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Comparative Analysis of Phytoconstituents and Caffeine Levels Of Acacia Nilotica (Subalata) And Coffea Arabica Varieties

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Abstract: *The indiscriminate use of whole plant or their parts to treat various diseases and to make coffee like concoction by local communities in Kenya could pose a serious threat to health. This is because one cannot assure that these plants are medicinally important and possess caffeine. Although some plants have been reported to contain valuable phytochemicals which are of medicinal value, no work has been done to compare phytochemicals and caffeine content in Acacia nilotica ssp. Subalata and coffee Arabica. The current study deals with the phytochemical analysis and caffeine content of Acacia nilotica bark extract in comparison with coffee Arabica varieties. The phytochemical study was done using standard procedure while caffeine content was determined using High Liquid Performance Chromatography (HPLC). Phytochemical screening revealed presents of phenols, carbohydrates, saponins, alkaloids, flavonoids, steroids and triterpernoids among the three plants. Tannins were found to be absent in Acacia nilotica ssp. Subalata extract. Quantification of phytochemicals in the two coffee varieties in comparison with Acacia nilotica ssp. Subalata was doneand all the values were significant (p≤0.5). The results from High Liquid Performance Chromatography showed thatRuiru 11 had the highest concentration of 161.75 ± 39.16 followed by Batian 27 with 127.39± 14.83 and Acacia nilotica ssp. Subalata with 68.91± 17.39.*

Keywords: *Acacia nilotica ssp. Subalata, coffee varieties, phytochemical analysis, caffeine content.*

I. Introduction

Since time immemorial, most communities in Kenya have been using whole plant or their parts to treat various diseases and also others go to extend of using it to make coffee like concoction. Therefore, one cannot assure that these plants are medicinally important and more importantly possess caffeine. Although some plants have been reported to contain valuable phytochemicals, no work has been done to compare phytochemicals and caffeine content in *Acacia nilotica* ssp. Subalata and coffee Arabica hence the gap. The indiscriminate use of these plants could be having a long term health effects to the locals hence the need to make comparison of phytochemicals and caffeine levels in these two plants. These aids in knowing whether the extensive use of *Acacia nilotica* ssp. Subalata as a medicinal plant and making beverage possess threat to human health or can be used as a substitute for coffee.*Acacia nilotica* subspecies subalata have bitter taste and this helps protect the plant against intruders such as animals which feed on them. Other bioactive compounds include flavonoids-anthocyanins, terpenes, tannins, steroids, oleosins, resins, phenols glycosides, phenols, catechins, pyrocatechol, gallic acid and alkaloids (Ali et al., 2012).Caffeine is one of the most valuable stimulant compounds found in over 63 plantsand it is mostly found in coffee, tea leaves (theine) and cocoa plants (Mumin*et al.*, 2006). It is a naturally occurring substance found in various parts of the plant including the bark, fruits, flowers, roots and leaves. The chemical formula of caffeine is $C_8H_{10}N_4O_2$, and its systematic name is 1, 3, 5-trimethylxanthine (Aurnaud, 1987).*Acacia nilotica* is found in several parts of the world. It grows in arid and semi-arid areas. It was first described by the CarlLinnaeus in 1773 as *Acacia nilotica*. There are almost 1380 species of acacia spread around the world with most of them found in Australia. It thrives well in tropics and subtropical regions. More than 40 species of acacia are found in Indiaand are referred to as 'Babul." In Africa it is found in North West countries such as Senegal, northern Africa, south west and east Africa. In east Africa, it is majorly spread in Ethiopia, Sudan and Kenya (Rajvaidhya*et al.*, 2012).*Acacianilotica* subspecies subalata in Kenya is found in Rift valley, northeastern and some parts of coastal region.The locals have used the plant as a multipurpose ranging from herbs to food additives. The *Acacia nilotica* subspecies Subalata occur in wooded grassland, savanna and dry scrub forests on deeply sandy soils along the riverine habitat and some organizations have planted these trees on farms across the vast plains in Kenya.Coffee belongs to kingdom plantae, order gentianales, family of Rubicae, genus Coffea and species arabica (Farah, 2012). It is grown in more than 50 countries in three continents including Africa where it is grown in countries such as Kenya, Ethiopia, Rwanda and Uganda. There are two types of coffee which are Arabica and Robusta. In Kenya, coffee Arabica is grown in highlands while Robusta coffees are grown in low lying regions (Mureithi, 2008). *Coffeaarabica*was first described by Antoine Jussieu as *Jasminumarabicum.* Carl Linnaeus later placed it in its own genus *Coffea* in the

year 1737.Coffee *(Coffeaarabica*) is among the mostly widely consumed beverage in the whole world. There are several coffee Arabica varieties in the world. In Kenya, *Coffeaarabica* has many varieties which include Ruiri 11, SL34, 38, Batian 27 and 28.Coffee is a plant which grows in a temperature range of between 15-24 °C and 24-30 °C for Arabica and Robusta respectively. Also this plant needs an annual rainfall of between 1500-3000 mm (Mureithi, 2008). Coffee is an important beverage where over 2.25 billion cups are consumed daily (Farah, 2012). Unfortunately, consumption takes place in developed countries whereas its production (90 %) takes in developing countries. It is the second most valuable commodity in the world after petroleum where there are over 25 million farmers in 50 countries practicing coffee production (Diminse*t al.,* 2011).Caffeine is the major component of coffee. Studies suggest that low to moderate intake of caffeine leads to increase in alertness, heartbeat, learning capacity and decrease in fatigue leading to clearer flow of thoughts, exercise performance, whether in tablet form or not (Bolton *et al*., 1981).The present study was conducted to screen and compare different phytochemicals present in ethanol, methanol and water extract of bark of Acacia nilotica ssp. Subalata and coffee varieties. The aim of the study also was to compare their caffeine levels in Acacia nilotica ssp. Subalata and caffeine varieties.

II. Materials and Methods

2.1 Plant material

This study used*Acacia nilotica*(Subalatasubsp.) bark and coffeearabica varieties (Batian 27 and Ruiru 11).About two kilograms of *Acacia nilotica* subsp. subalata's bark wasobtained in Baringo County, Kenya. Coffee varieties (Batian 27 and Ruiru 11) samples of about one kilogram each was obtained from Coffee Research Foundation (CRF) in Ruiru, Kenya using random sampling. *Acacia nilotica* subsp. subalatawas identified and authenticated botanically in the Department of botany JKUAT. Both plant samples were stored in air tight bags for further use.

2.1.1 Qualitative phytochemical analysis

Phytochemical analysis of saponins, tannins, alkaloids, phenols, steroids,flavonoids, carbohydrates and triterpernoidswas done through chemical test approach qualitatively and scored as present (+) or absent (-) based on either precipitation or colour change(Harbone, 1983).

2.1.2 Quantitative test of phytochemicals

The quantitative estimation of flavonoids, alkaloids, saponins, phenols and tannins of *Acacia niloticas*sspsubalata'sbark and coffee varietieswas performed according to standard protocols byOkwu and Ukanwa, (2007), Poornima and Ravishankar, (2009),Aliyu, (2008),Hussain*et al.*, (2011) and Price and Butler, (1977) respectively.

2.2 Determination of caffeine levels

Samples and standards were run in the High Performance Liquid Chromatography system. The conditions of the HPLC were as follows; Column, Reverse phase – ODS, 250 × 4.6 mm, flow rate (1 ml/min), photo iodide array detector (PAD) was set at 278 nm, pressure (150 khf/cm^2), mobile phase - water, acetic acid, methanol (59.5, 0.5 and 40) and sample volume of 10 µl (Eloff, 2004). Calibration curve of peak areas versus concentration of the standards was plotted and caffeine content of various samples was calculated by using regression equation of the best line of fit.

III. Results

Table 1: Phytochemical profile of BWMEE, PWMEE and PWMEE of *Acacia nilotica*Subalatassp, Batian 27 and Ruiru 11 KEY

Secondary metabolites	ANSBWE	ANSBME	ANSBEE	PWE	PBME	PBEE	PRWE	PRME	PREE
Tannins	-	-	-	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	-
Alkaloids	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	-	+	+	+
Steroids	+	+	+	+	-	-	+	+	-
Triterpernoids	+	+	+	+	-	-	+	+	-
Carbohydrates	+	+	-	+	+	+	-	+	-
Phenols	+	+	+	+	+	+	+	+	+

ANSBWE-*Acacia niloticas*spSubalata water extract, ANSBME- *Acacia niloticas*spSubalata methanol extract, ANSBEE- *Acacia niloticas*spSubalata ethanol extract, PBWE- Processed Batian 27 water extract, PBME- Processed Batian 27 methanol extract, PBEE- Processed Batian 27 ethanol extract, PRWE- Processed Ruiru 11 water extract, PRME- Processed Ruiru 11 methanol extract, PREE- Processed Ruiru 11 ethanol extract.+ = present and - = absent

Table 2: Quantitative phytochemical analysis of saponins, alkaloids, flavonoids, phenols and tannins

Phytochemicals	Concentration in %		
	Acacia nilotica ssp. Subalata	Batian 27	Ruiru 11
Alkaloids	25.13 ± 0.81	16.93 ± 0.31	17.00 ± 0.92
Saponins	11.60 ± 0.72	5.23 ± 0.40	4.47 ± 0.12
Flavonoids	4.93 ± 1.17	11.87 ± 0.42	12.00 ± 0.60
Phenols	14 .00 ± 0.18	22.00 ± 0.08	26.00 ± 1.09
Tannins	0.53 ± 0.05	2.52 ± 0.11	2.82 ± 0.16

Values are percentage mean ± standard deviation (n=3)

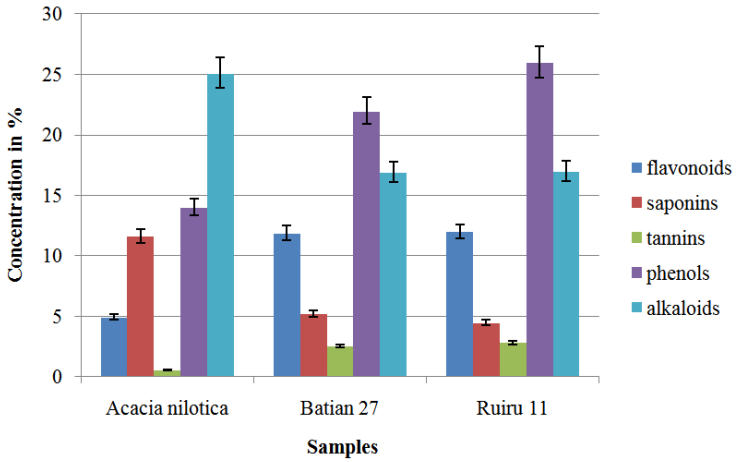

figure 1: comparison of phytochemicals in *acacia nilotica* ssp. subalata, batian 27 and ruiru 11

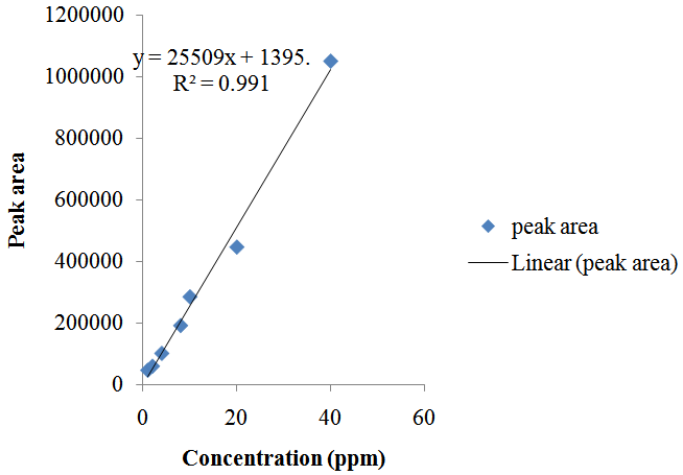

figure 2: shows caffeine calibration curve for determination of caffeine levels in *acacia nilotica* ssp. subalata, batian 27 and ruiru 11 using hplc

Table 3: Caffeine concentration (ppm) in *Acacia nilotica* ssp. Subalata, Batian 27 and Ruiru 11

Samples	Mean ± SD
Acacia nilotica ssp. subalata	68.91 ± 17.39
Batian 27	127.39 ± 14.83
Ruiru 11	161.75 ± 39.16

Table 4: Percentage levels of caffeine in *Acacia nilotica* ssp. Subalata, Batian 27 and Ruiru 11

Samples	Concentration %
Acacia nilotica ssp. Subalata	0.34 ± 0.09
Batian 27	0.64 ± 0.07

Ruiru 11	0.81 ± 0.20

Table 1: Shows the results of qualitative phytochemical screening of water, methanol and ethanol extracts of stem bark of *Acacia nilotica*ssp. Subalata and coffee varieties.The three extracts for the two samples contained alkaloids, flavonoids, saponins, carbohydrates, phenols, steroids and triterpenoids while tannins were absent in it for stem bark extract of *Acacia nilotica* ssp. Subalata. Saponins, carbohydrates, steroids and triterpenoids were absent in ethanol extract while carbohydrates was absent in both water and methanol extract of Ruiru 11 respectively. Steroids and triterpenoids were absent in both methanol and ethanol extract of Batian 27 while flavonoids was absent in ethanol extract.

Table 2: Shows the results of quantitative phytochemical analysis of three extracts prepared from *Acacia nilotica*subalata subspecies, Batian 27 and Ruiru 11. The three extracts contain saponins, tannins, phenols, flavonoids and alkaloids. *Acacia nilotica*subalata subsp. extract has the highest amount of saponins, alkaloids and flavonoids but has the lowest phenols and tannins. Ruiru 11 and Batian 27 have the highest amount of phenols and tannins respectively

Table 3: Reveals that Batian 27 has the highest concentration of 127.39 ± 14.83 ppm followed Ruiru 11 has 161.75 ± 39.16 ppm while *Acacia nilotica* ssp. Subalata has the lowest concentration of 68.91 ± 17.39 ppm

Table 4: Shows the amount of caffeine in the *Acacia nilotica* ssp. Subalata, Batian 27 and Ruiru 11 ranged between 0.34 ± 0.09 % to 081 ± 0.20 % with Ruiru 11 having the highest concentration of 0.81 ± 0.20 % and *Acacia nilotica* ssp. Subalata having the lowest value of 0.34 ± 0.09 %. The above values do not correlate well with documented values of between 2-5% in literature review. (http://www.polaris.nova.edu).

IV. Discussions

Over the past several decades, people have been resorting crude methods of making coffee like or tea like concoction in remote areas of Kenya. Some communities even go to extend of taking spoonful of sugar and wrap it in a paper or just put it on a metallic object and burn it. When it turns brown after burning it they put to the pot containing boiling water and colour changes from colorless to brown or black like coffee. The obvious reason why this people do this is because of poverty that crippling the country. Studies show that Kenya has almost 70 % of people living below a dollar a day. This makes it totally impossible to avail the basic needs to the table. In these remote regions people and school going children eat wild fruits and in most instances it poisons them to an extent of killing them. To effectively promote the use of *Acacia nilotica*ssp. Subalata as a source of herbal medicine, it is vital to study it further for potential sources for new drugs and as a food substitute. The medicinal properties of this plant are due to the presence of various phytochemicals which occur as secondary metabolites (Karthikeyan*et al.,* 2009)In the present study it showed presents of similar compounds with exception of tannins which were absent in *Acacia nilotica* ssp. Subalata. In terms of their quantities, it varied slightly in comparison with Batian 27 and Ruiru 11. The results from HPLC on caffeine concentration, the three plants had caffeine because they had similar retention time with the standard caffeine. Caffeine separation was done using the reverse phase column (Non polar C_{18} column). The mobile phase was methanol: water: acetic acid (40:59.5:0.5). Several standards of different concentrations were run followed by unknown samples of the two plant species. Quantification of caffeine content was made by making comparison of the caffeine peak area in both *Acacia nilotica* ssp. Subalata and coffee varieties samples compared to those of the standards.Caffeine is a very nice compound because it is used in preparation of analgesics, diet aids and cold remedies. It can also be applied in additives in carbonated drinks (PawarPruthviraj*et al* 2011). The current findings demonstrate that *Acacia nilotica* ssp. Subalata has the lowest caffeine than the two coffee Arabica varieties although there are some factors that contribute to the above results which are type of soil, type of varieties, altitude, growing conditions, processing techniques,maturity of the cherries, type of coffee beans and method of preparation applied (http://www.nobleharbor.com/tea/caffeine.html) and (http://www.ico.org/caffeine.asp). Now *Acacia nilotica* ssp. Subalata can be used to make coffee as it contain chemical compound caffeine as in Batian 27 and Ruiru 11(coffee).

The results of qualitatively phytochemical analysis of ethanol, water and methanol extract of stem bark of *Acacia nilotica* ssp. Subalata in the present study revealed the presence of alkaloids, saponins, flavonoids, phenols, steroids, triterpernoids and carbohydrates which was consistent with what Dishapande and Kadam, 2013. Tannins, phenols, triterpenoids, saponins, alkaloids, flavonoids, carbohydrates and steroids were presence in two coffee varieties which was consistent with a study by Gunalan*et al.*, 2012. The absence of tannins in this study correlates with study by Godghate*et al.*, 2014. The results of quantitative phytochemicals analysis *Acacia nilotica* ssp. Subalata bark and coffee varieties extract showed the presence of phenols, tannins, alkaloids and flavonoids which correlates with study by Alam*et al.*, 2007 who found tannins and phenols and Nayeem*et al.*,2011respectively.

V. Conclusion And Recommendations

From the above results it can be suggested that the three types of plants have almost the same number of phytochemicals with exception of tannins which are absent in *Acacianilotica*. *Acacia niloticas*sp. subalata contained minimal amounts of tannins, flavonoids, phenols compared to Batian 27 and Ruiru 11. A. nilotica ssp. subalata was particularly rich in alkaloids and saponins. The order of caffeine levels in the three samples was: Ruiru 11 followed by batian 27 and finally *Acacia nilotica* ssp. Subalata. Coffeearabica had the highest caffeine concentration while acacia niloticasubsp. subalata had the lowest caffeine concentration. Therefore, *Acacianilotica* can be used as an easily accessible source of natural coffee (Acacia coffee) as it can reduce common health problems hence efficiency to live a healthy and productive life. Further research needs to be done to determine the effect of caffeine from *Acacia nilotica* ssp. subalataon health particular people suffering from blood pressure.

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