts of the plant useful in traditional medicinal practices, in the management of diarrhea, convulsion and tonsillitis (Agbo *et al*., 2010; Agbafor *et al.,* 2014). Its roots have been reported to possess anti-inflammatory, antifungal, anti-trypanosomal and antibacterial activities (Nwodo and Uzochukwu, 2008; Nwodo *et al*., 2009; Agbo *et al*., 2010; Agbafor *et al*., 2014). Others include antimalarial (Nwodo et al., 2015; Joshua et al., 2016) and beneficial effects in management of benign prostate hyperplasia (Joshua et al., 2018). Flavonoids, saponins, terpenoids and steroids obtained from the column fractions of the root extracts have proved to be responsible for the production of significant anti-inflammatory activity (Agbo *et al.*, 2010).

The methanol extract of the roots have been reported to possess potent anti-ulcer activity (Ukwe *et al*, 2010). The different extracts prepared from the leaves of the plant have proved to be useful as antibacterial and antifungal agent due to its antimicrobial properties (Agbafor *et al.*, 2011). In this study, a preliminary study on the roots of *Z. portoriscensis* with respect to the proximate composition of the plant root sample, phytochemical composition, toxicity studies and characterization of the crude methanol extract, methanol and ethyl acetate fractions usinf FTIR was carried out.

Methods

Plant materials

The roots of *Zapoteca portoricensis* were collected from a habitat in Nsukka, Enugu State, Nigeria. The roots were identified and authenticated by Mr. Alfred Ozioko of the Bioresource Development and Conservation Program (BDCP) Research Centre Nsukka, Enugu state, Nigeria. The root samples were air-dried for three weeks to constant weight at room temperature (29°C- 35°C) and ground into uniform coarse form using a milling machine. The methanol extract was prepared by soaking 2000 grams of dried pulverized roots samples in 1.5 liters of methanol for 72 hours. It was filtered using Whatman Number 4 filter paper and the filtrate was concentrated using Rotary evaporator at regulated temperature. The methanol extract obtained was fractionated by column chromatography using 1.3 liters of methanol and 1.3 liters of ethyl acetate as solvents. Toxicological studies, phytochemical analyses and characterization were carried out on the crude methanol extract, methanol and ethyl acetate fractions while proximate analysis were carried out on the dried pulverized root samples.

Proximate analysis

Proximate analyses were carried out according to the procedure of Association of Official Analytical Chemist (AOAC, 1980).

Phytochemical analyses

Qualitative phytochemical analyses were carried out according to the methods of Trease and Evans (2002) and Sofowora (1993) while the Quantitative phytochemical analyses were carried out according to the methods of Nwaokonkwo (2009) and El-Olemyl *et al*., (1994).

Toxicity studies

The acute toxicity studies of the crude methanol extract, methanol and ethyl acetate fractions were estimated using the method of Lorke (1983). The chronic toxicity study was carried out according to the OECD Guideline (2009). Adult Swiss albino mice (20-30 g) of both sexes obtained from the animal holding unit of the Department of Zoology and Environment Biology, University of Nigeria, Nsukka were used for the toxicity study. The guide for the care and use of laboratory animals procedures were

followed in this study (Indian Council of Medical Research, 2001).

Characterization of extracts

The Foutrier Transform Infra-red (FT-IR) was used to characterize and identify the functional groups present in the plant extract and fractions.

Statistical analysis

Results were expressed as mean ± SD and test of statistical significance were carried out using One-way analysis of variance (ANOVA. The test of significance was determined at $p < 0.05$. The statistical product and service solutions (SPSS) was used.

Results and discussion

Proximate constituents of the Zapoteca portoricensis root sample

Proximate analysis of the plant root sample showed a high percentage of carbohydrate (84.05 ± 0.03%), relatively low percentage of protein (6.39 ± 0.21%), moisture (6.25 ± 0.14%) and ash (3.04 ± 0.11%) and very low percentage of crude fibre (0.18 ± 0.04%) and fats (0.05± 0.01%) (Table 1). This is an indication that *Z. portoricensis* roots may be ranked as carbohydrate rich and may serve as a good source of energy but it may not be considered a good source of protein and vegetable fats and oil. The low ash and moisture content suggests an indication of its low mineral content and stability against microbial growth. It therefore has good storage potentials (Iniaghe *et al*., 2009).

Phytochemical constituents

Qualitative phytochemical analyses of *Zapoteca portoricensis* roots revealed the presence of tannins, flavonoids, alkaloids, saponins, HCN, terpenoids, steroids, phenols, glycosides, reducing sugars and soluble carbohydrates (Table 2). The crude methanol extract had high amount of tannins and flavonoids, moderate amount of phenols, alkaioids, HCN, saponins, terpenoids, steroids and trace amount of glycosides. Methanol fraction had high amount of terpenoids and reducing sugars, moderate amount of tannins, flavonoids, alkaloids, saponins, HCN and phenols with steroids, glycosides and soluble carbohydrates in trace amount. In the ethyl acetate fraction, reducing sugars and soluble carbohydrates were not detected. However, it has moderate amount of tannins, flavonoids and phenols, and trace amount of alkaloids, saponins, HCN, terpenoids, steroids and glycosides. The quantitative phytochemical analyses revealed, the alkaloids, saponins, terpenoids, steroids, phenols, glycosides, reducing sugars and soluble carbohydrates levels in the methanol fraction were significantly ($p < 0.05$) higher when compared with crude methanol extract and ethyl acetate fraction (Table 3). The presence of these phytochemicals indicates that a well processed *Z. portoricensis* root may offer medicinal and chemoprotective benefits to its users (Agbo *et al*., 2010; Nwodo *et al*., 2014).

Toxicity profile of the extract and its fractions

The acute and chronic toxicity studies showed neither mortality nor behavioral changes in the test animals up to 5,000 mg/kg body weight dose. This may be a possible indication of the safety of the plant root to its users.

Characterization of extracts

Characterization of CME, MF and EAF of *Z. portoricensis* roots using the Foutrier Transform Infrared (FT-IR) revealed the following:

Crude methanol extract: IR (KBr) cm^{-1}3320 (N-H), 3246-3092 (OH), 2728 (C-H aliphatic), 1502(C=C aromatic), 1393 (C-OH), 1073 (C-C), 1005 (C-N), 730 (mono substitution).

Methanol fraction: IR (KBr) cm^{-1}3383 (OH), 2925 (C-H aliphatic), 1581(C=O aromatic), 1403 (C = C), 1087 (C-OH), 730 (mono substitution).

Ethyl acetate fraction: IR (KBr) cm^{-1}3261-3210 (OH), 2961 (C-H aliphatic), 1697(C= N), 1625 (C-C), 1470 (C = C), 1010 (C-N), 704 (mono substitution).

The functional groups identified were –OH (Hydroxyl group), C=O (ketone group, carboxyl group), C-OH (aldehyde group), C-N, C=N and NH (amides, amines and imino group respectively), C-H, C-C and C=C (alkyl, alkanes and alkenes respectively).The -OH identified at 3246-2283 stretch bands indicated the presence of Phenols, polyphenolic compounds and alcohols, such as in flavonoids and terpenoids, saponin and steroids. The C=O, identified at 1581 peak and C-OH, identified at 1073-1625 stretch bands indicated the presence of aldehydes, ketones, carboxyls and esters, such as in carbohydrates, reducing sugars, lipids glycosides and

steroids. The C-N, identified at 1005-1010 stretch band and C=N, identified at1697 peak indicated the presence of amines and amides, such as commonly found in alkaloid, hydrogen cyanide and glycosides. NH, identified at 3320 peak indicated the presence of imino groups, amines and amides, probably indicating the existence of peptide linkages and/or protein primary or secondary structure. The CH, alkyl group identified at 2728- 2921 stretch band, C-C, alkanes identified at1073-1625 stretch bands and C=C, alkenes identified at1403-1502 stretch bands, indicated the presence of hydrocarbons (aliphatic and aromatic chains), such as commonly found in all phytochemicals. These functional groups are the active components of the phytochemicals responsible for the medicinal and biological activities that prevent organs and tissues from diseases.

Conclusion

The presence of these functional groups indicates the presence of these phytochemicals and biologically active compounds. They may explain the potential medicinal and therapeutic activities of the plant roots and a possible indication of the safety of the plant root to its users.

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Table 1. Proximate composition of *Zapoteca portoricensis* roots sample

Parameter	Composition (%)
Ash	3.04 ± 0.01
Moisture	6.25 ± 0.00
Crude Fiber	0.18 ± 0.00
Fats and Oil	0.05 ± 0.00
Protein	6.39 ± 0.00
Carbohydrate	84.05 ± 0.01

Results are mean ± standard deviation of triplicate determinations

Table 2. Qualitative phytochemical screening of the extract and its fractions

Phytochemical (Test used)	Observation	CME	MF	EAF
Tannins (Ferric chloride)	Greenish-brown precipitate	+++	++	++
Flavonoids (NaOH) test	Intense yellow colour	+++	++	++
Alkaloids (Dragendorf)	Red precipitate	++	++	+
Saponins (Frothing)	persistence foaming	++	++	+
Hydrogen cyanide(HCN)	Wine red colour	++	++	+
Terpenoids	Reddish violet colour	++	+++	+
Steroids	Reddish brown colour	+	+	+
Phenols	Greenish colour	++	++	++
Reducing sugars	Brick-red precipitate	++	+++	-
Soluble carbohydrates	Purple interfacial ring	+	+	-
Glycosides	Orange colour	+	+	+

Keys: - = not detected, + = detected in low level; +++ = detected in moderate level; +++ = detected in high level. CME = crude methanol extract, MF = methanol fraction, EAF = ethyl acetate fraction

Table 3. Quantitative phytochemical constituents of the extract and its fractions

Group	CME	MF	EAF
Tannins	675 ± 0.04[c]	343.60 ± 0.08[c]	269.44 ± 0.66[a]
Flavonoids	597 ± 0.04[b]	530.35 ± 0.45[a]	11041.97 ± 0.00[c]
Saponins	0.49 ± 0.04[b]	0.62 ± 0.04	0.35 ± 0.01[a]
Cyanides (HCN)	1.41 ± 0.06[c]	1.31 ± 0.04[a]	1.37 ± 0.00[a]
Soluble carbohydrates	2.22 ± 0.04[b]	2.74 ± 0.03[c]	0.00 ± 0.00[a]
Steroids	0.38 ± 004[b]	0.42 ± 0.04[c]	0.22 ± 0.00[a]
Terpenoids	181.44 ± 181.46[a]	401.76 ± 401.77[b]	172.07 ± 44.09[a]
Reducing sugars	444.88 ± 45.62[a]	1393.47 ± 0.06[b]	0.00 ± 0.00[a]
Glycosides	46.58 ± 0.04[b]	109.62 ± 0.03[c]	39.58 ± 0.15[a]
Phenols	741.13± 0.04[b]	775.62 ± 0.03[c]	607.09 ± 0.00[a]
Alkaloids	1267.30± 0.03[b]	1398.38 ± 0.01[c]	824.63 ± 0.01[a]

Results are mean ± standard deviation of triplicate determinations. Values with different alphabets as superscript in a row are significant at $p < 0.05$. CME = crude methanol extract, MF = methanol fraction, EAF = ethyl acetate fraction

Table 4. Toxicity profile of the extract and its fractions

Phases	Dosages mg/kg b.w	Mortality for CME	Mortality for MF	Mortality for EAA	Behavioural Changes
			Phase I		
Group 1	10	0/3	0/3	0/3	Nil
Group 2	100	0/3	0/3	0/3	Nil
Group 3	1000	0/3	0/3	0/3	Nil
			Phase II		
Group 1	1900	0/3	0/3	0/3	Nil
Group 2	2600	0/3	0/3	0/3	Nil
Group 3	5000	0/3	0/3	0/3	Nil

(N = 3)