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Phytochemical Properties of a Namibian Indigenous plant; Eembe (*Berchemia discolor*)

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Abstract

The aim of this study was to investigate the phytochemical properties of Eembe (*Berchemia discolor*); one of the underutilized indigenous plant in Namibia. This species is distributed in the northern part of Namibia and has many general uses. The fruit is high in sugar and contains vitamin C. It is eaten fresh or dried and stored for use in the dry season. Dried fruit have a date-like flavour. Fresh fruit is fermented to make beer and wine. The bark is used medicinally and for basket dye. The wood is used for furniture, hut construction and other items. There have been no previous investigations on the bioactive metabolites of *B. discolor* in Namibia. *B. discolor* extracts (fruits, leaves and barks) were prepared by different organic solvents. Qualitative phytochemical analysis of the extracts was performed to investigate the presence of: Tannins, Alkaloids, Flavonoids, Saponins, Total Phenols, Steroids, Phlobatanins, Terpenoids, Cardiac Glycoside and Anthraquinone. The significance of Eembe (*B. discolor)* in traditional medicine and the importance of the distribution of its chemical constituents will contribute in elucidating the importance of this plant in ethnomedicine in Namibia.

Keywords: *Berchemia discolor;* Qualitative phytochemical analysis; ethnomedicine; bioactive compounds.

1. Introduction

Medicinal plants are of great importance to the health sector since they have been used for the treatment of different ailments and diseases for a long time. The medicinal value of these plants relies on some of the bio-chemically active components which produce, give or stimulate a physiological action(s) on the human body (Aiyelaagbe and Osamudiamen, 2009). Phytochemicals are the most important category of these bioactive components in medicinal plants especially: flavonoid, tannin, alkaloids and phenolic compounds.

The species *Berchemia discolor* (Klotzsch) Hemsl or bird plum belongs to Rhamnaceae and distributed in the Arabian Peninsula and Africa stretching from Ethiopia to South Africa. The form of this plant is a shrub or small tree (El Amin, 1900; Venter and Venter, 1996). The fruits are edible and the leaves are used to make beverages. Also, this plant is a good source of timber and of a dye material. In Namibia it is known as "Eembe" and is one of the most popular wild fruits in the northern parts of the country (Hailwa, 2002). This plant is native to Namibia and grows abundantly mainly in the western part of the Ohangwena Region. Communities collect bird plums for home consumption and for income generation (Musaba and Sheehama, 2009). The fruit contain high sugar in the pulp (30%), and seeds taste like walnuts, the vitamin C content of the fruit is 65 mg/100 g (Venter and Venter, 1996). The time of harvesting of Eembe fruits occurs between March and April in northern Namibia. This happens just at the end of the peak cultivation period for food crops. The harvesting time of wild fruits is particularly convenient for most farmers, since they can do this without disrupting their farming activities. In addition, the bird plum fruits can be dried, stored and processed later after farmers have finished with seasonal agricultural activities (Musaba and Sheehama, 2009).

Phytochemicals are sometimes referred to as phyto-nutrients, and are defined, as any chemical or nutrient that is exclusively produced by plants. However, in common usage phytochemicals have a more limited definition. They are usually used to refer to compounds found in plants which are not required for normal functioning of the body but which nonetheless have a beneficial effect on health or plays an active role in the amelioration of disease (James et al, 2007). A minority claim that many of the diseases affecting the people of industrialized nations are as a result of lack of phyto-nutrients in their diet. Phytochemicals have various beneficial functions in the body, such as promoting the function of the immune system, acting directly as anti-bacteria or viruses, reducing inflammation. Phytochemicals are also associated with the treatment and/or prevention of cancer and cardiovascular diseases (James et al, 2007).

The aim of this study was to investigate the phytochemical properties of different Eembe (*Berchemia discolor*) plant parts: fruit, leaves and bark. This study provides one of the first insights into the phytochemical profile of the *Berchemia discolor*.

2. Materials and methods

2.1. Plant material

Berchemia discolor (English: bird plum; Oshiwambo: Eembe) fruit and bark were collected from Nakalale village, south of Outapi town in Omusati region, north of Namibia. The samples were transferred to the Indigenous Knowledge Systems Technology (IKST) Food Biochemical analysis laboratory at the Department of Chemistry and Biochemistry, University of Namibia (UNAM). Leaves were collected from a *Berchemia discolor* tree growing at the UNAM Windhoek campus. Leave samples were washed in distilled water and dried at 40ºC over night. Leaves and bark material were grinded to powder and stored in aseptic plastic bags and stored at 4ºC.

2.2. Preparation of Plant Extract

Extractions were prepared according to Kubmarawa et al. (2008) and Masola et al. (2009).

2.2.1. Ethanol and methanol extractions

Samples of powdered leaf and bark material was weighed out to 25grams each and transferred to separate autoclaved 1liter bottles. Hundred grams of intact dried fruits were weighed and transferred to an autoclaved 1 liter bottle. 100ml of absolute ethanol were added to each bottle and was extracted at room temperature for 7 days. The extractions were vacuum filtrated and reduced to 25 ml using a rotavapor. The reduced extracts were stored at 4˚C.The same procedure was repeated but Methanol was used as extraction solvent. In additional 200ml of methanol was added to the seed extract since the fruit absorbed most of the solvent.

2.2.2. Water Leaf Extraction

One 25g sample of powdered dry leaf material and two 100g samples of Eembe fruit were weighed out and transferred to separate 1liter autoclaved bottles. To the leaf extract 100ml autoclaved purified water was added and in total 400ml autoclaved purified water was added to the fruit samples. The leaf extraction and one of the fruit extractions was incubated for 7 days at room temperature. The second fruit extract was only incubated for 48 hours at room temperature. The extractions were vacuum filtrated and reduced to 25 ml using a rotavapor. The reduced extracts were stored at 4˚C.

2.3. Phytochemical screening

2.3.1. Tannins (Lead acetate test)

The test for tannins was done according to Kumar et al. (2009); a sample volume of 0.5ml of extract was diluted with 2ml of the original extraction solution. To the dilution 4-6 drops of 1% lead acetate was added. A yellow precipitate indicated a positive result.

2.3.2. Alkaloids (Meyer's test)

The test for alkaloids was done according to Hettiarachchi (2009); a sample volume of 0.5ml of each extraction was allocated to a separate test tube and transferred to a steam bath. 5ml of 1% HCl was added to each test tube. The solutions were filtered. To 1ml of filtrate a few drops of Meyer's reagent was added. A change in turbidity or any precipitate indicates the presence of alkaloids.

2.3.3. Flavonoids (Ammonia test)

The test for flavonoids was done according to Edeago, et al. (2005) and Ayoola et al. (2008); a sample volume of 1ml of each extraction was allocated to a separate test tube; 5ml of 10% ammonia solution was added to each test tube. 1ml of concentrated H2SO4 was added to each tube and a yellow color indicated the presence of flavonoids.

2.3.4. Saponins (Foam test)

The test for saponins was done according to Hettiarachchi (2009) with the following modifications. One milliliter of extract was mixed with 1ml of 70% ethanol in a test tube with a 1cm diameter and a total capacity of no less than 15ml. 5ml of water at 60˚C was added. Solutions were shaken vigorously for 2 minutes. Volume of froth was recorded every 10 minutes by measuring the froth with a ruler.

2.3.5. Total Phenols (Ferric Chloride test)

The test for total phenols was done according to Hettiarachchi (2009) with the following modifications. One milliliter of 70% acetone was added to 1 ml of extracts to a final concentration of 50mg/ml. A few drops of 10% ethanolic ferric chloride solution were added to 1ml of this mixture. A color change to blue green/dark blue was indicative of the presence of phenolic compounds.

2.3.6. Steroids (Acetic anhydrate/H_2SO_4 test)

The test for steroids was done according to Hettiarachchi (2009) with the following modifications. Two milliliters of 70% acetone was added to 1ml of the extract, and then 6 drops of acetic anhydrite was added followed by 2 drops of concentrated H_2SO_4 to test solution. Color change from violet to brown or dark brown, was indicative of steroid presence in the solution.

2.3.7. Phlobatanins

The test for phlobatanins was done according to Egwaikhide and Gimba (2007) with the following modifications. Two milliliters 1% HCl solution was added drop-wise to the extract. If a red precipitate formed, this indicated the presence of Phlobatanins. If no precipitate formed, no phlobatanins were present in the solution.

2.3.8. Terpenoids (Salkowski Method)

The test for Terpenoids was done according to Egwaikhide and Gimba (2007) with the following modifications. Two milliliters chloroform was added to the extract and mixed well. Three milliliters conc. H_2SO_4 was carefully added to the mixture to form two layers. A red-brown color at the interface of the layers was an indication of the presence of terpenoids.

2.3.8. Terpenoids (Salkowski Method)

The test for Terpenoids was done according to Egwaikhide and Gimba (2007) with the following modifications. Measure 5ml or 200mg of extract into a test tube, then add 2ml chloroform and mix well. Carefully add 3ml conc. H2SO4 to form a layer. A red-brown color at the interface of the layers is an indication of the presence of terpenoids.

2.3.9 Cardiac Glycoside (Keller Killiani's test)

The test for cardiac glycoside was done following Keller Killiani's test (Ayoola, 2008). About 1ml of extract was dissolved in 5ml water. An amount of 2 ml glacial acetic acid solution containing one drop of ferric chloride solution was added, then carefully 1ml concentrated H_2SO_4 solution poured down the side of the test tube to form two layers. A color change to brown at the interphase and a slight green color indicates the presence of cardiac glycosides.

2.3.10. Anthraquinone (Borntrger's Test)

The test for Anthraquinone was done following Borntrger's Test (Hettiarachchi, 2006). About 1ml of extract was added to 2 ml 70% acetone, and then 4ml hexane was added and shaken well. The two layers were separated, and then 4ml diluted ammonium (10%) was added to the top lipiphilic layer add. A bright pink color will indicate anthraquinones.

3. Results and discussion

The phytochemical screening of the Eembe plant parts showed that this plant contains the phytochemical compounds from the groups of chemicals listed in table 1.

Table (1) Phytochemical compounds detected in *Berchemia discolor* extracts

Phytochemical category	S1	S2	S3	S4	S5	S6	S7	S8	S9
Alkaloids	-*	-	-	-	-	-	+**	+	+
Anthraquinone	-	+	-	-	-	+	-	-	-
Cardiac Glycoside	+	+	+	+	+	+	-	-	-
Flavonoids	+	+	+	+	+	+	+	-	-
Phlobatanins	-	+	-	-	-	+	-	-	-
Saponins	-	-	-	+	-	-	+	+	+
Steroids	-	-	+	-	+	+	-	+	-
Tannins	-	+	+	+	+	+	+	+	-
Terpenoids	+	+	+	+	+	+	+	+	+
Total Phenols	+	+	+	+	+	+	+	+	+

S1: F- E-OH; S2: B- E-OH; S3: L- E-OH
S4: F- M-OH; S5: L- M-OH; S6: B- M-OH
S7: F- H_2O; S8: L- H_2O; S9: B- H_2O
F: fruits; L: leaves; B: Barks; E-OH: Ethanol; M-OH: Methanol; H_2O: Distilled water
*: -: Absent; +: present

This study on Eembe (*Berchemia discolor*) fruits, leaves and barks extracts revealed the presence of terpenoids, total Phenols, Saponins and Alkaloids in the aqueous extract; flavonoid, tannins, terpenoids, total phenols and Cardiac glycoside in the methanol extracts; Cardiac glycoside, flavonoid, terpenoids and total phenols in the ethanol extracts (Table 2).

Table (2) Phytochemical screening for *Berchemia discolor* extracts (Extraction solvent)

Phytochemical category	H_2O			M-OH			E-OH		
	S1 F	S2 L	S3 B	S4 F	S5 L	S6 B	S7 F	S8 L	S9 B
Alkaloids	+[2]	+	+	-[1]	-	-	-	-	-
Anthraquinone	-	-	-	-	-	+	-	-	++[3]
Cardiac Glycoside	-	-	-	+	++	++	+	++	++
Flavonoids	++	-	-	++	++	+	+	+	++
Phlobatanins	-	-	-	-	-	++	-	-	++
Saponins	+++[4]	++	+	+	-	-	-	-	-
Steroids	-	++	-	-	++	+	-	++	-
Tannins	++	+	-	++	++	++	-	+	+
Terpenoids	++	+	+	++	+++	+++	++	+++	+++
Total Phenols	++	+++	++	++	+++	+++	+	++	++

S1: F- E-OH; S2: B- E-OH; S3: L- E-OH
S4: F- M-OH; S5: L- M-OH; S6: B- M-OH
S7: F- H_2O; S8: L- H_2O; S9: B- H_2O
F: fruits; L: leaves; B: Barks; E-OH: Ethanol; M-OH: Methanol; H_2O: Distilled water
[1] -: -: Absent; [2]+: weakly present; [3]++: present; [4]+++: strongly present

The leaves and fruits did not show the presence of Anthraquinone and Phlobatanins in any of the extracts that were tested for its presence; they are only found in Barks (Table 3).

Table (3) Phytochemical screening for *Berchemia discolor* extracts (Plant parts)

Phytochemical category	Fruits			Leaves			Barks		
	S1 H_2O	S2 M-OH	S3 E-OH	S4 H_2O	S5 M-OH	S6 E-OH	S7 H_2O	S8 M-OH	S9 E-OH
Alkaloids	+[2]	-[1]	-	+	-	-	+	-	-
Anthraquinone	-	-	-	-	-	-	-	+	++[3]
Cardiac Glycoside	-	+	+	-	++	++	-	++	++
Flavonoids	++	++	+	-	++	+	-	+	+
Phlobatanins	-	-	-	-	-	-	-	++	++
Saponins	+++[4]	+	-	++	-	-	+	-	-
Steroids	-	-	-	+++	++	++	-	+	-
Tannins	++	++	-	+	++	+	-	++	+
Terpenoids	++	++	++	+	+++	+++	+	+++	+++
Total Phenols	++	++	+	+++	+++	++	++	+++	++

S1: F- E-OH; S2: B- E-OH; S3: L- E-OH
S4: F- M-OH; S5: L- M-OH; S6: B- M-OH
S7: F- H_2O; S8: L- H_2O; S9: B- H_2O
F: fruits; L: leaves; B: Barks; E-OH: Ethanol; M-OH: Methanol; H_2O: Distilled water
[1] **-:** -: Absent; [2]+: weakly present; [3]++: present; [4]+++: strongly present

The various phytochemical compounds detected in this study (table 1) are known to have beneficial and medicinal importance as well as exhibiting physiological activities (Sofowara, 1993; Edeoga et al, 2005).

Saponins were found in the three extracts of *Berchemia discolor*; these compounds are used as a mild detergent and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hyperchloles-trolaemia, hyperglycaemia, antioxidant, anti-cancer, anti-inflammatory and weight loss etc. It is also known to have anti-fungal properties (Aiyelaagbe and Osamudiamen, 2009).

Tannins were found in leaves mostly then in the fruits, followed by the barks; these compounds are reported to exhibit antiviral, antibacterial, anti-tumor activities. It was also reported that certain tannin are able to inhibit HIV replication selectivity and is also used as diuretic (Heslem, 1989).

Cardiac glycosides were extracted totally by the organic solvents but not with the aqueous extraction. These compounds are known to work by inhibiting the Na^+/K^+ pump. They are used in the treatment of congestive heart failure and cardiac arrhythmia. They are also, used to strengthen a weakened heart and allow it to function more efficiently, though the dosage must be controlled carefully, since the therapeutic dose is close to the toxic dose (Denwick, 2002).

Flavonoids of *Berchemia discolor* were extracted from the three plant parts (fruits, leaves and barks) by organic solvents. Chin et al (2006) isolated five new prenylated flavonoids from the root bark of Berchemia discolor, collected in Tanzania, along with 10 known compounds, by bioactivity-guided fractionation. The isolated compound exhibited cytotoxic activity when evaluated against a small panel of human cancer cells (Chin et al., 2006). Flavonoids compounds possess anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities. They have been referred to as nature's biological response modifiers because of their inherent ability to modify the body's reaction to allergies, virus and carcinogens (Aiyelaagbe and Osamudiamen, 2009).

Anthraquinone are generally used as dyes and are also, known as antibacterial agents. The bark is used medicinally and for basket dye, this could be explained by the high contents of Anthraquinone and Phlobatanins of which it was only found in *Berchemia discolor* barks.

Steroids were found only in *Berchemia discolor* leaves and these compounds are important and interest in medicine due to their relationship with sex hormones (Okwu, 2001). Our results are in agreement with Okwu (2001) who reported on the use of the leaves of *Cleome rutidosperma* as vegetable for expectant mothers or breast feeding mothers to ensure their hormonal balance, since steroidal structure could serve as potent starters in synthesis of these hormones (Okwu, 2001). They are also routinely used in medicine because of their profound biological activities (Denwick, 2002).

Terpenoids, total Phenols were found in all *Berchemia discolor* extracts with higher concentrations in the leaves extracts than the fruits and barks extracts. Normally terpenoids rich plants are widely used in herbal medicine (Hayashi et al., 1993).

Phlobatanins were extracted from the bark of *Berchemia discolor* by organic solvents only (Table 3) and Alkaloids from all parts by aqueous extraction only. These compounds with Alkaloids, Anthraquinone, Cardiac glycosides, Phlobatanins, and Tannins contribute to the antimicrobial potency *Berchemia discolor*. This result agrees with the report of Ebena, et al. (1991), Trease and Evans (2005).

4. Conclusion

Berchemia discolor can be seen as a potential source of useful drugs since it seems as rich source of terpenoids, total Phenols, Saponins, flavonoid, tannins and Cardiac glycoside. Currently ongoing studies in the Indigenous Knowledge Systems Technology (IKST) Food Biochemical analysis laboratory at the Department of Chemistry and Biochemistry, University of Namibia includes the quantitative analysis of the phytochemical compounds, isolation and characterization of the chemical structures of these bioactive compounds. The antimicrobial activities of the different extracts of *Berchemia discolor* are also under investigation.

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