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Comparative analysis of total tannins in *Plectranthus barbatus* Andrews water, acetone and methanolic extracts in Kenya

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ABSTRACT

The purpose of this study was to identify the most suitable solvent for use in extracting tannins from *Plectranthus barbatus* Andrews leaves, a plant indigenous to various parts of Kenya. The plant was traditionally used by the Gusii community in Kisii and Nyamira Counties for establishing land demarcations, enhancing ripening of bananas and as a stomachache remedy. The plant's resistance to either wild or artificial fire, a feature predominant in plants with high tannin content makes it a good candidate for research. The tannin content and the most eco-friendly and economical solvent to use have not been established and this compound can also be used in preserving and tanning hides and skins. Furthermore, the plant contains nepetoidin B which is a powerful antioxidant capable of eliminating toxic free radicals present in chrome tanned leather.

The leaves of the plant were collected from three locations in Nyamira County, Kenya and positively identified at the Kenya National Museum Herbarium in Nairobi. Extraction was carried out using 80% methanol, 80% acetone and water followed by determination of total tannins using the hide powder method. The percentage tannin content was found to be 56%, 36% and 60% respectively and furthermore phytochemical screening revealed that the leaves contained tannins of hydrolysable type. There was a significant difference ($p < 0.05$) in the levels of tannins dissolved by the three solvents and the study concluded that water was the best solvent for use in extraction of tannins.

Keywords: *Plectranthus barbatus* Andrews, Hydrolysable tannins, Hide powder, Tannins, Non-tannins.

INTRODUCTION

Plectranthus barbatus Andrews belongs to the family lamiaceae also known as labiatae and it is the largest family of order lamiales which has herbs and shrubs with distinct four-sided stems and blue raceme inflorescence. The plant is also commonly known as *Coleus forskohlii* or Indian coleus and locally known as *Omoroka* (Kisii), *Mwaraka* (Embu) and *Mumbu (Digo)* is a tropical perennial plant. One of the most widely studied compounds derived from this plant is the labdane forskolin which has a range of diverse medicinal uses [1]. *Plectranthus barbatus* Andr grows perennially over the tropical and subtropical regions of the Indian subcontinent and is cultivated commercially for its use in pickles [2]. Pickles are salt solutions used to preserve perishable foods such as meat and vegetables. In addition the plant has also been studied for its antimalarial properties in Msambweni in Kenya [3].

Plectranthus barbatus Andr is distributed in Egypt, Ethiopia, Brazil, India, Sri Lanka and Tropical East Africa including Kenya, Uganda and Tanzania [1]. The crop is being commercially grown in South Africa, Zimbabwe, Rajasthan, Maharashtra, Karnataka and Tamil Nadu in an area of about 2500ha [1] however, in Kenya the crop is neglected. In Kenya *Plectranthus barbatus* Andr grows in all parts of Kisii and some parts of Elgon region, South Coast, Central province, Western Kenya, Eastern province and South Turkana [1].

The plant is known to contain tannins [4] however, the best solvent for use when isolating them has to be investigated. Tannins are large polyphenolic compounds with sufficient hydroxyls and other suitable groups such as carboxyls which contribute to their high molecular weight that allows them to form strong complexes with proteins and other macromolecules [5]. Its resistance to fire is a salient characteristic of plants that are rich in tannins and hence this together with the presence of a brown dye in the leaf water extract form a basis of this investigation. There are three main classes of tannins which include hydrolysable or pyrogallols (gallotannins and ellagitannins), condensed and complex tannins [6].

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Tannins can be concentrated in stem, bark and/or leaves and their primary role in plants is protection from decomposition, predation and wild fire [7].

Phytochemistry of *Plectranthus barbatus* reveals that it contains flavonoids together with two non-flavanoidphenolics, caffeic acid derivatives and nepetoidin A and B [1]. Previous studies demonstrated that nepetoidin B present in water extracts showed a strong free radical scavenging characteristic that was more valuable than the activity displayed by rosmarinic and gallic acids [8]. The role of phenolic compounds in the medicinal properties of *Plectranthus spp.* is not well understood [1] and therefore in this study the likelihood of their use together with other tannins in leather preservation and tanning industry is highly feasible hence the need of establishing the most economical and eco-friendly way of extracting them.

MATERIALS AND METHODS

Plant collection and identification

The leaves of *Plectranthus barbatus* Andrews in this experimental study were collected from three locations namely; Gesima, Riamoni and Esani in Nyamira County, Kenya during the month of October, 2014. The specimen of the plant was positively identified and compared at the Kenya National Museum herbarium in Nairobi. The leaves were shredded and the dried at room temperature of 22°C for a period of one month before they were ground by a suitable mill (Thomas Wiley Lab Mill Model 4). The resultant powder was sieved and classified according to the location of origin before it was stored in clear sealed polythene bags pending further analysis. 100 grams of each class of powder was then soaked in water, 80% methanol and 80% acetone for 72 hours in two litre conical flasks. The liquid extracts were then carefully filtered using Whatman No. 1, 15 cm filter papers into two litre beakers which were labelled respectively. The extracts were later reduced using a rotavapor Buch (W 240 N) at 50°C before further evaporation was done in 250 ml beakers using dry air oven (Gallen Kamp Oven BS – Size Two) at 65°C until solid extracts were collected. The correctly labelled samples were covered with an aluminium foil and stored at room temperature of 22°C.

Qualitative analysis

Test for tannins

i) Ferric chloride test: each sample extract was mixed with 1% ferric chloride solution and development of blue black and/or green colours were recorded as positive for tannins [9].

Tests for flavanoids

i) Ferric chloride test: Alcoholic extracts of each sample were mixed with few drops of neutral ferric chloride solution and development of green colour was recorded as positive for flavonoids [9].

ii) Lead acetate tests: Alcoholic solutions of the sample extracts were mixed with few drops of 10% lead acetate and development of a yellow precipitate indicated presence of flavonoids [9].

Tests for condensed and hydrolysable tannins

Each sample extract was mixed with few drops of aqueous potassium hydroxide and rapid development of red colour confirmed the

presence of condensed tannins and where there was no observable change the presence of hydrolysable tannins was concluded [10].

Quantitative analysis of non-tannins and total tannins in solid extracts

The hide powder method was used to determine the total tannins in the samples as outlined in the IULTCS official methods of analysis [11, 12]. The method is based on the absorption of materials from the extract by the hide protein. It is not based on a chemical analysis of a true tannin molecule. Subtraction of non-tannins from soluble solids gives total tannin content. The tannin content is expressed as a percentage of the soluble solids.

Determination of non-tannins

The quantity of wet hide powder that contained 6.25 g dry hide powder was weighed and immediately added to 100 ml of unfiltered tannin infusion plus 20 ml of distilled water present in a 300 ml wide mouth flask and the process was completed according to the test method SLC 116 [11].

Determination of tannins

The tannins were determined in duplicates by the difference between the percentages of the total soluble and the non-tannins according to SLC 117 [12].

Statistical analysis

The data was analyzed with two-way and one-way analysis of variance (ANOVA) test and presented using tables. (p<0.05).

RESULTS

The phytochemistry conducted on the ground *P barbatus* leaves showed that they contained hydrolysable tannins by giving blue- black precipitate when reacted with ferric chloride and the samples did not develop red colour on addition of a few drops of aqueous Potassium hydroxide. The results are summarized in the table 1 below.

Table 1: Results for phytochemical screening of *P barbatus* leaf water, acetone and methanolic solid extracts from Riamoni, Esani and Gesima locations as compared to *Mimosa*

Test	Method	Sample/ Result			
		<i>P barbatus</i>		<i>Mimosa</i>	
		Riamoni	Esani	Gesima	
Tannins	Ferric chloride test	+++	+++	+++	+++
Flavanoids	a) Ferric chloride test	+	+	+	X
	b) Lead acetate test	+	+	+	X
Saponins	Froth test	++	++	++	++
Hydrolysable tannins	Ferric chloride test	✓	✓	✓	X
Condensed tannins	Ferric chloride test	X	X	X	✓

Key:

+++ Present in high percentage.

X Absent

++ Present in moderate percentage.

+ Present in small percentage.

✓ Present.

Table 2: Average and separate percentage compositions of *Plectranthus barbatus* leaf water, acetone and methanolic solid extracts from Riamoni, Esani and Gesima locations

	Samples												
	Water extract				Methanol extract				Acetone extract				Mimosa
	Avr.	G	R	E	Avr.	G	R	E	Avr.	G	R	E	
% Tans	60	58	62	60	56	60	54	54	36	35	37	36	66
% Non-tans	33	30	34	35	34	35	36	31	24	22	25	25	24
% Moisture	7	5	6	10	8	7	10	7	16	18	14	16	9
% Total solids	93	95	94	90	92	93	90	93	84	82	86	84	91
% Tans in total solubles.	64.5	65	64.5	63	62	63	60	63.5	60	61	60	59	73
Tanning strength(T/NT)	1.8	1.9	1.8	1.7	1.7	1.7	1.5	1.7	1.5	1.6	1.5	1.4	2.8
Av PH	5.9	5.9	5.9	5.9	4.7	4.7	4.7	4.7	4.5	4.5	4.5	4.5	4.6

Key: G= Gesima location, R= Riamoni location, E= Esani location

DISCUSSION

The solid extracts from *P barbatus* leaves that concentrated by a Rotavapor- Re, Buchi W 240 N showed differences in terms of the percentage tannins and non-tannins extracted. The water extracts yielded the highest amount of tannins at 60% followed by methanol extracts at 56% and acetone extracts recorded the lowest amount of tannins at 36%. The non-tannins levels were 33%, 34% and 24% respectively. This means that most components in *P barbatus* leaves tannins are polar since water being the most polar of the three solvents recorded the highest yield while acetone being the least polar of the three solvents extracted the least amount of tannins and non-tannins. Tannins are polyphenolic compounds found in plants that are soluble in water and polar organic solvents [13]. 80% Methanol-water extracted the highest amount of non-tannins because methanol dissolves both polar and non-polar molecules but also if the concentration of polar components increases it hinders the solubility of non-polar components meaning that the highest percentage of compounds in the methanol-water solid extract were polar.

Acetone-water solvent usually extracts higher amounts of phenolics and condensed tannins [14] however, from the results it dissolved the lowest amounts of non-tannins at 24% and lowest content of tannins at 36%. This phenomenon would have been the case since the tannins in *P barbatus* are of hydrolysable type and most components had relatively high polarity. In addition, it was possible that there might have been unfavorable interactions between the compounds extracted by acetone-water solvent that subsequently blocked the activity of total tannins.

There was a significant ($p=0.0<0.05$) difference in the level of tannins and non-tannins dissolved in respect to the three types of solvents used although the same levels are not significantly different ($P=0.98>0.05$) between the three locations. There is significant interaction ($p=0.0<0.05$) between the locations and the solvents used in extraction meaning that the treatments and the sampling blocks are not independent.

These results clearly show that the type of tannins present in *P barbatus* were of hydrolysable type however, *Mimosa* that was used as a control revealed that it contained only condensed tannins although it was expected that it would also contain some fraction of hydrolysable tannins [15]. Water emerged to be the best solvent for hydrolysable tannin extraction from *P barbatus* leaves as compared to methanol and acetone. Consequently for commercial application in

tanning industry it would mean that the whole process will be much cheaper and more environmentally friendlier than when organic solvents are used for large scale extraction of tannins.

CONCLUSION

The results from phytochemical analysis of *Plectranthus barbatus* leaves indicated that tannins present in the samples are predominantly of hydrolysable type and that there was no variation among the samples collected from Riamoni, Esani and Gesima locations.

This research study has shown that water solid extracts yielded 60 % total tannins which was the highest among the three solvents involved in this study and therefore it is evident that water is the best solvent for large scale extraction of tannins in *Plectranthus barbatus* leaves. Furthermore, statistical analysis depicts that *Plectranthus barbatus* leaves can be collected from any of the three locations included in the study.

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