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Chemical and Antimicrobial Properties of Leaf Extracts of *Zapoteca portoricensis*

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ABSTRACT

This research was designed to evaluate the chemical (phytochemical, vitamin and mineral) and antimicrobial (antibacterial and antifungal) properties of leaves of *Zapoteca portoricensis*. Four extracts were prepared with deionized water, methanol, ethylacetate and diethylether. Phytochemical and vitamin analyses were performed according to standard methods while mineral content was measured with atomic absorption spectrophotometer. Agar disc diffusion method was used for antimicrobial study. Alkaloids, flavonoids, saponins, tannins, cardiac glycosides, terpenoids and anthraquinones which were present in the extracts, varied significantly ($p<0.05$) among the extracts. The level of Vitamins B_2, B_6, C, E and niacin, found in all the extracts did not show any significant difference ($p>0.05$), except B_6 and C. The values for B_6 and C in water extract were significantly higher ($p<0.05$) than those in ethylacetate and diethylether extracts. All the extracts contained Na, Ca, K, Mg, P, Zn and Fe, whose values did not vary significantly ($p>0.05$) in the extracts. The extracts inhibited all the microorganisms tested in a concentration dependent pattern, the water extract being significantly higher ($p<0.05$) than organic extracts. These results may be useful in explaining the medicinal applications of leaves of *Zapoteca portoricensis*.

Key words: Phytochemicals, ciprofloxacin, deionized water, antimicrobia, *Zapoteca portoricensis*

INTRODUCTION

The use of plants in the management and treatment of diseases started with life. It has been observed that many plants do indeed have medicinal value and extracts from these plants have been used to make modern drugs (Treben, 1998).

Zapoteca portoricensis, commonly called white stick (Izzo *et al*., 1995), is a perennial shrub with small oval green leaves. Different parts of the plant are used in Eastern Nigeria in the management/treatment of disorders such as constipation, convulsion, madness, prolonged labour, external wounds and skin infections. Phytochemical screening determines the presence of biologically active non-nutritive compounds that contribute to the flavor, colour and other characteristics of plant parts. These compounds (e.g., alkaloids, tannins, cardiac glycosides, terpenoids, saponins, anthraquinones, flavonoids, etc) are the major basis of pharmacological activities of medicinal plants (Oloyede, 2005). Morphine alkaloids are powerful pain relievers and narcotics (Teguja and Omak, 1993) while saponins are natural antibiotics which help in fighting infections and microbial invasion (Okwu and Ndu, 2006). Tannins have been shown to inhibit multiplication of HIV and herpes simplex virus (Okuda *et al*., 1991), whereas flavonoids are

antioxidants (Mau *et al*., 2002). Minerals play significant roles in many processes taking place in living systems. A great majority of elements act as key components of essential enzyme systems or vital biochemical functions (Slowinski and Sadowski, 2001) e.g., magnesium is an antioxidant (Okaka *et al*., 2006) and its presence may boost the immune system (Hassan *et al*., 2007).

Vitamins are essential nutrients that affect the health and development of living things. They occur naturally in plants and are required in trace amounts for normal functioning of the body (Burtis and Ashwood, 2003). Vitamin C is a powerful antioxidant. It favours absorption of iron in the intestine, protects against infections, neutralizes blood toxins and intervenes in the healing of wounds (Okwu and Ndu, 2006).

There is a continuous and urgent need to discover new Antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increases in the incidence of new and re-emerging infectious diseases and resistance to existing antibiotics. The results of investigation performed in the late 19 and 20th century and the advent of streptomycin and other antibiotics provide the ground for experimentation of a vast number of plants for antibiotic or antimicrobial activities that are useful to man (Varadarajan *et al*., 2008).

The various medicinal applications of leaves of *Zapoteca portoricensis* by traditional medicine practitioners have not been investigated. In this communication, phytochemical, mineral and vitamin contents of the leaf extracts are evaluated and the antimicrobial properties of the extracts are also examined, to establish pharmacological activity of the leaves.

MATERIALS AND METHODS

Collections and extraction of leaves material: Fresh leaves of *Zapoteca portoricensis* collected in March, 2010, from a bush in Abakaliki of Ebonyi State in Nigeria and identified by Prof. S.C. Onyekwelu of Applied Biology Department in Ebonyi State University, was used immediately for extraction. Extraction and all the investigations were concluded with in March 2010. The extraction procedures used by Agbafor (2004) were adopted, using four different solvents-deionised water, methanol, ethylacetate and diethylether. The aqueous extract was obtained by pounding 100 g of the fresh leaves and soaking the paste in 150 mL of deionized water for 30 min. The extract (green solution) was squeezed out of the mixture with a white muslin clot. In the extraction using ethylacetate, 150 g of fresh leaves, cut into smaller pieces, was soaked in 250 mL of ethylacetate for 24 h. After decantation, the solvent was removed with rotor evaporator. This was repeated using methanol and diethylether.

Collection of microorganisms: The microorganisms (*S. aureus, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, C. albicans, M. audouini, A. flavus, P. marneffei* and *T. tonsuraus*) used were obtained from Federal Medical Centre Abakaliki, Nigeria and identified at Microbiology Department, Ebonyi State University.

Phytochemical analysis: Qualitative determination of saponins, alkaloids, tannins, cardiac glycosides, terpenoids, flavonoids and anthraquinones was carried out on the four extracts using the method described by Sofowora (1993). The quantitative measurement of the phytochemicals was done according to standard procedures of Trease and Evans (1983) and Harborne (1973). Determinations were done in triplicates.

Mineral composition: The extracts were incinerated into ash, dissolved in 1 mL of 2 M HCl and diluted to 100 mL with deionized water. The resulting solution was used for the determination of Na, Ca, Mg, Mn, Fe, Zn, K and Cu using atomic absorption spectrophotometer (Buck scientific AAS 200 A) (Igwe *et al*., 2007). Phosphorous was measured by the vanadiumolybdate colorimetric method (Pearson, 1976).

Analysis of vitamins: The levels of vitamins C, B2, B_6, E and niacin in the four extracts were measured according to the methods described by Okwu and Ndu (2006). Values were also obtained in triplicates.

Antimicrobial activity: Antibacterial and Antifungal properties of the extracts were evaluated using agar disc diffusion assay according to the method of Bauer *et al*. (1966) and described by Varadarajan *et al*. (2008). Three different concentrations (5.0, 10.0 and 20.0 mg mL^{-1}) of each extract were used. Ciprofloxacin and griseofulcin were the standards for antibacterial and antifungal tests respectively. Experiments were carried out in triplicates.

RESULTS AND DISCUSSION

The phytochemical constituents of the extracts are given in Table 1. Alkaloids, flavonoids, saponins, tannins, cardiac glycosides, anthraquinones and terpenoids were found in all the extracts. Alkaloids and flavonoids contents in aqueous extract were significantly higher ($p<0.05$) than those in ethylacetate and diethylether extracts while there was no significant difference ($p>0.05$) in their values in aqueous and methanol extracts. Conversely, compositions of anthraquinones and terpenoids were significantly higher ($p<0.05$) in organic extracts than in the aqueous extract. Values of saponins, tannins and cardiac glycosides did not show any significant difference ($p<0.05$) in aqueous and organic extracts.

Table 3 presents the Vitamin composition of the extracts. It showed that all the extracts contained Vitamins B_2, B_6, C, E and niacin. The values for pyridoxine and ascorbate in water extract were significantly higher ($p<0.05$) than those in ethylacetate and diethylether extracts while their values did not show any significant difference ($p>0.05$) in aqueous and methanol extracts. The contents of other vitamins did not vary significantly ($p>0.05$) in all the extracts.

The minerals compositions of the extract are shown in Table 2. Na, K, Ca, Mg, P, Zn and Fe were found in all the extracts in different proportions. Cu and Mn were not detected in the extracts. The elemental composition of the extracts varied insignificantly ($p>0.05$).

Table 1: Phytochemical composition of the extracts

	Percentage composition (w/w)			
Phytochemical	Distilled water	Ethylacetate	Methanol	Diethylether
Alkaloids	20.76±2.31^a	9.06±1.70^b	13.33±2.11^a	8.55±1.40^b
Flavonoids	16.22±1.40^a	8.35±1.35^b	12.47±1.86^a	7.03±1.55^b
Saponins	5.77±2.00^a	8.13±1.72^a	10.55±1.51^a	9.67±2.06^a
Tannins	4.05±1.22^a	5.90±1.66^a	6.02±0.85^a	5.85±0.61^a
Anthraquinones	0.30±0.04^a	4.45±2.20^b	1.04±0.06^c	6.83±1.33^b
Cardiac glycosides	4.33±0.53^a	3.05±1.03^a	4.11±1.30^a	2.93±0.55^a
Terpenoids	0.82±0.02^a	9.60±1.33^b	4.40±1.23^c	9.92±1.41^b

Values are Mean±SD; n = 3. Values in the same row having different superscripts differ significantly ($p<0.05$)

Table 2: Mineral content of the extracts

	Composition (mg/100 g)			
Minerals	Aqueous	Methanol	Ethylacetate	Diethylether
Ca	74.1±2.7[a]	70.5±1.8[a]	62.8±2.4[a]	61.6±2.6[a]
K	1.11±0.05[a]	0.81±0.09[a]	0.63±0.02[a]	0.64±0.05[a]
Mg	10.33±0.22[a]	11.25±0.36[a]	8.55±0.12[a]	8.04±0.14[a]
P	28.52±0.81[a]	20..41±1.33[a]	18.32±1.50[a]	18.02±1.32[a]
Zn	0.08±0.004[a]	0.06±0.001[a]	0.06±0.001[a]	0.05±0.02[a]
Cu	ND	ND	ND	ND
Fe	0.05±0.001[a]	0.04±0.002[a]	0.02±0.001[b]	0.02±0.001[b]
Mn	ND	ND	ND	ND
Na	176.50±1.72[a]	162.42±3.11[a]	160.22±2.52[a]	160.0±1.24[a]

ND = Not detected. Values are Mean±SD; n = 3. Values in the same row having different superscripts differ significantly ($p<0.05$)

Table 3: Vitamin content of the extracts

	Composition (mg 100 g^{-1})			
Vitamins	Water	Methanol	Ethylacetate	Diethylether
B_2 (riboflavin)	36.05±2.80[a]	29.51±3.11[a]	28.22±2.50[a]	20.30±3.22[a]
B_6 (Pyridoxine)	92.71±3.73[a]	80.41±1.82[a]	46.30±3.65[b]	36.52±4.40[b]
C (Ascorbate)	98.50±2.33[a]	79.53±3.33[a]	40.51±1.83[b]	24.35±2.71[b]
E (Tocopherol)	40.6±3.52[a]	32.50±4.25[a]	48.34±3.40[a]	55.41±2.55[a]
Niacin	52.42±1.35[a]	38.77±1.31[a]	22.50±2.32[b]	18.6±1.53[b]

Values are Mean±SD; n = 3. Values in the same row having different superscripts differ significantly ($p<0.05$)

All the extracts inhibited the growth of all the microorganisms tested (Table 4). The inhibitory zones were concentration dependent. Zones inhibition of the aqueous extract were significantly higher ($p<0.05$) than the corresponding values for the non-polar solvents (ethylacetate and diethylether). The values did not vary significantly ($p>0.05$) between polar solvents (water and methanol) and between non-polar solvents. The extracts were most effective against the fungus, *A flavus* and least against the bacterium, S. pyog.

The zones of inhibition produced by all the extracts were lower than those produced by the corresponding standard drugs at 1.0 mg mL^{-1}.

The differences in the concentrations of the phytochemical classes in the solvents underline their solubility in the respective solvents. The concentrations of the phytochemicals in *Zapoteca portoricensis* are higher than those found in *Aspilia Africana* and *Bryophyllium pinnatum* (confirmed medicinal plants). The medicinal value of plants lies in their constituent chemical substances that produce definite physiological actions on the human body (Iniaghe *et al*., 2009). Traditional medicine practitioners in Eastern Nigeria use leaf extracts of *Zapoteca portoricensis* in the management/treatment of various diseases. The medicinal uses may be attributed to the identified constituent phytochemicals. Alkaloids have analgesic effects (Okwu and Ndu, 2006). Morphine alkaloids are powerful pain relievers and narcotics (induces sleep or drowsiness). Atropine, cocaine and other alkaloids are known stimulants of the central nervous system. (Teguja and Omak, 1993). Tannins prevent urinary tract infection by preventing bacteria from adhering to the walls. Tannins have been shown to be useful in the management of HIV infection and herpes. Combination of tannin and anthocyanins can breakdown cholesterol in the bloodstream

Table 4: Antimicrobial activity of the extracts

	Zone of inhibition (mm)												
	Water (mg mL^{-1})			Ethylacetate (mg mL^{-1})			Methanol (mg mL^{-1})			Diethylether (mg mL^{-1})			Standard
Microorganisms	5.0	10.0	20.0	5.0	10.0	20.0	5.0	10.0	20,0	5.0	10.0	20.0	1 mg mL^{-1}
S. aureus	6.2±1.2^{a}	11.5±1.0^{b}	14.0±1.3^{b}	3.4±0.5^{c}	5.0±1.2^{a}	7.8±1.3^{a}	5.6±1.2^{a}	9.2±1.0^{a}	12..3±1.2^{b}	2.2±0.2^{c}	3.4±0.4^{c}	6.1±1.0^{a}	20.2±1.0
S. pyogenes	6.0±1.0^{a}	10.6±1.3^{b}	12.4±1.1^{b}	3.5±1.0^{c}	4.6±0.4^{c}	6.3±1.2^{a}	5.4±1.0^{a}	8.5±1.4^{a}	10.7±0.5^{b}	2.8±1.0^{c}	3.9±0.6^{c}	5.2±0.7^{a}	22.5±1.5
E. coli	6.9±1.3^{a}	12.7±1.5^{b}	15.6±1.2^{b}	3.8±1.3^{c}	6.7±1.3^{a}	7.2±04^{a}	5.3±1.2^{a}	8.5±1.4^{a}	13.4±1.3^{b}	2.9±0.3^{c}	5.6±1.2^{a}	6.5±1.3^{a}	21.4±12
K. pneumonia	6.5±0.6^{a}	11.9±1.0^{b}	14.5±1.2^{b}	2.6±1.4^{c}	4.3±1.2^{c}	7.5±0.5^{a}	5.9±1.0^{a}	10.0±1.0^{b}	13.0±1.0^{b}	2.5±0.3^{c}	4.1±1.3^{c}	7.8±1.2^{a}	23.7±0.8
P. aeruginosa	7.4±1.2^{a}	13.3±1.2^{b}	16.5±1.0^{b}	3.0±1.2^{c}	7.2±1.3^{a}	9.1±1.4^{a}	6.4±1.0^{a}	11.3±1.5^{b}	14.1±1.2^{b}	3.2±1.2^{c}	7.8±1.0^{a}	9.2±1.4^{a}	20.6±1.0
C. albicans	8.4±0.5^{a}	13.6±1.3^{b}	18.3±1.0^{b}	4.8±1.0^{c}	6.5±1.2^{a}	8.8±0.5^{a}	7.0±0.6^{a}	10.5±1.2^{b}	15.3±1.4^{b}	3.6±0.3^{c}	6.1±0.2^{a}	8.0±0.4^{a}	32.0±0.6
M. audouini	8.5±1.2^{a}	13.0±0.2^{b}	17.2±1.0^{b}	4.8±0.6^{c}	6.0±0.5^{a}	8.0±1.3^{a}	7.3±0.2^{a}	10.2±1.2^{b}	14.7±1.4^{b}	4.5±0.4^{c}	6.3±0.2^{a}	8.4±0.3^{a}	29.1±0.4
A. flavus	9.6±0.6^{a}	14.5±1.3^{b}	19.6±1.2^{b}	4.7±0.4^{c}	7.0±0.6^{a}	9.2±1.2^{a}	8.7±0.2^{a}	12.0±0.6^{b}	16.5±1.3^{b}	4.0±0.2^{c}	8.0±0.2^{a}	9.5±1.3	22.5±0.4
P. marnoffei	7.5±1.0^{a}	11.0±1.0^{b}	5.8±0.3^{a}	3.5±0.5^{c}	6.2±0.4^{a}	8.1±0.4^{a}	7.0±0.3^{a}	10.2±0.5^{b}	13.5±1.2^{b}	3.0±1.0^{c}	4.5±0.7^{c}	7.0±0.4^{a}	26.0±0.5
T. tonsuraus	8.9±0.4^{a}	13.0±1.2^{b}	16.2±0.5^{b}	4.0±0.3^{c}	6.5±0.2^{a}	8.5±1.3^{a}	7.6±0.3^{a}	11.3±0.0^{b}	14.7±0.4^{b}	3.3±1.0^{c}	5.2±0.5^{a}	7.1±0.3^{a}	27.3±1.0

Values are Mean±SD (n = 3). Values in the same row having different superscripts differ significantly ($p<0.05$)

and in atherosclerotic plaques. Tannins, along with Vitamin C help build and strengthen collagen (Okuda *et al*., 1991). Saponins serve as natural antibiotics which help body to fight infections and microbial invasions. They also enhance the effectiveness of certain vaccines, lower cholesterol and knock out some tumor cells, particularly lung and blood cancers (Okwu and Ndu, 2006). Flavonoids act as antioxidants in biological systems. Other properties of flavonoids include protection against allergies, inflammation, free radicals, platelet aggregation microbes, ulcers, hepatoxins, viruses and tumors (Okwu and Ndu, 2006). The other phytochemicals have various health implications. The presence of alkaloids (possibly morphine alkaloids) may form the basis for the application of the extracts in the management of madness while tannins with vitamin C may be related to wound healing which collagen synthesis is a vital component.

The presence of the observed Vitamins in the extracts is a boost to the therapeutic potentials of the leaves. Vitamin B_6 (pyridoxine) helps in the breakdown of blood sugar and dilation of blood vessels (Trumbo *et al*., 2004); Vitamin B_2 (riboflavin) is essential for energy production and in its coenzyme forms (FMN and FAD), serves as hydrogen transport systems (Murray *et al*.,2000); Vitamin C, an antioxidant, facilitates wound healing, production of collagen, formation of red blood cells and boosts immune system (Monsen, 2000); Vitamin E is an antioxidant and plays a role in cellular respiration (Burtis and Ashwood, 2003); niacin (nicotinic acid) is converted to NAD and NADP which are coenzymes for various oxidoreductases (Burtis and Ashwood, 2003). The values of Vitamins in the aqueous extract were insignificantly higher ($p>0.05$) than those contained in *B. diffusa* and *C. nudiflora*, plants of established pharmacological activities (Ujowundu *et al*., 2008). The relatively high content of Vitamin C in the leaves of *Zapoteca portoricensis* may explain some of their therapeutic applications, especially in wound healing.

The concentration of Na, Ca, P, K, Mg and Fe found in *Zapoteca portoricensis* were high when compared the values found in *Garcina kola* and *Aframomum melagueta* (Okwu, 2005). These minerals play significant roles in several biological processes. Bone growth and turnover are influenced and regulated by the metabolism of Ca, phosphate and Mg; Fe is important in the formation of hemoglobin. Mg and K are also involved in inducement of calmness (Burtis and Ashwood, 2003). The presence of Zn in the extracts may further explain the utilization of the leaves in wound healing. Zn has inhibitory effect on bacterial growth and is involved in immune response. There is increased demand for Zn during cell proliferation and protein synthesis (Williams and Leaper, 2005).

The results obtained from the antimicrobial activity of the extracts show that the leaf extracts possess antibacterial and antifungal properties and can be source of antibiotics, having inhibited microbial growth. This inhibition was dose and species dependent as shown by the different zones of inhibition. This is inline with the work of Iwalokun *et al*. (2007). The antimicrobial property of the extracts may be related to the presence of some phytochemicals. According to Okwu and Ndu (2006), saponins natural tendency to ward off microbes makes them good candidates for treating fungal and yeast infections. Terpenoids have been implicated as the phytoconstituent responsible for the antibacterial activity of mushrooms (Iwalokun *et al*., 2007). Many medicinal plants exhibit antimicrobial activity by mechanisms of action characterized by cell membrane lyses and inhibition of protein synthesis, proteolysis enzymes and microbial adhesions (Cowan, 1999).

Although the zones of inhibition of the various extracts were less than the corresponding standard drugs (ciprofloxacin and griseofulcin), the extracts have broad spectrum of action as reflected by their activity against all tested microorganisms. The antifungal activity of the extracts may be the basis for the use of the leaves for treatment of skin infections while the large inhibitory

zone against *P. aeruginosa* (which causes wound infection) has aligned with the application of the leaves in wound healing. The aqueous extract consistently showed higher ($p<0.05$) chemical constituents and antimicrobial activity than others. This may be due to high proportion of the active compounds being soluble in water and may explain why the local users frequently prefer aqueous preparations of the leaves to organic ones.

The results of this study showed that extracts of leaves of *Zapoteca protoricensis* have pharmacological activity. The presence of vitamins and minerals makes the leaves nutritionally interesting. Efforts to purify and identify the specific phytochemicals in the leaves are on going.

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