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Free Radical Scavenging Activity of Alkaloid and Flavonoid Fractions of *Kalanchoe pinnata* Leaves

Taofik Olatunde Uthman*, Rabiat Yetunde Salman, Ismail Abubakar Lamorde

Department of Biochemistry and Chemistry, Faculty of Natural and Applied Sciences, Nile University of Nigeria, Abuja, Nigeria

Email address:

taofik.sunmonu@nileuniversity.edu.ng (Taofik Olatunde Uthman)

*Corresponding author

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Abstract: *Kalanchoe pinnata* is an important medicinal plant in Nigeria with numerous pharmacological activities. The present study investigates the antioxidant activity of alkaloid and flavonoid fractions of *Kalanchoe pinnata* leaves. Proximate and phytochemical analyses of the plant were conducted followed by evaluation of the antioxidant activity of the alkaloid and flavonoid fractions using 1,1-diphenyl-2-picryl-hydrazyl, 2,2'-azinobis(3-ethylbenzothiazolline)-6-sulfonic acid, phosphomolybdenum, cupric ion reducing antioxidant capacity, ferric reducing antioxidant power and nitric oxide inhibition assays. The proximate composition of *Kalanchoe pinnata* leaves confirmed its edibility particularly in respect of moisture (86.73%) and carbohydrate (7.05%) content. The results revealed the presence of phenol (12.50 mg TAE/g), flavonoid (18.10 mg QE/g), flavanol (17.70 mg CE/g), alkaloid (26.98 mg/g), tannin (4.03 mg/g) and saponin (3.69 mg/g). Antioxidant assays indicated that both fractions exhibited antioxidant activity that is comparable to ascorbic acid and rutin with nitric oxide having the highest scavenging potential of 98.97% inhibition. GC-MS analysis of the alkaloid and flavonoid fractions revealed the presence of 18 and 22 compounds respectively. The antioxidant activity of *Kalanchoe pinnata* may be attributed to 5-hydroxymethyl furfural and oleic acid detected in the alkaloid and flavonoid fractions respectively. Overall, the data generated form this study portrayed *Kalanchoe pinnata* as a potential antioxidant agent.

Keywords: Free Radicals, Oxidative Stress, Alkaloid, Flavonoid, Antioxidants

1. Introduction

Over the years, medicinal plants have been employed for the treatment of a variety of ailments. These plants have been reported to contain several constituents that bring about pharmacologic actions [1]. Through this approach, the importance of medicinal plants in drug development was identified and has led the way for researchers to explore new medications from plant sources. In synthesizing different drugs, medicinal plants serve as raw materials for the extraction of active ingredients, thereby serving as a fundamental lead for modern drug development [2]. Due to ease of accessibility and relative abundance, medicinal plants have a favorable future with their therapeutic potential yet to be fully investigated. Furthermore, the folkloric application of medicinal plants in the management of diseases has prompted researchers to explore new medications from plant sources [3].

The plant of interest in the present study is *Kalanchoe pinnata.* It is a member of the *Crassulaceae* family with many common names including canterbury bells, love plant, life plant and miracle leaf. In the Caribbean region specifically, the plant is commonly known as a "Master Herb" or a "Cure for all" by a large community of herbal practitioners. In Nigeria, *Kalanchoe pinnata* is known as *ewe abamoda* or *odundun*, *harfifi*, *odaa opue*, and *danweshin* in Yoruba, Hausa, Igbo and Edo speaking people respectively. All around the world, various pharmacological activities have been reported for *Kalanchoe pinnata.* In Nigeria, the plant is used for the treatment of cough, asthma, bronchitis, wounds, insect bites, ulcers, burns, and diarrhea. In India, it is used to treat epilepsy, cholera, asthma, chest colds, conjunctivitis, fever, pile, constipation, menstrual disorder and antihemintic

[4-6]. Its wound healing, analgesic, anti-inflammatory and hemostatic effects have been exploited in Brazil [7]. In Ayurvedic medicines, the plant is employed for treating conditions such as nausea, hematemesis, hemorrhoids and ophthalmia [8].

Several ailments affecting humans have been attributed to free radicals in the body [9]. This factor has heightened the search for potent antioxidant agents from natural products. Most times, crude and whole extracts of plants are usually consumed regardless of the constituents. This may further impose serious health risks to consumers especially for plants that contain toxic substances. Therefore, there is a need to characterize medicinal plants with a view to identifying and isolating relevant phytochemicals responsible for the treatment of specific ailments. Hence, the present study is designed to investigate the *in vitro* antioxidant properties of the alkaloid and flavonoid fractions of *Kalanchoe pinnata* leaves.

2. Materials and Methods

2.1. Chemicals and Reagents

Folin-Coicalteu reagent, sodium carbonate, aluminum chloride, ethanol, sodium acetate, methanol, hydrochloric acid, acetic acid, sulfuric acid, sodium phosphate, ammonium molybdate, cupric chloride, neocuproine, ammonium acetate, rutin, potassium persulfate, 2,2'-azinobis(3-ethylbenzothiazolline)-6-sulfonic acid (ABTS), 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), n-hexane, ethanol, ammonium hydroxide, ethyl acetate, petroleum ether, sodium hydroxide. All other chemicals and reagents were of analytical grades.

2.2. Plant Collection and Authentication

Fresh leaves of *Kalanchoe pinnata* were collected from a farm settlement in Abuja, Nigeria. Identification and authentication of the plant were done at National Institute for Pharmaceutical Research and Development (NIPRD) Abuja. A voucher specimen with number NIPRD.H.2891 was prepared and kept in the Herbarium for future reference.

2.3. Determination of Proximate Composition of Kalanchoe pinnata Leaves

The proximate composition of *Kalanchoe pinnata leaves* was done according to standard methods described in AOAC [10]. The nutrients evaluated were moisture, ash, protein, fat, fiber and carbohydrates.

2.4. Preparation of Plant Material

Fresh *Kalanchoe pinnata* leaves were thoroughly cleansed under running tap water in the laboratory after which they were dried in an oven for 3 hours at 30°C. The dried leaves were then milled using a laboratory blender and the resulting powder was stored in an air-tight container and kept in a refrigerator for the experiments.

2.5. Quantitative Phytochemical Analysis of Kalanchoe pinnata Leaves

The phytochemicals present in *Kalanchoe pinnata* leaves were quantified using standard methods as follows; total phenol [11], total flavonoid [12], alkaloid [13], total flavanol [14], tannin [15], saponin [16].

2.6. Extraction of Alkaloid Fraction

The alkaloid fraction of *Kalanchoe pinnata* leaves was extracted using the method of Harborne [17] with minor adjustments [18]. The powdered leaf sample (100 g) was defatted in 250 mL n-hexane for 24 hours before extraction with 100 mL 10% acetic acid in ethanol. The clear filtrate was concentrated under vacuum at 45°C (IKA Rotary evaporator) after being filtered with muslin cloth and then filter paper (Whatman No. 1). The concentrated extract was precipitated using concentrated ammonium hydroxide and the resulting precipitate was washed with dilute ammonium hydroxide to obtain the alkaloid fraction. The fraction was kept in the refrigerator for subsequent analysis.

2.7. Extraction of Flavonoid Fraction

The flavonoid fraction of *Kalanchoe pinnata* was extracted according to the method described by Chaves *et al*. [19]. The sample (100 g) was extracted using 500 mL of 80% ethanol solution and left for 24 hours before filtering (Whatman No. 1). The filtrate was concentrated in a water bath and the resulting extract was stored in a refrigerator for further analysis.

2.8. Evaluation of Antioxidant Activity of Alkaloid and Flavonoid Fractions of Kalanchoe pinnata Leaves

The antioxidant activity of alkaloid fraction of *Kalanchoe pinnata* leaves in comparison with ascorbic acid and rutin was evaluated using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay [20], 2,2'-azinobis (3-ethylbenzothiazolline)-6-sulfonic acid (ABTS) assay [21], phosphomolybdenum (PM) assay [22] and cupric ion reducing antioxidant capacity (CUPRAC) assay [23]. For the flavonoid fraction, antioxidant activity was evaluated based on DPPH assay [20], ferric reducing antioxidant power (FRAP) assay [24] and nitric oxide inhibition assay [25]. In all cases, the antioxidant activity of the flavonoid fraction was compared with ascorbic acid and rutin.

2.9. Gas Chromatography-Mass Spectrometer (GC-MS) Analysis

The GC-MS analysis of the alkaloid and flavonoid fractions was done using a QP 2010 Ultra instrument under computer control at 70 eV. The components in the fractions were identified by comparing their retention indices and mass spectra fragmentation patterns to those in the computer library as well as published literature NIST.LIB and WILEY.LIB. The detected components in the sample were matched using library sources. The National Institute of

Standards and Techniques database, which contains over 8,000,000 patterns, was used to interpret the mass spectrum of the GC-MS. Comparing the average peak area of each component to the total areas, the relative percentage quantity of each component was calculated.

2.10. Statistical Analysis of Data

The data presented are average of three replicates ± standard deviation. Multiway analysis of variance was used to analyze the data, and Tukey's multiple range test was used to compare mean values using SPSS version 20.0.

3. Results

3.1. Proximate Composition of Kalanchoe pinnata Leaves

The proximate composition of *Kalanchoe pinnata* leaves is presented in Table 1. Moisture had the highest concentration of 86.73%, followed by carbohydrate 7.05%, crude fiber 2.21%, crude protein 1.87%, ash content 1.13%, and finally crude fat 1.01%.

Table 1. *Proximate Composition of Kalanchoe pinnata leaves.*

Nutrients	Concentration (%)
Moisture	86.73 ± 1.16
Fat	1.01 ± 0.04
Ash	1.13 ± 0.03
Protein	1.87 ± 0.01
Fibre	2.21 ±0.04
Carbohydrate	7.05 ± 0.01

Results are expressed in mean ± standard deviation for 3 determinations.

3.2. Phytochemical Constituents of Kalanchoe pinnata Leaves

Table 2 shows the results of phytochemical analysis carried out on *Kalanchoe pinnata* leaves. The data revealed that alkaloid (26.98 mg/g) has the highest concentration, followed by flavonoid (18.10 mg QE/g), flavanol (17.70 mg CE/g), phenol (12.50 mg TAE/g) and tannin (4.03 mg/g). Saponin had the lowest concentration of 3.69 mg/g.

Table 2. *Phytochemicals present in Kalanchoe Pinnata leaves.*

Phytochemicals	Concentration
Alkaloid	26.98 ± 1.21 mg/g
Flavonoid	18.10 ± 1.02 mg QE/g
Flavanol	17.70 ± 1.01 mg CE/g
Phenol	12.50 ± 0.83 mg TAE/g
Tannin	4.03 ± 0.18 mg/g
Saponin	3.69 ± 0.21 mg/g

Results are expressed in mean ± standard deviation for 3 determinations.

3.3. Antioxidant Activity of Alkaloid Fraction of Kalanchoe pinnata Leaves

The antioxidant activity of alkaloid fraction of Kalanchoe pinnata leaves in comparison with ascorbic acid and rutin are presented in Tables 3 to 6 for DPPH, ABTS, PM and CUPRAC assays respectively. The results indicate that alkaloid fraction exhibited antioxidant activity in a concentration dependent manner. CUPRAC assay produced the most effective result followed by ABTS, PM and DPPH. At 0.2 mg/ml concentration, the alkaloid fraction reduced cupric ion by 85.90% and inhibited ABTS, phosphomolybdenum and DPPH radicals by 82.90%, 79.83% and 74.72% respectively.

Table 3. *Percentage inhibition of DPPH radical by alkaloid fraction of Kalanchoe pinnata leaves in comparison with ascorbic acid and rutin.*

Concentration (mg/mL)	Samples/Percentage inhibition		
	Alkaloid	Ascorbic acid	Rutin
0.025	62.23 ± 1.01^{a}	77.34 ± 0.93^{b}	83.30 ± 1.31^{c}
0.05	67.30 ± 1.34^{a}	78.06 ± 0.93^{b}	85.00 ± 1.08^{c}
0.1	68.20 ± 1.01^{a}	80.25 ± 1.73^{b}	87.00 ± 0.98^{c}
0.2	74.72 ± 2.01^{a}	87.50 ± 1.62^{b}	89.00 ± 1.11^{b}

Results are presented as mean ± standard deviation for three readings. Values with different superscripts for each concentration are different significantly ($p<0.05$).

Table 4. *Percentage inhibition of ABTS radical by alkaloid fraction of Kalanchoe pinnata leaves in comparison with ascorbic acid and rutin.*

Concentration (mg/mL)	Samples/Percentage inhibition		
	Alkaloid	Ascorbic acid	Rutin
0.025	72.00 ± 0.98^{a}	81.10 ± 0.91^{b}	83.40 ± 0.87^{b}
0.05	80.75 ± 1.58^{a}	82.14 ± 0.92^{a}	85.67 ± 1.45^{a}
0.1	81.40 ± 1.51^{a}	83.15 ± 1.15^{a}	86.81 ± 2.41^{a}
0.2	82.90 ± 3.83^{a}	86.45 ± 2.02^{a}	89.25 ± 3.85^{a}

Results are presented as mean ± standard deviation for three readings. Values with different superscripts for each concentration are different significantly ($p<0.05$).

Table 5. *Percentage inhibition of phosphomolybdenum by alkaloid fraction of Kalanchoe pinnata leaves in comparison with ascorbic acid and rutin.*

Concentration (mg/mL)	Samples/Percentage inhibition		
	Alkaloid	Ascorbic acid	Rutin
0.025	68.07 ± 1.08^{a}	62.60 ± 0.41^{b}	62.00 ± 0.87^{b}
0.05	70.60 ± 1.18^{a}	63.14 ± 1.22^{b}	63.08 ± 1.45^{b}
0.1	76.01 ± 1.11^{a}	68.33 ± 1.15^{b}	65.10 ± 2.41^{b}
0.2	79.83 ± 2.03^{a}	69.72 ± 2.20^{b}	69.10 ± 2.85^{b}

Results are presented as mean ± standard deviation for three readings. Values with different superscripts for each concentration are different significantly ($p<0.05$).

Table 6. *Cupric ion reducing antioxidant capacity of alkaloid fraction of Kalanchoe pinnata leaves in comparison with ascorbic acid and rutin.*

Concentration (mg/mL)	Samples/Percentage inhibition		
	Alkaloid	Ascorbic acid	Rutin
0.025	75.44 ± 0.89^{a}	78.09 ± 1.91^{a}	80.32 ± 1.87^{a}
0.05	79.61 ± 1.95^{a}	83.14 ± 1.92^{a}	82.67 ± 1.45^{a}
0.1	80.81 ± 1.81^{a}	85.15 ± 2.15^{a}	87.25 ± 2.81^{a}
0.2	85.90 ± 1.83^{a}	86.95 ± 2.12^{a}	89.88 ± 2.59^{a}

Results are presented as mean ± standard deviation for three readings. Values with different superscripts for each concentration are different significantly ($p<0.05$).

3.4. Antioxidant Activity of Flavonoid Fraction of Kalanchoe pinata Leaves

The DPPH radical scavenging activity of flavonoid fraction of Kalanchoe pinnata leaves is presented in Figure 1.

The result indicated that the plant exhibited significant antioxidant activity in a concentration-dependent manner. The flavonoid fraction at the highest tested concentration of 0.5 mg/mL inhibited DPPH radical by 95.36% compared to ascorbic acid and rutin standards which inhibited same radical by 89.43% and 88.34% respectively.

As illustrated in Figure 2, the transformation of Fe^{3+} to Fe^{2+} in the presence of flavonoid fraction was better than the reference compounds. Although the reducing power was higher than those of ascorbic acid and rutin, the results revealed that the reductive capability of the flavonoid fraction was concentration dependent. The highest reducing ability of 96.46% was observed at 0.5 mg/mL.

The reduction of nitric oxide by the flavonoid fraction of Kalanchoe pinnata leaves produced the most effective antioxidation result (Figure 3). The data indicated that at 0.5 mg/mL concentration, the fraction exhibited a percentage inhibition of 98.97% better than ascorbic acid and rutin which inhibited nitric oxide to the extent of 84.24% and 86.75% respectively. Significant nitric oxide inhibition was evident at all the tested concentrations when compared with the standards (ascorbic acid and rutin). The inhibition produced by flavonoid fraction occurred in a concentration dependent manner.

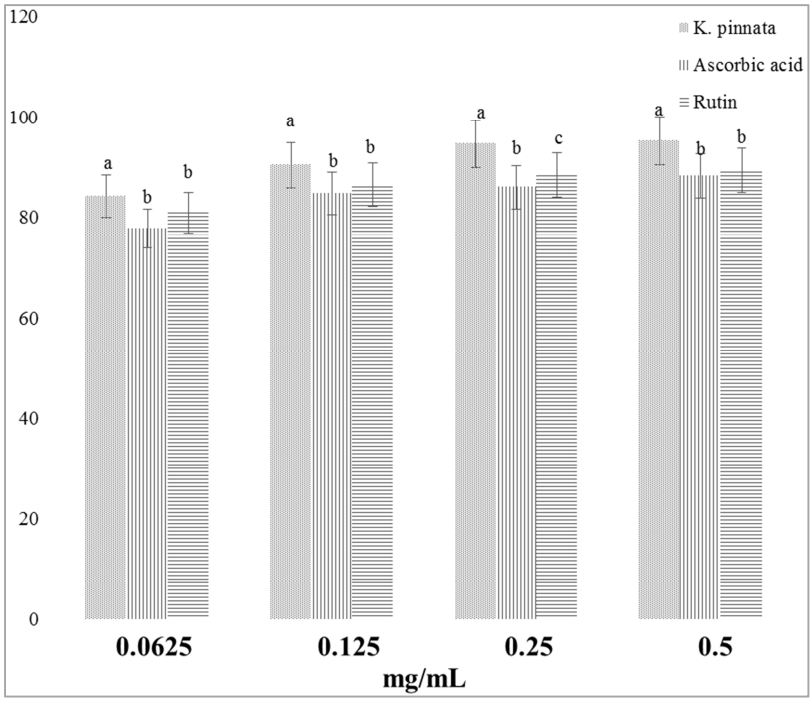

Figure 1. *Percentage inhibition of DPPH radical by flavonoid fraction of Kalanchoe pinnata leaves. Results are presented as mean ± standard deviation for three determinations. Bars with different letters for each concentration are significantly different (p<0.05).*

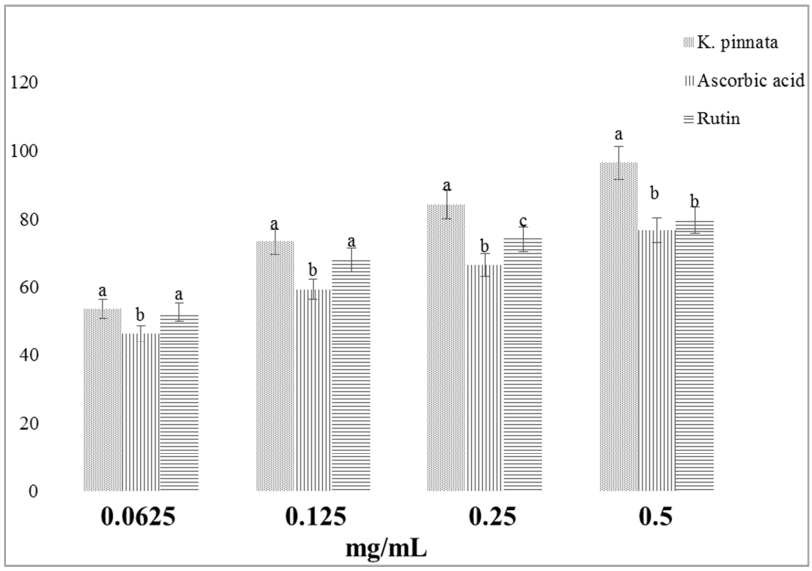

Figure 2. *Ferric reducing potential of flavonoid fraction of Kalanchoe pinnata leaves. Results are presented as mean ± standard deviation for three determinations. Bars with different letters for each concentration are significantly different (p<0.05).*

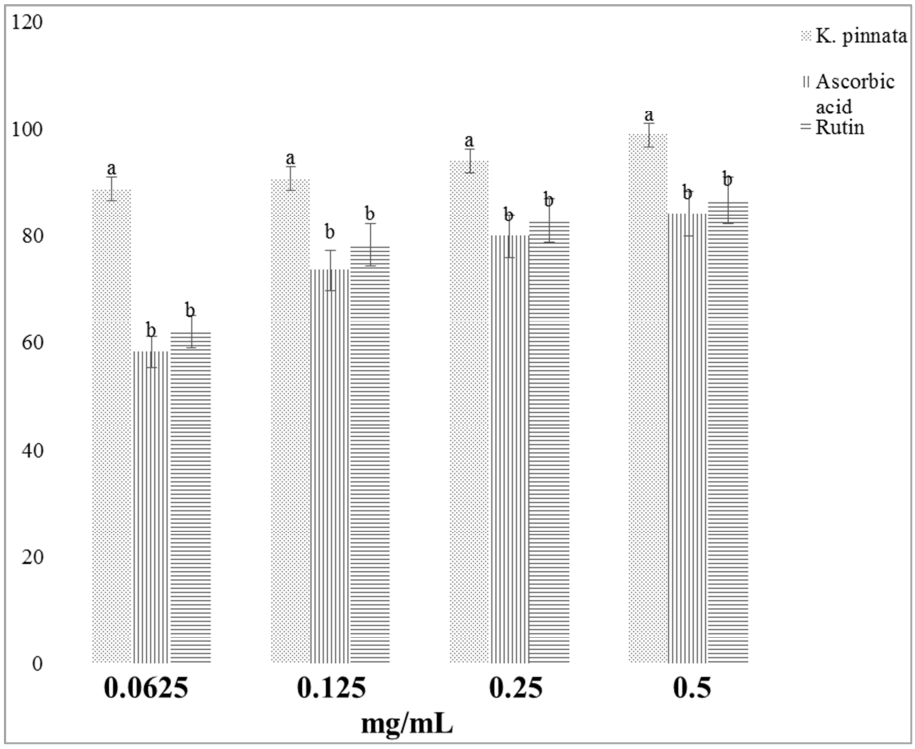

Figure 3. *Percentage inhibition of nitric oxide by flavonoid fraction of Kalanchoe pinnata leaves. Results are presented as mean ± standard deviation for three determinations. Bars with different letters for each concentration are significantly different (p<0.05).*

3.5. Chemical Composition of Alkaloid and Flavonoid Fractions of Kalanchoe pinnata Leaves Based on GC-MS Analysis

GC-MS chromatogram of alkaloid and flavonoid fractions (Figures 4 and 5) revealed the presence of 17 and 22 compounds respectively (Tables 7 and 8). From the information presented in Table for 7 for alkaloid, the highest retention time of 31.331 minutes was recorded for 2,4,6-cycloheptatrien-1-one and lowest retention time of 11.46 minutes for D-(+)-Glucose. With respect to composition, β-D-Glucopyranose had the highest concentration (16.14) followed by Benzo[h]quinoline and 1,2,3-Triazol. For flavonoid (Table 8), the highest retention time of 28.23 minutes was recorded for Pseduosarsasapogenin-5, 20-dien methyl ether and lowest retention time of 9.48 minutes from Banol phenol. Oleic acid had the highest composition of 24.18% followed by palmitic acid (13.32%) and Octadecanoic acid methyl ester (10.75).

Table 7. *Compounds identified in alkaloid fraction of Kalanchoe Pinnata leaves by GC-MS.*

S/N	Name of compound	Retention time	Area
1.	D-(+)-Glucose	11.46	1.32
2.	1H-Imidazole	11.59	0.56
3.	5-Hydroxymethylfurfural	12.289	2.94
4.	Thiophenol	18.496	1.11
5.	2-Chloro-5-tmethoxybenzimidazole	18.535	0.34
6.	Beta-D-Glucopyranose	18.785	16.14
7.	Benz[b]-1,4-oxazepine-4(5H)-thione	21.687	1.11
8.	2-(n-Propyl)oxybenzylidene acetophenone	24.387	0.43
9.	2-Methyl-7-phenylindole	24.923	0.20
10.	Ethenone	25.827	0.27
11.	Tetrasiloxane	26.328	1.30
12.	1,2,3-Triazol	28.014	15.34
13.	2-Ethylacridine	28.393	8.52
14.	Silicic acid	28.828	1.85
15.	Benzo[h]quinoline	29.721	16.09
16.	Benzene	31.125	7.77
17.	2,4,6-Cycloheptatrien-1-one	31.331	0.63

Table 8. *Compounds identified in the flavonoid fraction of Kalanchoe pinnata leaves by GC-MS.*

S/N	Name of compound	Retention time (min)	Composition (%)
1.	Banol phenol	9.48	3.62
2.	Dodecyl alcohol	10.97	1.40
3.	Phenol 2,4-di-tert-butyl-	11.49	0.79
4.	Lignocerol	14.05	1.00
5.	Palmitic acid	17.51	13.32
6.	Hexadecanoic acid	18.40	2.42
7.	Ethyl iso-allocholate	19.36	0.68
8.	Linoleic acid	19.62	6.72
9.	Oleic acid	19.73	24.18
10.	Beta Carotene	19.67	0.88
11.	Octadecanoic acid methyl ester	20.96	10.75
12.	Z,Z-8,10-Hexadecadien-1-ol	20.16	1.27
13.	6,9,12,15-Docosatetraenoic acid	20.25	1.36
14.	Oleic acid amide	20.46	4.07
15.	Oleic acid, ethyl ester	20.55	4.89
16.	Ethyl stearate	20.90	2.87
17.	Dihomo-.gamma.-linolenic acid	22.72	1.98
18.	Erucyl amide	22.82	8.28
19.	Crodamide	23.17	2.48
20.	Cinnamic acid	23.57	1.09
21.	2,6-Nonadienoic acid, 7-ethyl-9-(3-ethyl-3-methyloxiranyl)-3-methyl-, methyl ester, [2R-[2.alpha.(2E,6E),3.alpha.]]-	25.92	1.95
22.	Pseduosarsasapogenin-5,20-dien methyl ether	28.23	3.99

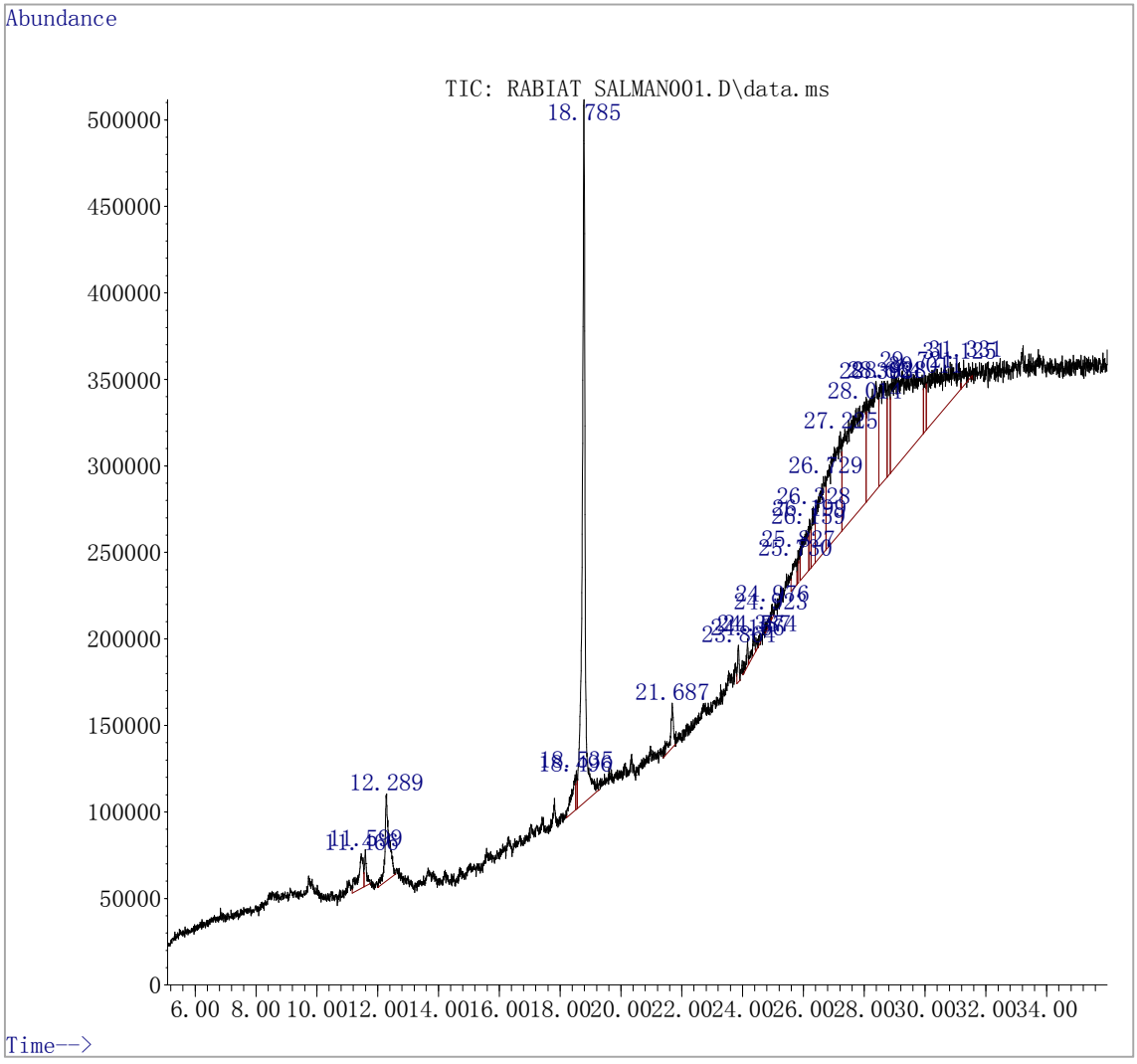

Figure 4. *GC-MS chromatogram of alkaloid fraction of kalanchoe pinnata leaves.*

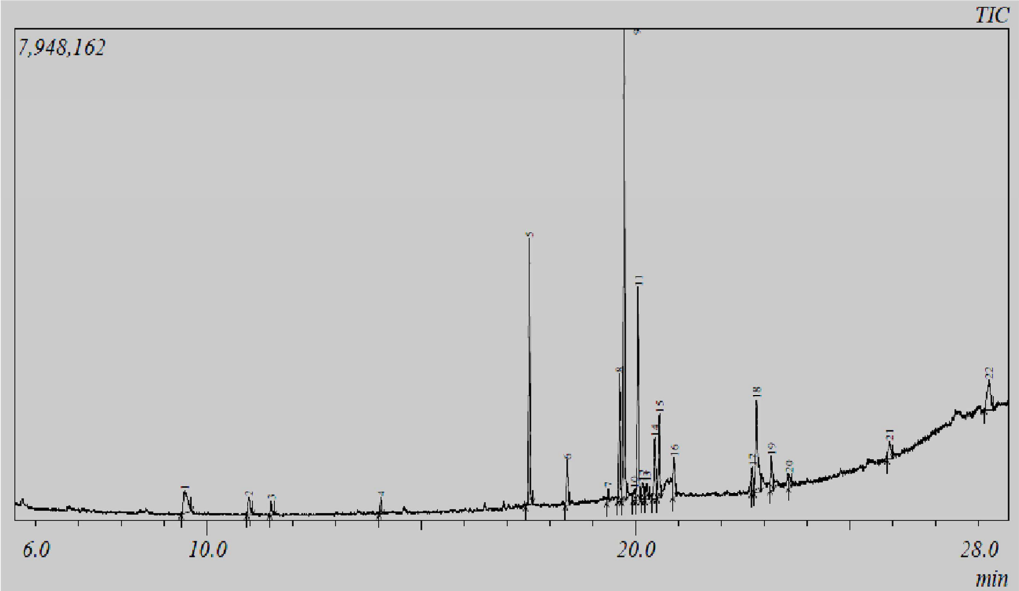

Figure 5. *GC-MS chromatogram of flavonoid fraction of Kalanchoe pinnata leaves.*

4. Discussion

The nutritive value and biological activity of plants are generally attributed to the nutrients and phytochemicals they contain. *Kalanchoe pinnata* leaves have an appreciable amount of nutrients, thus confirming their dietary value. The presence of nutrients in the leaves could be part of the contributing factors which suggest the use of the plant as both food and medicine. This further indicates that *Kalanchoe pinnata* could be prescribed as a dietary supplement since it is a good source of human nutrition [26]. However, the high moisture content is an indication of its susceptibility to spoilage [26].

Phytochemicals have been reported to possess significant antioxidant properties that prevent occurrence of several human diseases such as cancer, ageing, cardiovascular and inflammatory diseases [27]. They scavenge free radicals, chelate metals, and prevent lipid peroxidation as part of their antioxidant functions. Phenolic compounds are also known as powerful chain-breaking antioxidants. In terms of their ability to operate as effective radical scavengers and metal chelators, phenols have attracted a lot of attention as potential natural antioxidants [28]. The antioxidant activity of phenolic compounds is mainly due to their ability to donate hydrogen and quench singlet oxygen [29]. *Kalanchoe pinnata* is rich in phenolic compounds, which suggest that it could be employed as an antioxidant agent. Alkaloids and flavonoids present in *Kalanchoe pinnata* may be responsible for the antioxidant activities [30]. Flavonoids prevent oxidation of low-density lipoprotein by free radicals which could lead to accumulation of cholesterol in the arteries. This is made possible through chelating and scavenging action of the hydroxyl group at C3 of flavonoids [31]. Alkaloids also function as antioxidants through detoxification of reactive oxygen species produced by different stresses [32].

In recent times, there has been an increase in interest and search for compounds with antioxidant properties from plants [33, 34]. To achieve this, several assays are employed to screen samples for antioxidant activity as a single test may not produce reliable results [35]. DPPH is a stable organic radical that has been widely employed to determine antioxidant activity of compounds [36] while ABTS is a protonated radical with a characteristic absorbance at 734 nm which decreases with the scavenging of proton radicals [37]. The scavenging ability of alkaloid and flavonoid fractions on free radicals compared favourably with ascorbic acid and rutin, which is an indication of the antioxidant activity of *Kalanchoe pinnata*. The DPPH radical scavenging activity of the flavonoid fraction of *Kalanchoe pinnata* leaves agrees with a previous study that confirmed the antioxidant activity of flavonoids and phenols using DPPH and ABTS radicals [22].

Based on GC-MS analysis, many important bioactive compounds including 5-hydroxymethyl furfural (5-HMF) were detected in the alkaloid fraction of *Kalanchoe pinnata*. Previous studies reported that 5-HMF displayed unique antioxidant potential by inhibiting AAPH-induced hemolysis in a dose-dependent manner by scavenging ABTS and DPPH free radicals [38]. Furthermore, 1,2,3-Triazole which was also found in alkaloid fraction of *Kalanchoe pinnata* is used as a bioisostere to produce more complicated chemical compounds such as mubritinib and tazobactam [39]. Mubritinib is a protein kinase inhibitor used for treatment of cancer while Tazobactam works by inhibiting the action of bacterial β-lactamases [40]. The antioxidant activity of flavonoid fraction of *Kalanchoe pinnata* leaves may be attributed to the presence of oleic acid. This finding is consistent with a recent study which reported that palmitic and oleic acids displayed significant antioxidant potential by reducing malondialdehyde concentration and enhancing antioxidant enzymes [41]. These two compounds were

detected in the flavonoid fraction of *Kalanchoe pinnata* and may be responsible for the observed antioxidant activity of the plant. Other compounds like octadecanoic acid methyl ester, linoleic acid and erucyl amide have also been reported to exhibit antioxidant activity [42, 43]. The presence of these important bioactive compounds in the alkaloid and flavonoid fractions of *Kalanchoe pinnata* makes the plant a potent antioxidant agent that may be used in drug development.

5. Conclusion

Several phytochemicals including alkaloid, flavonoid, flavanol, phenol, tannin and saponin were detected in the leaf extract of *Kalanchoe pinnata.* Proximate analysis indicated that *Kalanchoe pinnata* leaves may be a good source of moisture and carbohydrates. The alkaloid and flavonoid fractions of *Kalanchoe pinnata* leaves exhibited antioxidant activity in a dose-dependent manner that is comparable to ascorbic acid and rutin. GC-MS analysis of the two fractions revealed the presence of several bioactive compounds including 5-hydroxymethyl furfural, oleic acid and palmitic acid to which the antioxidant activity of the plant may be attributed. Based on the results generated in this study, *Kalanchoe pinnata* leaves exhibited significant antioxidant activity, and may be explored for the development of antioxidant drugs.

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