

EFFECT OF EXTRACTION METHOD ON THE PHYTOCHEMICAL CONSTITUENTS OF *VERNONIA AMYGDALINA*

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ABSTRACT

Quantitative analysis to assess the levels of alkaloids, tannins, flavonoids, saponins and cardiac glycosides, as well as qualitative analysis of steroids was carried out with *Vernonia amygdalina* leaf extracts. The extracts were cold water extract and cold ethanolic extract at room temperature as well as soxhlet ethanolic extract at 78°C. The results show that there were significant increases ($p < 0.05$) in tannins, saponins and steroids (qualitative) in soxhlet ethanolic extract and cold ethanolic extract compared to the cold water extract. Significantly higher ($p < 0.05$) values were however obtained for flavonoids and cardiac glycosides in the cold water extract compared to the cold ethanolic extracts. The alkaloid content of the three extracts however did not differ significantly at 5 per cent level. The results show that the extractant and the method of extraction influenced the concentrations of the selected phytochemicals investigated in *Vernonia amygdalina*.

KEYWORDS: *Vernonia amygdalina*, leaf extracts, phytochemicals, extraction method.

INTRODUCTION

Vernonia amygdalina is a woody shrub found mainly in the tropics. It is also called bitter leaf due to its characteristic bitter taste and flavour. Its characteristic odour and bitter taste are due to its antinutritional factors such as alkaloids, saponins, tannins, and glycosides (Ohigashi, *et al*, 1991). Its proximate composition as well as mineral content has been documented (Bonsi *et al*, 1995). Phytochemical analysis of the leaves showed the presence of some compounds of importance which may have useful medicinal roles whether in raw or processed forms (Jisika, *et al*, 1992). Phytochemical screening of the methanolic and petroleum ether extracts showed negligible concentrations of flavonoids in both extracts as well as the presence of cardiac glycosides and polyphenols. The methanolic extract also had high contents of alkaloids and saponins as well as tannins and reducing compounds which were not detected in the petroleum ether extract (Ugwuibe, 2004). A number of antinutritional factors or phytochemicals have been extracted from plant materials. Trypsin inhibitors from African yam bean flours extracted using 0.01M NaOH as extractant at pH 9.50, and extraction time of 1.0 and 3.0 hours were found adequate to extract the maximum amount of the inhibitors from raw and heat processed flours respectively (Onyeike and Ayalogu, 1999).

MATERIALS AND METHODS

Collection and treatment of samples

Fresh mature leaves of *Vernonia amygdalina* were collected from the Endocrine Research Farm in the University of Calabar, Calabar, Cross River State. They were sundried, blended with an electric blender and used for the various extractions.

The cold water extract was obtained by soaking 1kilogram of the blended leaves overnight in 2.5 litres of distilled water, and then squeezing out the extract that was subsequently dried at room temperature in a wide tray.

The cold ethanolic extract was obtained by overnight soaking 1kilogram of the blended *Vernonia amygdalina* leaves in 2.5litres of 98% ethanol and then filtering. The filtrate was evaporated to dryness at room temperature by exposure to air in a wide tray.

The soxhlet ethanolic extract was obtained by soxhlet extraction of 400grams of the blended *Vernonia amygdalina*

leaves at 78°C using a soxhlet apparatus. The extract was then dried at room temperature.

Estimation of phytochemical contents

Qualitative Test for steroids: 2millilitres (2mL) of acetic anhydride was added to 0.5grams of ethanolic extract of each sample with 2ml of 1N H₂SO₄. The color changed from violet to blue in the samples.

Determination of Alkaloid content: The method of Harborne (1973) was adopted. 5 g of the sample was weighed into a 250ml beaker and a 200ml of 10% acetic acid in ethanol was added and allowed to stand for 4hours. This was filtered and the extract was concentrated in a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid which was dried and weighed. The percentage alkaloid was calculated.

Determination of Tannin content: Tannin content was determined by the method of Van burden and Robinson (1981): 500mg of the sample was weighed into a 50ml plastic bottle, 50 ml of distilled water was added and shaken for 1hr in a mechanical shaker, this was filtered into a 50ml volumetric flask and made up to the mark; 5ml of the dilute sample was pipetted out into a test tube and mixed with 2ml of 0.10 M FeCl₃ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm within 10 minutes.

Determination of saponin content: Saponin content was determined by the method of Obadoni and Ochuko (2001). The samples were ground and 20g of each was put into a conical flask and 100cm³ of 20% aqueous ethanol was added. The samples were incubated in a water bath at 55°C for 4hours with continuous stirring. The mixture was filtered and the residue re extracted with another 200ml in 20% ethanol. The combined extracts was reduced to 40ml in a water bath at 90°C, the concentrate was transferred into a 20ml separatory funnel and 20ml of diethyl ether added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded, the purification process was repeated and 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride and

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the remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as a percentage.

Determination of flavonoid content: The flavonoid content of the extracts was estimated by the method of Boham and

Kopical-abyazan (1974): 10 g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol. The whole solution was then filtered through Whatman No. 42 filter paper. The filtrate was later transferred into a crucible and evaporated into dryness over a water-bath and weighed to constant weight.

Table 1: Phytochemical constituents of *Vernonia amygdalina*

Group	Alkaloids (%)	Tannins (%)	Flavonoids (%)	Saponins (%)	Cardiac glycosides	Steroids
Cold water extract	0.09±0.01	0.67±0.10	1.20±0.02	2.02±0.02	113.40±2.54	+
Cold ethanolic extract	0.08±0.01	3.10±0.60	0.50±0.01	4.10±0.06	64.80±1.86	++
Soxhlet ethanolic extract	0.10±0.04	6.30±0.54	0.60±0.02	4.08±0.02	81.00±2.28	++

Values are means ± standard deviations of triplicate determinations

Data Analysis: This was done using ANOVA and student's T-test

RESULTS

The phytochemical contents of *Vernonia amygdalina* extracted using different solvents are presented in Table 1. From the results, while the alkaloid content (%) of the three extracts showed no significant differences ($p>0.05$), the concentration of tannins increased significantly ($p<0.05$) in the cold ethanolic extract and soxhlet ethanolic extract (6.30±0.54) compared to the control (0.67±0.10). The tannin content of the soxhlet ethanolic extract was however significantly higher ($p<0.05$) than that of the cold water extract.

The content of flavonoids (%) was significantly higher ($p<0.05$) in the cold water extract (1.20±0.02) compared to the cold ethanolic extract (0.50±0.01) and the soxhlet ethanolic extract (0.50±0.01).

The saponin content (%) of both the cold ethanolic extract (4.10±0.06) and the soxhlet ethanolic extract (4.08±0.02) were significantly higher ($p<0.05$) than that of the cold water extract (2.02±0.02).

The content of cardiac glycosides as presented in Table 1 show that the cold water extract had a value (113.40±2.54) that was significantly higher ($p<0.05$) compared to cold ethanolic extract (64.80±1.86) and the soxhlet ethanolic extract (81.00±2.28).

Qualitative assessment of the extracts showed the presence of steroids in the three extracts with higher concentrations in the cold ethanolic and soxhlet extracts compared to the cold water extract.

DISCUSSION

The phytochemical screening and quantitative estimation of the percentage crude yield of chemical constituent of plant leaves show that they are rich in alkaloids, flavonoids, tannins and saponins; and also possess medicinal properties as well as physiological activity (Sofowara, 1993). Flavonoids are most commonly known for their antioxidant activity. They have been referred to as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anti-cancer activity (Balch and Balch, 2000). Quantitative analysis of the leaf extracts of *Vernonia amygdalina* obtained using cold water extraction and cold ethanolic extraction (at room temperature), as well as soxhlet

ethanolic extraction (at 78 °C) show the presence of alkaloids, tannins, flavonoids, saponins, cardiac glycosides and steroids. This agrees with the report that phytochemical analysis of the leaves of *Vernonia amygdalina* show the presence of some compounds of importance which may be useful in medicinal roles whether in raw or processed forms (Jisika, *et al*, 1992). The result presented in Table 1 show that there was no significant difference ($p>0.05$) in alkaloid content in the three extracts. The cold water extract had significantly higher ($p<0.05$) values for flavonoids (%) and cardiac glycosides (%). Cardiac glycosides as drugs are used in the treatment of congestive heart failure and cardiac arrhythmia. They work by inhibiting the Na^+/K^+ pump. This causes an increase in the level of sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca^{++} ions available for contraction of the heart muscle, improves cardiac output and reduces distention of the heart. These glycosides are found as secondary metabolites in several plants, but also in some animals. Some of these compounds (ouabain and some frog poisons) are used in Africa as arrow-poisons for hunting (http://en.wikipedia.org/wiki/Cardiac_glycoside).

The tannin content was significantly higher ($p<0.05$) in the ethanolic extracts, with a much higher value in the soxhlet extract compared to the cold ethanolic extract. Saponin and steroid content (qualitative) were higher in both ethanolic extracts compared to the water extract. In particular the saponins are used in veterinary vaccines as adjuvant (e.g., foot-and-mouth disease vaccines, helping to enhance the immune response). They are also mild detergents and are used commercially, as well as for research. In laboratory studies, they can be used at 0.04%-0.2% to make holes in the plasma membrane as well as the membranes of internal organelles. Therefore it is used in intracellular histochemistry staining to allow antibody access to intracellular proteins. Because of its reversible nature on cells and its ability to permeate cells without destroying cell morphology, it is used in laboratory applications to treat live cells in order to facilitate peptide or reagents such as antibodies entering cells instead of the harsher detergents (<http://en.wikipedia.org/wiki/Saponins>). The high steroid content of the ethanolic extracts is of particular importance. Steroidal compounds are of interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001).

The results show significant changes in the content of the extracts depending on the solvent used as well as the method of extraction and agrees with earlier reports of the

phytochemical screening of the methanolic and petroleum ether extracts which showed negligible concentrations of flavonoids in both extracts as well as the presence of cardiac glycosides and polyphenols. The methanolic extract also had high contents of alkaloids and saponins as well as tannins and reducing compounds which were not detected in the petroleum ether extract (Ugwuibe, 2004).

CONCLUSION

In conclusion, it is obvious that the chemical composition of *Vernonia amygdalina* extracts is influenced greatly by the method of extraction as well as the solvent used.

The differences in phytochemical composition in the extracts investigated in this research could be the result of differences in polarities of extractants as well as temperature used for the extraction.

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