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The Quantitative Phytochemical analysis of crude khat extracts(muguka) from Embu County, Mbeere North subcounty, Gitiburi location, Thura sublocation, Kamugu village.

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ABSTRACT: Studies on crude khat extracts have shown that khat is unstable and undergoes decomposition reactions after harvesting, during drying, extraction or storage of the plant material. This has created a general thought that freezing and freeze-drying protocols are the best options to preserve biological samples for long periods of time without primary compounds destructionand as a result, almost all pharmacological studies and chemical analysis of khat have been conducted using the fresh leaves of the plant. Other reports have documented that room temperature drying alone could not cause appreciable change in the concentration of cathinone; an alkaloid constituent liable for euphoric and hallucinogenic effects of the plant. As a result, there is confusion among researchers working on khat related projects about khat preservation without significant loss of the alkaloids.
Thus, the objective of this work was to investigate the phytochemicals of fresh and dried khat; a subset of *muguka*sample, from Embu County, Kenya.

KEYWORDS: Khat, *Muguka*, Spectrophotometric.

I. INTRODUCTION

Khat (catha edulis forsk) is an evergreen shrub whose leaves or twigs are chewed in social gatherings for recreation and stimulatory effects. (Patel, 2019) The name Khat is mainly used in the western literature but in areas where the plant is endemic, it is known by a variety of names, such as *qat* in Yemen, *jaad* in Somalia and *miraa* or *muguka* in Kenya. (Patel, 2015). In Kenya, locals chew stems and twigs as *miraa*or fresh shoots and leaves as *muguka* which are Meru and Embu variants respectively. Khat is a natural amphetamine that contains psychoactive alkaloids; cathinone and to a lesser extent cathine, which are extracted by the action of enzymes in saliva (Dhaifalah & Santavý, 2004). These compounds are Psycho active and cause euphoric, hallucinogenic and rewarding effects, a behaviour associated with khat dependency and plant abuse. (Engidawork, 2017). In Kenya, *Muguka*a subset of khat and is the Embu variant where locals chew tender leaves and young shoots. It's grown as a legal plant and remains the major cash crop contributing to around 60% of the total horticultural exports. (Kithinji, 2019). The juice from chewed Fresh leaves and young shoots is swallowed and the accumulated ball of residue known as *quid*is spat out at the end of the chewing session.

Existing literature has reported that khat is unstable and undergoes decomposition reactions after harvesting, during drying, extraction or storage of the plant material (Brenneisen & Geisshüsler, 1985). This has created a general thought that freezing and freeze-drying protocols are the best options to preserve biological samples for long periods of time and as a result, almost all pharmacological studies and chemical analysis of khat have been conducted using the fresh leaves of the plant. Other reports have documented that room temperature drying alone could not cause appreciable changes in the concentration of cathinone (Chappell & Lee, 2010). As a result, there is confusion among researchers working on khat related projects about khat preservation without significant loss of the alkaloids. Current literature shows that different Khat sample preservative conditions like air drying, freeze drying, oven

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drying, sun drying and freezing still yielded cathinone at73% 57%, 42% and 36%, respectively (Atlabachew et al., 2013, Chappell& Lee, 2010).

The total phenolic, flavonoid, and tannin contents were analysed using spectroscopic methods (Ultra violet Spectrophotometer UV8400 series) based on the Beer-Lambert Law which states that the amount of light absorbed is directly proportional to the concentration of the solute in the solution and thickness of the solution under analysis. Alkaloids and saponins were analysed using gravimetric method(precipitation) at the Jomo Kenyatta University of Agriculture and Technology (JKUAT) government of kenya (GK) laboratory.

II. EXPERIMENTATION

The following chemicals and glass wares were used for the analysis: 1N Folin ceocalteau, 5% Sodium nitrite, 10% aluminium chloride, 4% sodium hydroxide, Rutin, 80% Ethanol, 5% sodium carbonate, Gallic acid, polyvinylpolypyrrolidone, Dimethyl ether, n-butanol, ammonia, distilled water and acetic acid. Glass wares and apparatus included; beakers, conical flasks, 1ml micropipette, measuring cylinders and weighing balance.

Preparation of Crude Extract.

20 kgs of fresh Muguka plant comprising of young shoots and tender leaves were harvested from Embu County, Mbeere North subcounty, Nthawa ward and Kamugu village located 0.62800^0S,37.54269^0E at 1,127M altitude level and transported via road to JKUAT botany herbarium about 120 Kms away. The plant identification and authentication were carried out at Jkuat Botany herbarium by Mr. John. K. Muchuku for East African Herbarium, department of national museums of Kenya. A voucher specimen (collection number MPM-JKUATBH/001/CE/A-2021) was deposited for future reference.

The fresh sample was first prepared and the remaining plant material was air dried at room temperatures in Botany Laboratories for one week. The dried material was ground at Engineering department in Jkuat and two sample portions of 0.1grammes each for aqueous and methanolic extraction were taken the remaining powder was reserved at room temperatures in the botany laboratory just in case the study did not meet the broad objective.

Aqueous extraction.

0.1gm of cleaned fresh khat sample was crushed using mortar and pestle and both the fresh and ground plant material was soaked in 4.9 mls of distilled water and frequently agitated using a rotary shaker at 120 rpm for 48 h. It was then filtered using a What-man No. 1 filter paper of 90 mm diameter and the filtrates were frozen at $80^{0c.}$The concentrated extract was then transferred into a freeze-drying flask lyophilized and the dried extract kept in a tightly sealed container at 4° C.

Methanolic extraction.

0.1gm of cleaned fresh khat sample was crushed using mortar and pestle and both fresh and dried material was soaked in 4.9 mls of 80% methanol solvent, sealed with aluminum foil and frequently agitated using a rotary shaker at 120 rpm for 48 h. It was then filtered using a Whatman No. 1 filter paper 90 mm diameter and the filtrates were concentrated using a rotary evaporator at a temperature of 20° C to make the stock sample. The concentrated paste was then transferred into a smaller beaker and stored at 4° C.

Total Phenolic and Tannin Content

The total phenolic content of the crude extracts was evaluated by the Folin-Ceocalteau method with some modifications using Garlic acid as standard (Ruto et al., 2022). The 0.1ml of sample and the standards (2.5, 5, 7.5, 10, 12.5mg/ml) were treated with 0.5mL the Folin-Ceocalteau, left to stand for 5 mins, treated with 2.5ml of 5% sodium carbonate, vortexed and allowed to stand for 40mins. The absorbance was measured at 769 nm using compact, double-beam UV-VIS spectrophotometer (UV 1800, Shimadzu) (Baba & Malik, 2015; Parimelazhagan, 2016).

The total phenolic content expressed in mg of garlic acid equivalents (GAE) /g of dry weight extract (DW).

The same procedure was repeated for tannins after 500ul of dry sample was extracted using 100mg of polyvinylpolypyrrolidone for 4 hours at 4℃.

Total Flavonoid Content

The total flavonoid content was determined using the Aluminium chloride method with Rutin as the standard (Baba & Malik, 2015) and the absorption readings done at 510 nm using compact, double-beam UV-VIS spectrophotometer (UV 1800, Shimadzu). The standards 0, 8, 16, 24, 32 and 40mg/ml were prepared and together with the sample, were treated with 150uml of 5% sodium nitrite, vortexed and incubated for 5min and 150microlitres of 10% aluminium chloride vortexed

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and incubated for 6min. 2 ml of 4% sodium hydroxide was added to all the test tubes and all topped to 5ml with distilled water. The calibration curve was used to determine the total flavonoid content and was expressed as mg of Rutin equivalent (RE)/g of dry weight of sample.

Determination of Total Saponin Content

100cm^3 of 20% aqueous ethanol was added to 5g of sample in a 250cm^3 conical flask. The mixture was heated over a hot water bath for 3hrs with stirring at every 60 minutes. The extract was filtered and reextracted again with another 20% ethanol. The extracts were combined and evaporated to a quarter over a water bath. 20cm^3 of dithylether was added to the extract in a separating funnel, agitated and recovered. The sample was purified with 60cm^3 of n-butanol and dried in the oven (Edewor & Owa, 2016; Ezeonu & Ejikeme, 2016).
% Saponin= (weight of saponin)/ (weight of sample) x 100.

Determination of Alkaloids

200cm^3 of 10% acetic acid in ethanol was added to 2.5g of sample in a 250cm^3 beaker and allowed to stand for 4 hrs. The extract was filtered and concentrated to a quarter of the original volume followed by dropwise addition of 15drops of ammonium solution to form a white precipitate (Edewor & Owa, 2016; Ezeonu & Ejikeme, 2016). After 3hrs, the supernatant was discarded and precipitate washed with 20cm^3 of ammonia solution, filtered and the residue was dried in the oven and the % alkaloid expressed as;
% Alkaloid= (weight of alkaloid)/ (weight of sample) x 100

III. RESULTS

The total flavonoid, phenol and tannin contents were determined using the formula;
C= (c x V)/m
Where C is the concentration of the phytochemical in mg/ gram of sample, c is the concentration given by the spectrophotometer, V is the volume of extract used during analysis, and m is the mass of dry sample measured for analysis.

Calibration Curves

Figures 1 and 2 shows the standard curves used for the analysis of flavonoids, phenols and tannins.

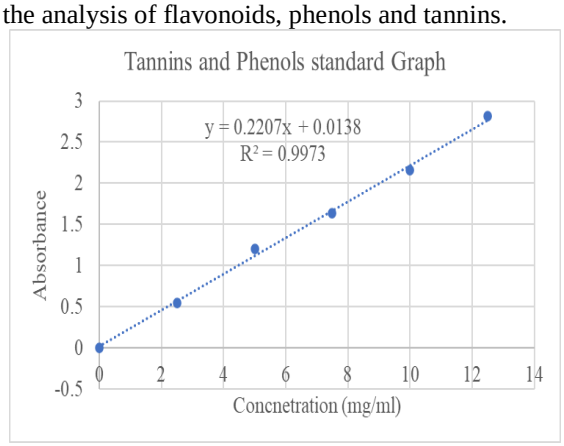

Figure 1: Standard Graph of Tannins and Phenols

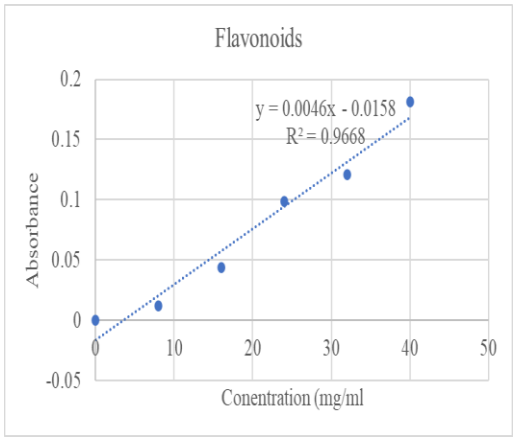

Figure 2: Standard Graph of Flavonoids

IV. OBESERVATIONS FROM THE TESTS CONDUCTED

Table 1 below shows the total flavonoid, phenol, and tannin content in both fresh and dried khat.
Table 2 shows percentage content in alkaloid and saponin in both fresh and dried khat.

Table 1: Total Flavonoid, Phenol, Alkaloid and Saponin Content

Sample Name	Total Flavonoid Content in mg (RE)/g of dry weight (DW)	Total Phenol Content in mg (GAE) /g of dry weight (DW).	Total Tannin Content in mg/g of dry weight
Methanol Fresh Khat	**19161.17±0.02**	**5474.75±0.006**	**5589.75±0.009**
Methanol Dried Khat	**12552.95±0.025**	**5758.00±0.010**	**5589.75±0.034**

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Water khat	**fresh**		**4690.60±1.20**
	8666.57±1.08	**4740.22±0.28**	
Water khat	**dried**		**3165.54±0.022**
	3670.11±1.97	**3249.67±0.008**	

Key; GAE - Garlic Acid Equivalents; DW – Dry Weight; RE - Rutin equivalent

Table 2: Percentage content of fresh and dried khat

Sample Name	Percentage Alkaloid content (%)	Percentage Saponin content (%)
Methanol Fresh Khat	**33±0.02**	**29.75±0.006**
Methanol Dried Khat	**36±0.025**	**26.75±0.010**
Water khat	**fresh**	
	30±1.08	**25±0.001**
Water khat	**dried**	
	32.11±1.97	**23 ±0.012**

V. CONCLUSION

The standard graphs were good representatives for the analysis because of the good r2 values.

It was observed that the total flavonoid content was relatively higher in methanol and aqueous extracts of khat. However, methanol extracts gave the highest values. Methanol is a more polar solvent hence extracts polar and relatively polar compounds unlike water which extracts only water-soluble compounds.

The percentage content of alkaloids was relatively higher in both fresh and dried khat samples than saponins. The high contents explain the effect of khat-on-khat consumers.

It is also evident that although both khat extractionandpreservation methods had slight difference in terms of percentage alkaloid yield results, the original constituents of the material were still present with no significant difference.

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