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ORIGINAL RESEARCH ARTICLE

Qualitative and Quantitative Phytochemical Analysis of *Aloe barbadensis* Miller Leaf Extracts

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ABSTRACT
The medicinal, folkloric, and other uses of aloe vera cannot be over-emphasized. In this study, we qualitatively and quantitatively analysed the phytochemical composition of aloe vera. Fresh leaves of aloe vera were extracted by percolation method using three different solvents: ethanol, diethyl ether, and distilled water. The extracts labeled as sample A, B, and C were analysed for important phytoconstituents using conventional qualitative methods while for the quantitative determination fresh leaves of the aloe vera were dried at room temperature and then crushed into powder. The dried powdered extract was then further used for the quantitative determination. The results of the qualitative analysis showed that alkaloids, flavonoids, glycosides, cardiac glycosides, saponins, saponin glycosides, and tannins were detected, while steroids, balsam, anthraquinones, and volatile oil were not detected. The quantitative analysis indicated a high concentration of alkaloids (31.067 g/100 g), tannins (25.66 g/100 g), and saponins (10.67 g/100g) while glycosides (0.060 g/100 g) had the least concentration. The result indicates the potential health and cosmetic benefits of aloe vera as well as its potential benefits in the food industries.

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INTRODUCTION

Aloe barbadensis Miller (Aloe vera) gets its name from the Arabic word '*alloeh*', which means a lustrous bitter substance, and 'vera', word which means 'true' in Latin. The Aloe vera plant belongs to Liliaceae family and is considered one of the oldest and most commonly used medicinal plants. It was historically used as a laxative or a stomachic in the form of juice extract and as a healing agent of burns skin ailments, and wounds for more than 20 centuries (Mukherjee *et al*., 2013).

Aloe barbadensis Miller plant shows a high water content, ranging from 99% to 99.5%, while the 0.5–1.0% solid material contains over 200 different potentially active compounds, including enzymes, minerals, vitamins, simple and complex polysaccharides, phenolic compounds, and organic acids (Misir *et al*., 2014).

The chemistry of the aloe vera has been studied for many years. Although for the analysis of the phytochemicals in aloe vera, different methods such as fluoro-photometry, Gas chromatography – mass spectrometry (GC-MS), thin layer chromatography, size exclusion chromatography, High-performance liquid chromatography (HPLC), Liquid chromatography–mass spectrometry (LC/MS), atomic-absorption spectrometry, counter current chromatography, capillary electrophoresis and micellar electrokinetic chromatography have been adopted; the GC-MS (Salisu and Shema, 2020) and HPLC (Mukherjee *et al*., 2013) methods has been widely applied to analyse the components in aloe vera. Chemical analysis reveals that Aloe vera contains various carbohydrate polymers, notably glucoman-

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nans, along with a range of other organic and inorganic components (Mukherjee *et al*., 2013). Although aloe vera is not well known when its medical applications were first discovered, it has a long association with folkloric application. Three preparations of the aloe vera used mainly in medicine are quite different in their chemical composition and therapeutic properties, aloe vera latex; aloe vera gel; and aloe whole leaf (aloe extract). Aloe vera latex is used for its laxative effect; aloe vera gel is used for skin ailments, such as wound healing, psoriasis, genital herpes, and by oral administration in diabetics and hyperlipidaemic patients and to heal gastric ulcers; and aloe vera extract is potentially useful for cancer and AIDS. It has been investigated that the aloe vera gel extract has various pharmacological properties such as promoting and healing wounds and burns, in addition to having anti-inflammatory, antifungal properties, hypoglycemic and gastro-protective properties (Reynolds and Dweck, 2019).

Aloe vera has been used for centuries in health, beauty, medicine, and skin care (Gupta *et al*., 2012). Other therapeutic benefits of aloe vera include treating teeth and gums, treating constipation, antioxidant and antibacterial properties, and protecting against radiation (Maan *et al*., 2018). Aloe vera is also used commercially in yogurt, beverages, and some desserts (Mukherjee *et al*., 2013). Today, Aloe vera is used as a traditional remedy for a variety of conditions and is found in dietary supplements and food products. Aloe vera gel can also be found in different skin products, including lotions and sun blocks (NCCAM, 2012).

Aloe vera is praised for its healing properties, but the scientific bases for these actions have not been adequately ascertained. Therefore, this study was conducted to fill the above knowledge gap by performing a phytochemical analysis of the plant to identify and quantify its phytochemical constituents. This may be helpful for researchers in the field to identify the potential health and cosmetic benefits of aloe vera as well as its potential benefits in the food industries.

MATERIALS AND METHODS

Sample collection

Aloe vera plants were collected from the Federal University Dutsinma Biological Garden, Katsina. This plant has been identified and authenticated at the herbarium of the Faculty of Biological Sciences of the Federal University Dutsinma in Katsina. The plants were dried at room temperature and then used further for extraction.

Extraction of plant material for qualitative analysis

For the qualitative analysis, a fresh sample of aloe vera leaves was cut into small pieces. The leaves were crushed with a pestle and mortar. Fifty grams (50g) each was weighed into three different Erlenmeyer flasks (250mL each) and labeled as samples A, B, and C. Hundred milliliter (100 mL) of diethyl ether was added to sample A, 100 mL of ethanol was added to sample B, and a 100 mL of distilled water was added to sample C, and the three samples were corked. The leaves were soaked in these solvents for 24 hours, decanted and heated to concentrate the plant extract. The aqueous plant extract was then filtered using a fine muslin cloth and the solutions were extracted. The extract concentration of the resulting filtrate was 100%. The standard methods for the identification of the components of the samples were adopted (Maan *et al*., 2018).

Extraction of plant material for quantitative analysis

For the quantitative determination, fresh leaves of the Aloe vera plant were collected and dried at room temperature and then crushed into powder using laboratory sterile mortar and pestle. The dried powdered extract was then further used in the determination of the quantity of the phytochemicals present in the plant. This method was reported in a study conducted by Usman *et al*., (2020).

The Qualitative Phytochemical Screening of Aloe vera Leaf Extracts

Determination of alkaloids

About 2 mL of each extract sample was stirred with 2 mL of 10% hydrochloric acid. A 1 mL portion was treated with a few drops of Wagner's reagent and a second 1 mL portion of the extract was similarly treated with Meyer's reagent. Presence of alkaloid was confirmed by HPLC method (Mukherjee *et al*., 2013).

Determination of flavonoids

Sodium hydroxide (NaOH) solution (10%) was added to 3 mL of filtrate of the each extract sample. The yellow color observed indicates the presence of flavonoid compounds (Bibi *et al*., 2021).

Determination of tannins
A solution of 0.1% ferric chloride ($FeCl_3$) was added in drops into 5 mL of extract after it has been boiled with 20 mL of $CHCl_3$, the appearance of brownish colour shows the presence of tannin (Bibi *et al.*, 2021).

Determination of saponins
About 5 mL of the aqueous extract was taken and heated with 5 mL of distilled water and filtered. Few drops of olive oil were added to 10 mL of the filtrate. Emulsion formation was taken as an indication of the presence of saponins (Bibi *et al.*, 2021).

Determination of glycosides
A two milliliter (2 mL) solution of 50% H_2SO_4 was added to 5 mL of the extract in a test tube. The mixture was heated in boiling water for 15 minutes. It was cooled, neutralized with 10% NaOH, 5 mL of Fehling's solution was added and the mixture was brought to a boil. A brick-red precipitate was observed, indicating the presence of glycosides (Bibi *et al.*, 2021).

Determination of cardiac glycoside
A five milliliter (5 mL) of each aqueous extracts was treated with 2 mL of glacial acetic acid containing a drop of $FeCl_3$ solution. This was under layered with 1 mL of concentrated H_2SO_4. A reddish-brown ring at the interface indicated a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Bibi *et al.*, 2021).

Determination of saponin glycosides
In this test, 2.5 mL Fehling's solutions A and B were added to 2.5 mL of the extract. A blue-green precipitate indicates the presence of saponin glycosides (Bibi *et al.*, 2021).

Determination of steroids
In this test 2 mL of acetic anhydride and 2 mL of sulphuric acid were added to 5 mL extract from each sample. The sudden colour change from violet to blue at interface indicates the presence steroids (Bibi *et al.*, 2021).

Determination of anthraquinones
Ten milliliter of benzene and 5 mL of 10% ammonia solution was added to 0.5g of the extract. The mixture was shaken and the presence of pink, red or violent color in the bottom indicates the presence of anthraquinones (Bibi *et al.*, 2021).

Determination of balsams
The extract was mixed with an equal volume of 90% ethanol. Two drops of alcoholic ferric chloride solution were added to the mixture. A dark green color indicates the presence of balsams (Bibi *et al.*, 2021).

Determination of volatile oils
A dilute hydrochloric acid (HCL) was mixed with 1 mL of the extract. Formation of a white precipitate indicates the presence of volatile oils (Bibi *et al.*, 2021).

The Quantitative Phytochemical Screening of Aloe vera Leaf Extracts

Alkaloid Determination
Alkaloid content was determined by following the method used by Luyang *et al.*, (2015). Five grams of powdered plant extract were added to 100 mL of methanol water (1:1). The mixture and solvent were evaporated. The resultant residue was mixed with 20 mL of 0.0025M H_2SO_4 and partitioned with ether to remove unwanted materials. A strong base (NH_3) was added to the aqueous fraction solution, followed by extraction with excess chloroform to obtain the alkaloid fraction or separated by filtration. The chloroform extractions were repeated several times and the extracts were concentrated to dryness. The alkaloids were weighed and the percentages were calculated relative to the initial weight of the powder.

$$\%\ Alkaloid\ = \frac{weight\ of\ alkaloid\ residue}{weight\ of\ sample} \times 100$$

Determination of tannins
Tannins content was estimated by following the method used by Muhammad *et al.*, (2012). A powdered sample (0.1) of the plant extract was placed in a 100cm^3 Erlenmeyer flask and a 50cm^3 volumetric flask. The residue was washed several times and a mixed solution of distilled water containing 0, 1, 2, 3, 4 and 5cm^3 of standard tannic acid and 10cm^3 of Na_2CO_3 solution was added and made up to volume with distilled water. The optical density was measured at 720 nm after allowing the flask to stand for 10 minutes. A calibration curve extrapolating the tannic acid concentration in the samples was plotted. A blank sample was prepared and read at the same wavelength. A standard was prepared using 0-5 μg/mL tannin acid and measured. Tannin content was calculated.

$$\%\ Tannin\ = \frac{absorbance\ of\ sample \times avarage\ gradient\ factor \times dillution\ factor}{weight\ of\ sample \times 10{,}000} \times 100$$

Determination of saponins

Saponins content was estimated by following the method reported by Chinelo *et al.*, (2013). Five grams of powdered plant extract was added to a 250 mL flask containing 30 mL of 50% alcohol. The mixture was refluxed for 30 minutes and immediately filtered hot through coarse filter paper. Two grams (2g) of charcoal was added. The contents were boiled hot and filtered. The extract was cooled (some saponins could be isolated) and an equal volume of acetone was added to complete the saponin precipitation. The isolated saponins were collected by decantation, dissolved in a minimal amount of boiling 95% alcohol and hot filtered to remove insoluble material. The saponin precipitated when the filtrate was cooled to room temperature. The separated saponins were collected by decantation, suspended in about 2 mL of alcohol and filtered. The filter paper was immediately transferred to a desiccator containing anhydrous calcium chloride to dry the saponin. They were weighed for the extract used.

$$\%\ Saponin\ = \frac{Weight\ of\ dried\ extract}{weight\ of\ sample} \times 100$$

Determination of glycosides

Glycosides content was determined by following the method adopted by Shamsu and Abubakar (2016). One gram (1g) of the extract was added into 10 mL of 70% alcohol and the mixture was filtered. From the filtrate, 8 mL of the mixture was added to 8 mL of 12.5% lead acetate to precipitate resins, tannins and pigments. The mixture was shaken well, made up to another volume of 100 mL with distilled water and then filtered. The filtrate (50 mL) was pipetted into another 100 mL volumetric flask and 8 mL of 4.7% disodium hydrogen phosphate (Na_2HPO_4) solution (to precipitate excess lead) was added. The mixture was made up to volume with distilled water and mixed. The mixture was then filtered through Whatman filter paper. 10 mL of Baljet's reagent was added to 10 mL of the purified filtrate. A 10 mL blank of distilled water was also added to 10 mL of Baljet Reagent. The two were left undisturbed for 1 hour (maximum coloring time). Color intensity was read at 495nm against a blank (20 mL distilled water) using a spectrophotometer. The color remained stable for several hours. The percentage of total glycosides in digitoxins was simply calculated using E^1cm 1% of given digitoxins (which is equivalent to 170).

$$\%\ Glycosides\ = \frac{A}{17} \times 100 \times g\%$$

Where A = Absorbance of the color at 495 nm.

RESULTS

The Qualitative Phytochemical Screening of Aloe vera Leaf Extracts

The samples A, B, and C revealed the presence of medicinally active phytochemical constituents like alkaloids, tannins, saponins, glycosides, cardiac glycosides, saponin glycosides and flavonoids. For the investigations of each phytochemical, tests were carried out with different solvents to confirm the presence or absence of the phytochemicals. The results of this analysis are presented in Table 1, 2, and 3 below.

Phytochemical components of the diethyl ether extract (Sample A)

The result for the phytochemical screening of the diethyl ether extract detected large amounts of alkaloids and tannins, moderate amounts of saponins, and trace amounts of flavonoids, glycosides, saponin glycosides, and cardiac glycosides. However, steroids, balsams, anthraquinones and volatile oils were not detected.

Table 1: Result for the phytochemical screening of the diethyl ether extract

S/N	Phytoconstituents	Qualitative value
1.	Alkaloids	+ + +
2.	Flavonoids	+
3.	Saponins	+ +
4.	Saponin glycosides	+
5.	Glycosides	+
6.	Tannins	+ + +
7.	Cardiac glycosides	+
8.	Anthraquinones	ND
9.	Balsams	ND
10.	Volatile oils	ND
11.	Steroids	ND

Key: + + +Detected in large amount, + +Moderate amount, +Trace amount, ND Not detected

Phytochemical components of the ethanol extract (Sample B)

Phytochemical screening of the ethanol extract detected large amounts of alkaloids, moderate amounts of tannins, and trace amounts of saponins, flavonoids, glycosides, saponin glycosides, and cardiac glycosides. However, no steroids, balsams, anthraquinones or volatile oils were detected.

Table 2: Result for the phytochemical screening of the ethanol extract

S/N	Phytoconstituents	Qualitative value
1	Alkaloids	+++
2	Flavonoids	+
3	Saponins	+
4	Saponin glycosides	+
5	Glycosides	+
6	Tannins	++
7	Cardiac glycosides	+
8	Anthraquinones	ND
9	Balsams	ND
10	Volatile oils	ND
11	Steroids	ND

Key: +++Detected in large amount, ++Moderate amount, +Trace amount, ND Not detected

Phytochemical components of the distilled water extract (Sample C)

Phytochemical screening of the aqueous extract detected moderate amounts alkaloids, and trace amounts of saponins, tannins, flavonoids, glycosides, saponin glycosides, and cardiac glycosides. However, no steroids, balsams, anthraquinones or volatile oils were detected

Table 3: Result for the phytochemical screening of the distilled water extract

S/N	Phytoconstituents	Qualitative value
1	Alkaloids	++
2	Flavonoids	+
3	Saponins	+
4	Saponin glycosides	+
5	Glycosides	+
6	Tannins	+
7	Cardiac glycosides	+
8	Anthraquinones	ND
9	Balsams	ND
10	Volatile oils	ND
11	Steroids	ND

Key: +++Detected in large amount, ++Moderate amount, +Trace amount, ND Not detected

The Quantitative Phytochemical Screening of Aloe vera Leaf Extracts

The results for the quantitative phytochemical analysis were presented in Table 4. The results showed a high concentration of alkaloids (31.067 g/100 g), a high concentration of tannins (25.66 g/100 g), a moderate concentration of saponins (10.67 g/100 g) and a low concentration of glycosides (0.060 g/100 g). Other phytochemicals such as flavonoids, saponin glycosides, and cardiac glycosides were detected in a trace amount.

Table 4: Results of the quantitative phytochemical constituent of aloe vera leaf extracts

S/N	Phytoconstituents	Quantitative value (g/100 g)
1.	Glycosides	0.060
2.	Saponins	10.67
3.	Tannins	25.66
4.	Alkaloids	31.067

DISCUSSION

The presence of alkaloid, saponins, glycosides, cardiac glycosides, saponin glycoside, tannins and flavonoids coupled with the absence of steroid, balsam, anthraquinones and volatile oils shown in the qualitative phytochemical screening conducted in this study was similar when compared with the result of other studies conducted using the same aqueous extract (Andama *et al.*, 2014). However, after the qualitative analysis, it was discovered that the phytochemicals present in all the three extracts were almost the same. It is based on that, a fresh, dried powdered extract was made further and used in the determination of the quantity of the phytochemicals present in the plant.

Results of the current studies unveil the vast medicinal, nutritional, therapeutic and cosmetic potential of aloe vera. It also shows that aloe vera can be a potential source of useful compounds that can be used as lead to synthesize new antimicrobial drugs. The presence of phytochemicals in aloe vera justifies the traditional medicinal uses of this plant by the local communities (Usman *et al.*, 2020).

Alkaloids (31.067 g/100 g), a secondary metabolite observed in the leaf extract of Aloe vera has the biological property of toxicity against cells of foreign organisms. Its activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Usman *et al.*, 2020). Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002).

Saponins (10.67 g/100 g) present in Aloe vera extracts have proved the usefulness of this plant in managing inflammation which revealed the inhibitory effect of saponins on inflamed cells (Usman *et al.*, 2020). The quantity of alkaloid and Saponin found in this study is relatively high compared with values obtained from

other researches (Usman *et al.*, 2020; Anon, 2018).

Glycosides (0.060 g/100 g) and Tannins (25.66 g/100 g) are believed to have antimicrobial potential and they also play a great role in curing a variety of diseases such as heart arrhythmia, anti-inflammatory effect and heart congestion. Both compounds found in Aloe vera leaf extract like Saponins, Alkaloids, Tannins and Glycosides are known to be biologically active and therefore aid in the antimicrobial activities of the plant (Igbinosa *et al.*, 2011).

CONCLUSION

Findings from this study showed that aloe vera contained many secondary metabolites. The study further revealed that more than 60% of the phytochemicals of aloe vera leaves are alkaloids, tannins and saponins, therefore justify their widespread use in traditional medicine. Further studies on the isolation and structural elucidation of the active components, toxicological and nutritional studies of the plant extract are recommended.

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