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COMPARATIVE PHYTOCHEMICAL, PROXIMATE AND VITAMIN SCREENING OF THE LEAVES OF *ALOE CHINENSIS* AND *ALOE BARBADENSIS* (ALOECEAE)

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ABSTRACT: The comparative phytochemical screening of the leaves of *Aloe chinensis* and *Aloe barbadensis* (Aloeaceae) revealed the presence of phytochemicals, vitamins and minerals at different proportions in the two *Aloe* species investigated. Using ANOVA, phytochemicals such as Tannin, Flavonoids and Alkaloids were significantly higher in *Aloe chinensis* (1.36, 0.72, and 0.73mg/100g respectively) than in *Aloe barbadensis* (1.28, 0.65 and 0.64mg/100g respectively) while HCN, Saponin and Sterol were higher in *Aloe barbadensis* (0.76, 1.42 and 0.06 mg/100g respectively) than in *Aloe chinensis* (0.68, 1.36 and 0.02mg/100g respectively). Phenol was at equal proportions of 0.06 in both specie. Similarly, vitamins such as Riboflavin and thiamin were found to be at equal proportions in *Aloe chinensis* and *Aloe barbadensis* (0.03% each) while ascorbic acid and niacin showed higher proportions in *Aloe chinensis* (15.70% and 14.61% respectively) than in *Aloe barbadensis* (13.85% and 12.76% respectively). Minerals such as Sodium and calcium were significantly higher in *Aloe chinensis* (0.29% and 0.23% respectively) than in *Aloe barbadensis* (0.22% and 0.21% respectively). These substances may be attributed to the health related properties of the *Aloe* species which include anti-cancer, anti-inflammatory and anti-allergic activities which probably constitute the high demand of the *Aloe* species in and around us.

KEY WORDS: Comparative, Phytochemicals, Aloe leaves, *Aloe chinensis, Aloe barbadensis,* Aloeceae

INTRODUCTION

Aloe is a genus containing about 400 species of flowering succulent plants. The genus is native to Africa and is common in South Africa's Cape Province, the mountains of Tropical Africa and neighboring areas such as Madagascar, the Arabian Pennisula and the Islands of Africa. They were once classified in the Liliaceae but now have their own own family Aloeceae (Barcroft and Myskja, 2003).

Note that the plant sometimes called "American Aloe" (*Agave Americana*) belongs to Agavaceae, a different family and is different from *Aloe* in that *Aloe* contains a gel like sap and do not die after blooming.

The genus *Aloe* belongs to a larger class of plants known as Xeroids, so called because of their ability to open and close their stomata to ensure that water is retained when necessary within the plant. This ability to conserve precious water allows members of the Xeroid group to survive long periods of dry weather even drought conditions when their plants would undoubtedly wither and die (Barcroft and Myskja, 2003).

Aloes are monocotyledons having petaloid sepals and a compound pistle. They have fibrous root system. Flowers are tubular, frequently yellow, pink, red or orange and are borne on densely clustered simple or branched leafless stems. Many species appear stemless with a rosette growing directly at ground level, other varieties may have a branched or unbranched stem from which the fleshy leaves spring. They vary in colour from grey to bright green and are sometimes striped or mottled.

Aloe leaf structure according to Barcroft and Myskja (2003) is made up of the following layers (1) The rind (2) The sap (3) The mucilage and (4) The gel.

The green rind (about 15 cell thick), is very important due to its physical protective properties. It is this layer that contains all the protective and photosynthetic materials and where the synthesis of all the naturally occurring nutrients found in *Aloe* takes place. The layer contains vascular bundles running the length of the leaf and closely associated with these bundles are elongated cells known as aloin cells in which phenolic substances are either synthesized or stored or both.

Below the rind layer is a sap which has a yellowish colour and a very bitter taste. Below this sap is the mucilage layer which effectively holds in the next layer-the gel or parenchyma. This gel is the largest section of the leaf and is full of gelatinous gel. When exposed, this resembles a raw fillet and is transparent. The inner gel is sterile and is where most of the nutrients are stored.

Historical uses of various *Aloe* species by humans are well documented. Of the 400 species of *Aloe*, only a few are used traditionally. These include *Aloe barbadensis* Mill. first described by Carl Linnaeus (Akinyele and Odiyi, 2007) and *Aloe chinensis* described by Harworth (2004) as a probable variety of *Aloe barbadensis. Aloe chinensis* is apparently however, a distinct species and is so recognized in the British Pharmacopoeia, its leaves are not more than half the length of those of *Aloe barbadensis* and are more or less copiously spotted on the upper and lower surfaces.

Aloe species are frequently cultivated as ornamental plants both in gardens and in pots. Many are highly decorative and are used by collectors of succulents. They are generally highly medicinal hence there is an increase in their cultivation even around the living homes.

Despite the numerous economic and medicinal importance of *Aloe* species, not much is known especially on the phytochemical screening of *Aloe barbadensis* and *Aloe chinensis* hence this research is designed to investigate

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the phytochemicals as well as the vitamins and minerals in *Aloe chinensis* and *Aloe barbadensis* in order to compare their levels in both species.

MATERIALS AND METHODS

Sample Collection: The leaves of *Aloe barbadensis* and *Aloe chinensis* were collected from No 6 bus stop Nekede, Owerri West L.G.A, Imo State, Nigeria and were identified by Mr, Ikpeama Ahamefula of Reliable, Research Laboratory Services, Umuahia, Abia State, Nigeria.

Sample Preparation: The fresh leaves of *Aloe barbadensis* and *Aloe chinensis* were sliced and crushed properly using pestle and mortar. Note: The gel is part of their leaves.

Quantitative Determination of the Chemical Constituents

Methods according to Harbone (1973) were used.

Alkaloid Determination: 5g of the leaf samples were extracted with 10ml of petroleum ether. The petroleum ether was removed by using rotary aspirator. 1g of the extract was suspended in 19ml of double distilled water, and the pH adjusted to 7.6. After shaking for 1 hour, the suspension was centrifuged. 1 ml of the supernatant was diluted to 50ml with phosphate buffer. The absorbence was read with a spectrophotometer at wavelength of 580nm.

Saponin Determination: 0.1g of the sample was boiled with 5ml of double distilled water for 5mins, decanted and filtered while still hot. 2 ml of olive oil was added to it, and shaken for 30 sec. The absorbance was read in a spectrophotometer at the wavelength of 620nm and zeroed with a blank.

Tannin Determination: 0.5g of the sample was introduced into a test tube, and 10ml of 2M HCl added and shaken for 5mins. The content of the test tube was transferred into a volumetric flask, made up to 50 ml and then filtered. 5ml of the filtrate was introduced into a test tube, and 3ml of 0.1 M $FeCl_3$ in 0.1M HCl and 3ml of 0.008M Pottasium ferocyanide ($K_3FeCCN)_3$) were added.

The absorbance was read at 720nm within 10mins with a spectrophotometer, and zeroed with a blank. A calibration curve was constructed and used for the calculation of the concentration of Tannins.

Flavonoid Determination: In 250ml beaker, 10g of the sample was dissolved in 70ml of double distilled water and heated for 15mins. 6g of activated carbon was added to the solution, mixed thoroughly and allowed to stand for 30mins. The solution was filtered with a 6 millipore mesh screen fitted glass funnel, containing an abeston pad. The flask and the residue were washed with six 25ml portions of double distilled water and the filtrate collected in a 400ml beaker. 2 drops of Hcl were added and evaporated on a steam bath to 40ml and transferred to a 50ml volumetric flask. It was diluted with water and then mixed. The absorbance was read with spectrophotometer at 233nm wavelength and zeroed with the blank.

Sterols Determination: 5g of the sample extract was dissolved in 100ml of double distilled water. The solution was eluted with ammonium hydroxide solution (pH 6) and sephadex-100. 2ml of the fraction was collected in a test tube and 2ml of chloroform added. 3ml of ice cold solution of acetic anhydride was added with 3 drops of sulphuric acid and shaken thoroughly. The absorbance was read using a spectrophotometer at the wavelength of 210nm.

Hydrogen Cyanide Determination: 5g of the sample was ground into a plate and dissolved in 50ml distilled water in a conical flask. It was allowed to stay overnight. The solution was later filtered. 2ml of the filtered solution was poured inside a conical flask and 4ml alkaline filtrate solution was added and incubated in water bath for 5mins for color development (reddish brown) and absorbance was taken at 490nm. Also a blank was prepared using 2ml distilled water. The cyanide content was extrapolated using a cyanide standard curve calculation:

$$HCN\ (mg/100g) = \frac{Vf}{Va} \times \frac{1}{100} \times \frac{100}{10} \times \frac{100}{1}$$

Where Vf = Total volume of extract
Va = Volume of extract used
W = Weight of sample used.

Terpenes Determination: The calorimetric method of ADAC (1980) (10^{th} edition) was used.

Standard hydrate was prepared. A measuring weight of reacting sample was mixed with 100ml of saturated NaCl solution and 35ml of ethanol and 2ml of AOAC followed by 100ml distilled water. The mixture was distilled in all glass distiller. 100ml distillate was collected. 5ml of the color reagent (Folic Danis reagent) was dispersed into labeled 50ml volume flasks. 5ml of concentrated H2SO4 was added to each while being cooled under running water. After that the flasks were allowed to attain room temperature between 2ml of distilled water from each sample was added to the flask of their respective labels. All the flasks were placed in a water bath of boiling water for 20mins after which they were cooled to room temperature under running water. Each was distilled to mark with 6.5% alcohol. They were shaken every few minutes until the solution was cleared. They were allowed to stand for 30 minutes before their absorbance was measured at 725nm. Absorbance was measured, reagent blank and zeroed. The terpene content was given as

$$T/100g = \frac{100}{W} \times \frac{Au}{As} \times \frac{C \times Vf}{Va}$$

Where W = weight of sample analyzed
Au = Absorbance of test sample
As = Absorbance of standard solution
Vf = Total volume of filtrate
Va = Volume of filtrate analyzed
C = Concentration of standard

Phenol Determination: The fat free sample was boiled with 50ml of ether for the extraction of the phenolic components for 15mins. 5ml of the extract was pipette with a 50ml flask; the 10ml distilled water was added. 2ml of ammonium hydroxide solution and 50ml of concentrated amyl alcohol were also added. The samples were then made up to mark and left to react for 30mins for colour development. The absorbance of the solution was read at 505nm using a spectrophotometer. Standard solutions of phenols were prepared at 0.00, 2.00, 4.00, 6.00, 8.00 and 10.00 respectively with the same treatment.

Calibration curves of the absorbance values versus concentration of the standard were constructed and the value of phenol in the sample calculated.

Determination of Vitamins: The -complex vitamins (Thiamin, Niacin and Vitamin C) were determined using Skalar Analyser 918 solar model.

Abscorbic Acid Determination: 5g of the sample was weighed into an extraction tube and 200ml of EDTA/TCA (2:1) extracting solution were mixed and the mixture shaken for 30mins. This was transferred into a centrifuge tube and centrifuged at 3000 r.p.m for about 20 minutes. It was transferred into a 100ml volumetric flask and made up to 100ml mark with the extracting solution. 20ml of the extract was pipetted into a volumetric flask and 1% starch indicator, this was added and titrated with 20% $CuSO_4$ solution to get a dark end point (Barakat *et al*, 1973).

Thiamin Determination: 5g of the sample was homogenized with ethanolic hydroxide (50ml). it was filtered into a 100ml flask. 10ml of the filtrate was pipette and the colour developed by addition of 10ml of potassium dichromate and read at 360nm. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard solution was prepared using thiamic acid to get 100ppm and serial dilution of 0.0, 0.2, 0.4, 0.6, 0.8ppm was made. This was used to plot the calibration curve.

Niacin Determination: 5g of the samples were treated with 50ml of 1N Sulphuric acid and shaken for 30mins. 3 drops of ammonia solution was added to the sample and filtered. 10ml of the filtrate was pipetted into a 50ml volumetric flask and 5ml of potassium cyanide were added. This was acidified with 50ml of 0.02N H_2SO_4 and absorbance measured in the spectrophotometer at 470nm wavelength. This was used to plot the calibration curve.

Riboflavin Determination: 5g of the sample was extracted with 100ml of 50% ethanol solution and shaken for 1hr. This was filtered into a 100ml flask. 10ml of extract was pipette into 50ml volumetric flask. 10ml of 5% potassium permanganate and 10ml of 30% H_2O_2 were added and allowed to stand over a hot water bath for 320mins. 2ml of 40% $NaSO_4$ was added forming a yellowish pale colour. This was made up to 50ml mark and absorbance measured at 510nm.

Determination of Minerals: The samples for the determination of the mineral elements of interest was subjected to acid digestion and subsequently, the different elements were determined using appropriately methods as described below by James (1995).

Digestion: 10ml of volume of each sample was dispensed into an evaporated to 50ml on a water bath and transferred quantitatively to 100ml standard volume flask. It was made up to volume with deionized water.

Calcium and Magnesium Determination: A Calcium and magnesium ion content of the digested sample was carried out by complex iometric titration. A measured aliquoterate (10ml) was dispensed into separate conical flask. Pinch doses of the masking agents potassium cyanide, potassium ferocyanide, hydrodiamine hydrochlorate were measured into the contents of each flask. 20ml of ammonia buffer was added to one of the flasks to raise the pH of the solution to 10.0 while 10ml of NaOH solution was added to the other to raise the pH to 12.0. To the flask at pH 10.0, E_2: Chrome black T indicator was added and titrated against 0.02N EDTA solution.

The other flask at pH 12.0 (for calcium alone), selechrome dark blue indicator was added and titrated and magnesium form complexes with EDTA reagent, blank was titrated as control. The calcium and magnesium contents of the sample were calculated using the standard determination. 1ml of 1N EDTA has an equivalence of 24mg magnesium and 20.04mg calcium.

Sodium and potassium Determination: Sodium and potassium were determined by flame photometry. The photometer was set up according to the manufacturer's instruction. 1ml of prepared potassium or sodium standard solution was aspirated into the machine and sprayed over the non-luminous butane gas flame. The sodium and potassium emission (having been appropriately filtered) form the different concentrations were recorded and made into standard curve. Subsequently, the optical density emissions recorded from each of the sample were matched against those in the curve, thus using the curve to extrapolate the quantity of the sample.

Phosphorus Determination: Phosphorus in the sample was determined by the Vanadomohylodate (yellow) spectrometry

1ml extract from each sample was dispensed into a test tube. Similarly, the same volume of standard phosphorus solution as well as water was put into other test tubes to serve as standard and blank respectively. The contents of each test tube were mixed with equal volume of the Vanadonohydodate colour reagent. They were left to stand for 15 minutes at room temperature before their absorbance was measured in jenway electronic spectrophotometer at wavelength of 420nm. Measurement was given with the blank at zero.

Phosphorus content was given by the formular

$$Pmg/100g = \frac{100}{W} \times \frac{Au}{As} \times \frac{C \times Vf}{Va}$$

Where W = weight of sample analyzed
Au = Absorbance of test sample
As = Absorbance of standard solution
Vf = Total volume of filtrate
Va = Volume of filtrate analyzed

RESULTS

The tables below showed the phytochemical screening results of the leaves of the two *Aloe* species investigated.
investigated.

Table 1: Phytochemical Composition of the Leaves of the Two *Aloe* Species Investigated

Phytochemical	*Aloe Barbadensis* (Mg/100g)	*Aloe Chinensis* (Mg/100g)	P-value
Tannin	1.28 ± 0.000	1.36 ± 0.007	0.0424
Hydrogencyanin	0.76 ± 0.007	0.68 ± 0.000	0.0424
Saponin	1.42 ± 0.000	1.36 ± 0.014	0.1051
Flavonoid	0.65 ± 0.007	0.72 ± 0.000	0.0424
Phenol	0.06 ± 0.007	0.06 ± 0.000	0.5000
Sterol	0.06 ± 0.007	0.02 ± 0.000	0.0900
Alkaloid	0.64 ± 0.007	0.73 ± 0.000	0.0431
Terpenoid	-	-	-

Values are means ± SD of double analysis

Table 2: Mineral Composition of the two *Aloe* species investigated

Minerals	*Aloe barbadensis* (%)	*Aloe Chinensis* (%)	P-value
Sodium	0.22 ± 0.000	0.29 ± 0.013	0.087
Phosphorus	0.34 ± 0.000	0.32 ± 0.007	0.126
Potassium	1.24 ± 0.000	0.31 ± 0.014	0.090
Magnesium	0.24 ± 0.114	0.32 ± 0.000	0.790
Calcium	0.21 ± 0.03	0.23 ± 0.000	0.012

Values are means ± SD of double analysis

Table 3: Vitamin Content of the two *Aloe* species investigated

Vitamin	*Aloe barbadensis* (%)	*Aloe Chinensis* (%)	P-value
Riboflavin	0.03 ± 0.001	0.03 ± 0.003	0.270
Vitamin C	13.85 ± 0.007	15.70 ± 0.000	0.002
Thiamin	0.03 ± 0.001	0.031 ± 0.002	0.107
Niacin	12.76 ± 0.05	14.61 ± 0.000	0.015

Values are means ± SD of double analysis

DISCUSSION

The result showed that the leaves of *Aloe barbadensis* and *Aloe chinensis* contain the same phytochemicals, vitamins and minerals but in different proportions. Just like the phytochemical screening experiment by Arunkumar and Muthuselvam (2009) where they observed that the juice of *Aloe* leaves contain Tannin, Saponin and flavoids although on the contrary, positive result for terpernoid contant and negative for steroids were observed. On the other hand, Davis *et al* (1994), concurred with the result that *Aloe* species contain sterols. Similarly, Bruneton (1995) observed that *Aloe* species contain sterol and tannin.

Statistical analysis using the ANOVA showed that the proportion of tannin, flavonoids and alkaloid were significantly ($P<0.05$) higher in *Aloe chinensis* than in *Aloe barbadensis* but proportions of hydrogen cyanin, saponin and sterol were significantly higher in *Aloe barbadensis* than *Aloe chinensis.*

Tannins according to Okwu (2004) are organic substances of diverse composition with pronounced astringent properties that hastens the healing of wounds and inflamed mucous membrane. They also inhibit pathogenic fungi (Gills, 1992) and so consumers of spoted *Aloe (Aloe chinensis*) may enjoy more, the benefits of tannin.

Flavonoids, also significantly higher in *Aloe chinensis* represents the most common and widely distributed group of plant phenolics and prevents oxidative cell damage, have strong anti-cancer activity and protects against all stages of carcinogenesis (Del-Rio *et al*, 1997). Flavonoids according to Okwu (2001) provide anti-inflamatory action and so, *Aloe chinensis* and *Aloe barbadensis* can be used in the treatment of arthritis in herbal medicine. Several studies have also shown that in addition to their antioxidant protective effect, falvonoid inhibit the initiation, promotion and progression of tumours (Urguiaga and Leighton, 2000).

Phenols found in both leaves, according to Davis (1994), reduced inflammation by up to 37% in croton oil induced oedema in mice suggesting that specific plant sterols may also contribute to the anti-inflammatory activity of *Aloe* gel.

Saponin also found to be higher in *Aloe barbadensis* are said to prevent disease invasions of plants by parasitic fungi and harmful cholesterol levels helping to explain benefits of *Aloe chinensis* and *Aloe barbadensis* for heart (Ibe, 2007). Saponins may prevent cancer by protecting DNA from damages. They are anti-viral in *in-vitro* studies and directly inhibit colon cancer.

Results in table 3 showed that vitamin C and Niacin levels in *Aloe chinensis* were higher as compared to *Aloe barbadensis* while riboflavin and thiamin proportions were at equal levels. Yan (2009) stated that the leaves of *Aloe* species contain a variety of vitamins and minerals which can promote the metabolism of protein, sugar and fats; regulate the physiological functions and maintain the osmotic pressure and acid-base balance. Vitamins in *Aloe* species are conductive to the skin care and can enhance immune functions of blood fluid. They can be helpful to the anti-cancer drugs to enhance their ability against bacterial and viral tumour or dangerous cells. Niacin is active in preventing the disease pellagra while a deficiency of thiamin in the diet is the cause of the disease beri-beri (Hunt *et al*, 1980). A deficiency of riboflavin does not lead to any specific and identifiable disease and one is apt therefore to underestimate the importance. The symptoms are inflammation of the tongue, lesions at the eyes and lips, congestion of conjuctival blood vessel (Taylor, 1972).

CONCLUSION

The outcome of this investigation has elucidated the nutritive composition of the two *Aloe* species as good medicinal plants. They can serve as potent nutritive food supplements and drugs.

Although the composition levels of most of the phytochemicals and vitamins are higher in *Aloe chinenisis*, both leaves can still serve the same purpose notwithstanding. The investigation further revealed that there is a variation in terms of phytochemicals, minerals and vitamins. This is an indication that all *Aloe* species, though may contain the same chemicals, have different proportions of these chemicals.

RECOMMENDATION

Since all the phytochemicals, vitamins and minerals tested for were present in both species but at significantly different proportions, we recommend that further studies should be made to investigate if the age of leaves and age of plant as a whole has any influence on these compositions.

Also, more investigations should be made on possible application of these *Aloe* species in agriculture

probably in fighting diseases and pathogens of plants.

Furthermore, the type of research investigation should be extended to all the species of *Aloe* so as to provide a comparative and comprehensive table of their chemical compositions in order to advise producers of *Aloe* species the particular species with maximum economic advantage.

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