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# Phytochemical Composition of *Kalanchoe pinnata* and *Bidens pilosa* Leaves Associated with Management of Diabetes

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**Abstract** **Background:** Diabetes is responsible for rapidly increasing morbidity globally such that it has been listed among the four priority non-communicable diseases. Global prevalence of diabetes was 8.5% of the adult population by 2014 but is steadily rising. It is estimated that global prevalence of diabetes will be 472 million by 2030 with diabetic neuropathy affecting up to 236 million people. Newer interventions based on natural compounds are required since the available options are marred with diverse side effects. Plants' natural bioactive compounds are capable of preventing development of diabetic complications via different mechanisms making them potential alternatives for its management. *Kalanchoe pinnata* and *Bidens pilosa* have been used in folkloric medicine to treat diseases including diabetes. **Objective:** Our study aimed at determining phytochemicals present in these two plants and their potential for use in management of diabetes. **Material and Methods:** Extracts from the two plants were prepared by maceration in different solvents followed by determination of presence of ten phytochemicals. **Results and Discussion:** Different polyphenolic compounds, glycosides and saponins were detected in aqueous extracts of both plants. Higher concentrations of flavonoids and phenolic acids were detected in aqueous extracts from *B. pilosa* (30.11±0.2 mg of QE/100 g and 92.7±0.1 mg of GAE/100 g) compared to *K. pinnata*. **Conclusion:** The presence of these phytochemicals qualify these two plants as candidates for development of interventions for managing type 2 diabetes.

***Keywords:*** *diabetes, complications, management, Kalanchoe pinnata, Bidens pilosa, phytochemicals*

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## 1. Background

There is a global concern on the escalating burden of diabetes especially in developing countries [1]. Diabetes was directly responsible for 1.5 million deaths globally in 2012 besides 2.2 million deaths that occurred due to associated complications [2]. It was estimated that 10.8 million people from sub Saharan region of Africa were diabetic in 2006 and it is predicted to increase to 18.7 million by 2025 [3]. A study in Kenya depicted the prevalence of diabetes as 4.2% among adults aged between 17 and 68 years [4]. Further studies have demonstrated prevalence of 5.3% within the Kenyan urban settings with the higher burden being observed among adults aged 45-54 years [1]. Type 1 diabetes is associated with autoimmune destruction of pancreatic β-cells resulting in insulin deficiency whereas type 2 characterized by insulin resistance with relative β-cell failure despite normal or elevated insulin levels [5]. Management of type 2 diabetes is geared towards improving insulin sensitivity and secretion by the β-cells in addition to controlling appetite and body weight [6,7] all of which are benefits offered by phytochemicals. Poorly controlled diabetes has potential for causing complications like blindness, terminal nephropathies, neuropathies and cardiovascular diseases [6,8]. Diabetic complications always develop due to persistent hyperglycemia that induces chronic glucotoxicity resulting in impairment of different metabolic pathways [9]. Progression of diabetes triggers upregulation of polyol, glycation, protein kinase C, hexosamine and alpha-ketoaldehyde pathways in order to restore glucose levels to normal but all these metabolic reactions results in generation and accumulation of reactive oxygen species (ROS) that include singlet oxygen, superoxide ion, hydroxyl ions and hydrogen peroxide [9,10]. These molecules are highly reactive and toxic with potential for causing severe oxidative damage to body tissues through

covalent binding and lipid peroxidation [10]. It is a challenge to manage diabetic complications with less side effects using currently available interventions and this has given rise to focus on medicinal plants which provide natural antidiabetic activity with reduced or no side effects [11]. Plants are rich in natural antioxidant ability to scavenge for free radicals [12] and this has resulted in concerted efforts to unearth potential herb-based interventions to combat chronic diseases like diabetes, cancer, inflammations among others. Over the past, traditional medicine has offered useful remedies for healing different human diseases due to their ability to produce assorted bioactive compounds [13,14]. These phytochemicals occur naturally in different parts of the plant at varied concentrations as primary or secondary compounds. Primary compounds are usually chlorophyll, proteins and common sugars while phenolic compounds, alkaloids, terpenoids, among others constitute secondary compounds [15,16]. Presence and ubiquity of these phytochemicals in plants qualifies them as candidates for discovery and development of novel interventions against type 2 diabetes [17]. Different plant parts contain polyphenolic compounds, flavonoids, terpenoids, saponins, polysaccharides and alkaloids whose moieties and secondary metabolites with potential for reversing or delaying development of diabetic complications by glycemic control, reducing formation of ROS, increasing secretion of insulin from β-cells and inhibiting formation of advanced glycation end products (AGEs) [18]. Flavonoids inhibit formation and propagation of free radicals thus lower oxidative stress besides their ability to cause regeneration of pancreatic β-cells thereby prevent diabetic complications [19]. Flavonoids have been demonstrated to up-regulate two peroxisome proliferator-activated receptors (PPARα and PPARγ) resulting in glycemic and lipids regulation required for management of diabetes [20]. Alkaloids exerts their effect on glycemic control by increasing availability of blood glucose to peripheral tissues and regulating oxidative status resulting in prevention of development of diabetic complications [21,22]. Phenolic acids are excellent radical scavengers that prevent development of diabetic complications due to formation and accumulation of AGEs [23,24]. Ability of phenolic acids to regulate blood glucose and lipids coupled with neutralization of free radicals responsible for oxidative tissue damage thus preventing development of diabetic complications has been demonstrated in previous studies [25].

The genus *Kalanchoe* that belongs to the family crassullaceae that consists of over 125 species [26] although only two; *K. pinnata* and *K. brasiliensis* are useful in ethnomedicine for treatment of different conditions including diabetes [27]. Leaves of *K. pinnata* have been used by traditional healers to treat common colds, diabetes, hypertension, renal calculi, asthma, prostate diseases and urinary tract infections when leaves are boiled and ingested by the patient from Trinidad, Tobago and Africa [28]. Its leaves are also consumed by natives from southern Maharashtra, India for glycemic control [29]. Despite reported usage of this plant parts in folkloric medicine there is need to determine phytochemical composition that are important in management of diabetes. In addition, geographical and climatic conditions could alter phytochemical composition.

Black jack (*Bidens pilosa*) is a plant that belongs to the Asteraceae family. It was originally found in South America but it has spread throughout the tropics and subtropics where it is widely distributed invading both cultivated and undisturbed lands [30]. Since *B. pilosa* is highly invasive in nature, it is most commonly considered as weed [31]. This plant has been utilized as food and ethnomedicine besides incorporation in tea by different communities globally making it an important subject for research. Every part of the plant can be used individually or in combination with another as dried powder or tincture topically or orally [32,33]. Extracts from *B. pilosa* have been shown to be efficient anti-hyperglycemic agents in experimental mice [34] making them good candidates for glycemic control studies. The ability of Black jack to control obesity which is currently a pandemic associated with type 2 diabetes [35] is an indication that it possess a high therapeutic value. To the best of our knowledge no similar studies have been conducted on the two plants from this region despite their promising benefits. This study aims at comparing phytochemical composition in leaves from both *K. pinnata* and *B. pilosa* and their potential to manage diabetes including development of associated complications.

## 2. Materials and Methods

### 2.1. Plant Material Collection

Both plants; *K. pinnata* and *B. pilosa* were collected from farmlands located at Gathaithi, Murang'a County, Kenya and submitted to the department of Photochemistry at the National Museums of Kenya for identification prior to any processing. Thereafter phytochemical extraction and analysis were conducted at the Institute of Primate Research (IPR).

### 2.2. Phytochemical Extraction

Fresh *K. pinnata* leaves, stems and roots were separated then washed thoroughly with distilled water. However, we concentrated on leaves thereafter since they appeared to be richer in phytochemicals than stem and roots (unpublished data). *K. pinnata* leaves were thoroughly washed with copious amount of water then rinsed using distilled water. Juice from the leaves was extracted and immediately lyophilized ready for grinding into fine powder that was used for phytochemical extraction and analysis. Whole plant; *B. pilosa* was harvested followed by separation of leaves, stem and roots. We selected to focus on leaves since preliminary assays indicated that they had more phytochemicals than the other plant parts (unpublished data). Leaves of *B. pilosa* were separated immediately after plant collection, washed thoroughly and completely dried away from direct sunlight before pulverizing them into fine powder that was used for phytochemical extraction. Lyophilized powered (20 g) from *K. pinnata* was extracted by maceration using 100 ml distilled water, ethanol (Merck, Germany), petroleum ether (PE) and dichloromethane (DCM) for 48 hours while shaking continuously. This was followed by evaporation of ethanol, PE and DCM in order to obtain extracts for

phytochemical testing. On the other hand 10 g of *B. pilosa* powder was macerated in 100 ml of distilled water, ethanol and chloroform for 48 hours before evaporation of the two solvents; ethanol and chloroform in order to remain with the extracts.

## 2.3. Phytochemical Analysis

We determined the presence of ten phytochemicals; alkaloids, flavonoids, phenolic compounds, steroids, terpenoids, glycosides, tannins, balsams, saponins and anthraquinones in the extracts from the two plants using methods described by Ebbo *et al* [36].

Quantification of total phenolic compounds was conducted using Folin-Ciocalteu reagent (Merck, Germany) method in triplicates. Gallic acid (Merck, Germany) was used as standard from which different concentrations; 10, 20, 40, 60, 80 and 100 µg/ml were prepared. One milliliter of each Gallic acid standard and plant extract was put into a test tube before adding 5 ml distilled water and 0.5 ml Folin-Ciocalteu's reagent. This was mixed and allowed to stand for 5 minutes followed by addition of 1.5 ml 20% sodium carbonate and making up the volume to 10 ml with distilled water. After incubation for 2 hours, absorbance of test and standard was determined at 750 nm against a reagent blank. Optical densities of the standards were used to prepare the standard calibration curve. Total phenolic compounds present in the plant extracts was expressed as mg of Gallic acid equivalent (GAE)/100 g of dry mass [37].

Quantification of flavonoids was performed using aluminum chloride method in triplicates. Briefly 0.5 ml of each quercetin (Merck, Germany) standard (100, 200, 400, 600, 800 and 1000 µg/ml) and plant extract was diluted in 4.5 ml of 70% ethanol before adding 0.3 ml $NaNO_2$ in a test tube. After 5 min of incubation, 0.3 ml 10% $AlCl_3$ was added and incubated further for 5 min. This was followed by addition of 2 ml 1M NaOH and making up the volume to 10 ml with distilled water. After incubation for 15 min, absorbance was measured at 510 nm using a spectrophotometer. Absorbance of the standards was used to plot the standard calibration curve. Total flavonoid content was expressed as mg of Quercetin equivalent (QE)/100 g of dry mass [37,38].

# 3. Results

In addition to distilled water, ethanol and chloroform were also used to extract phytochemicals from *B. pilosa* leaves thereby producing three different extracts from this plant. Ethanol, PE and DCM were used for extraction of phytochemicals from *K. pinnata* leaves in addition to aqueous method. In general, all extraction methods yielded flavonoids and phenolic acids (Table 1). Aqueous extract from both plants contained all phytochemicals except anthraquinones. Ethanolic extract from *B. pilosa* appeared to contain all phytochemical tested except anthraquinones and terpenoids. On the other hand only saponins and anthraquinones were absent in ethanolic extract of *K. pinnata*. Chloroform extract from *B. pilosa* possessed flavonoids, phenolic acids and terpenoids. Extraction using DCM yielded all phytochemicals from *K. pinnata* except cardiac glycosides, saponins, terpenoids and anthraquinones. Only four phytochemical; flavonoids, phenolic acids, alkaloids and tannins were detected in pet ether extracts of *K. pinnata* (Table 1).

**Table 1. Qualitative analysis of phytochemicals from the two plants**

| | *B. pilosa* | | | *K. pinnata* | | | |
|---|---|---|---|---|---|---|---|
| Phytochemical | Aqueous | Ethanol | Chloroform | Aqueous | Ethanol | Pet Ether | DCM |
| Flavonoids | + | + | + | + | + | + | + |
| Phenolic acids | + | + | + | + | + | + | + |
| Alkaloids | + | + | - | + | + | + | + |
| Tannins | + | + | - | + | + | + | + |
| Balsams | + | + | - | + | + | - | + |
| Cardiac glycosides | + | + | - | + | + | - | - |
| Steroids | + | + | - | + | + | - | + |
| Terpenoids | + | - | + | + | + | - | - |
| Saponins | + | + | - | + | - | - | - |
| Anthraquinones | - | - | - | - | - | - | - |

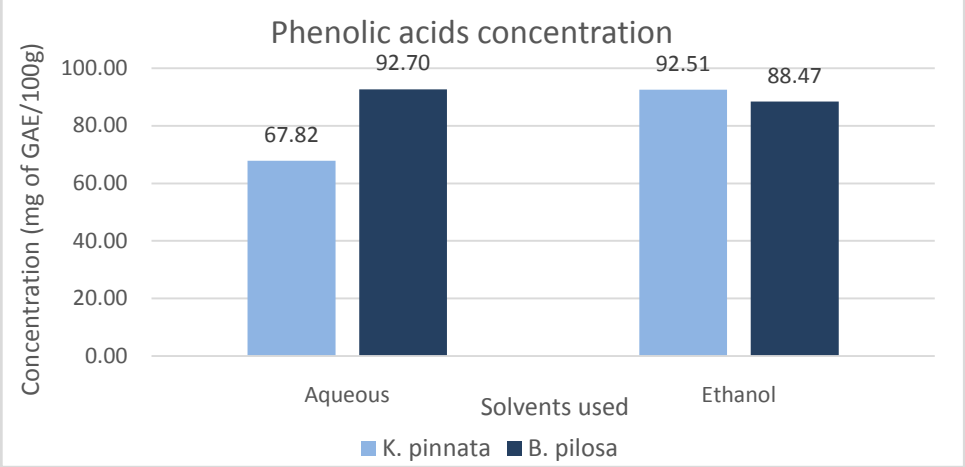


**Figure 1.** Comparison of total phenolic acids from *K. pinnata* and *B. pilosa*

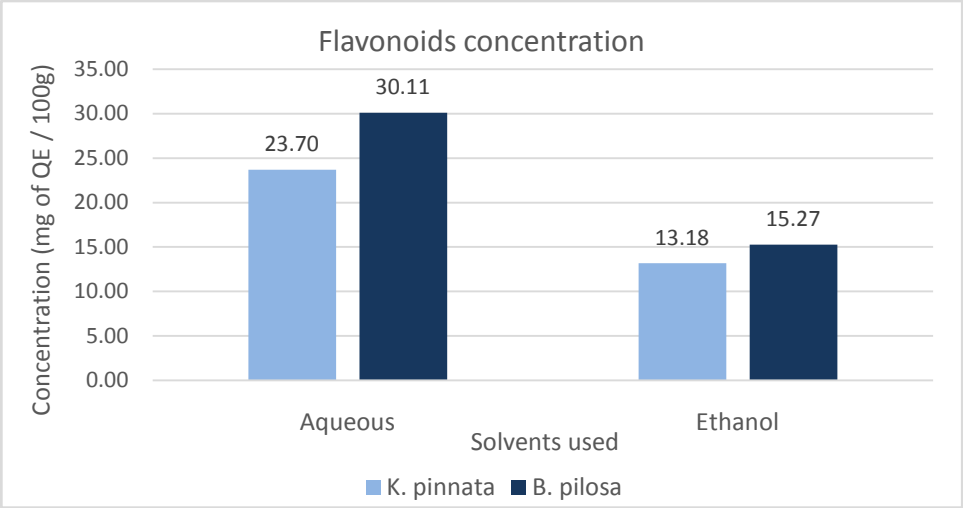


**Figure 2.** Comparison of total flavonoids concentrations between *K. pinnata* and *B. pilosa*

A higher concentration of Total phenolic acids was detected in aqueous from *B. pilosa* (92.7±0.10 mg of GAE/100 g) compared with *K. pinnata*. On the other hand, ethanolic extracts from *K. pinnata* showed higher concentrations of total phenolic acids (92.51±0.03 mg of GAE/100 g) than *B. pilosa* (Figure 1).

Aqueous extracts generally showed the highest concentration of flavonoids in both *B. pilosa* (30.11±0.20 mg of QE/100 g) and *K. pinnata* (23.7±0.16 mg of QE/100 g). However, flavonoid content appeared to be higher in both aqueous (30.11±0.20 mg of QE/100 g) and ethanolic (15.27±0.73 mg of QE/100 g) extracts from *B. pilosa* compared to *K. pinnata*. (Figure 2).

## 4. Discussion

Diabetes is rapidly developing into a grave global public health problem causing alarming morbidity and mortality. There is an urgent need for concerted efforts to prevent, control and manage diabetes in order to curb the morbidity that exerts huge pressure on the national economy especially in developing countries [2]. Persistent poor glycemic control consistent with chronic diabetes adversely affects nerves, kidneys, retina and blood vessels [39]. Plants secondary metabolites offer greater and effective opportunity for discovery and development of interventions against type 2 diabetes [40] thereby ameliorating its associated morbidity and mortality. Both *K. pinnata* and *B. pilosa* from different regions of the world have been extensively studied but little is known about the species found in Kenya. This study sought to determine the presence of phytochemicals present in *K. pinnata* and *B. pilosa* leaves collected from Murang'a County, Kenya with reference to their potential role in management of diabetes. Both plants were quite similar in their phytochemical composition particularly when water was used for extraction which indicated presence of flavonoids, phenolic acids, alkaloids, tannins, balsams, cardiac glycosides, steroids, terpenoids and saponins (Table 1). Like in previous studies, anthraquinones were not detected in any of the extracts from the two plants [41]. The above phytochemicals have been described as essential in management of diabetes through varied mechanisms [18]. Only anthraquinones were absent in aqueous extracts from both *K. pinnata* and *B. pilosa*. Ethanolic extracts of *K. pinnata* did not contain saponins while terpenoids were absent in similar extract from *B. pilosa* (Table 1). This study demonstrated high levels of phenolic acids in aqueous extracts of *B. pilosa* (92.7±0.10 mg of GAE/100 g) compared to *K. pinnata* (Figure 1). Higher concentration of phenolic acid was reported in ethanolic extracts from *K. pinnata* (92.51±0.03 mg of GAE/100 g) than *B. pilosa*. On the other hand, higher levels of flavonoids were detected in aqueous and ethanolic extracts of *K. pinnata* than in *B. pilosa* (Figure 2). Flavonoid concentration was higher in both aqueous (30.11±0.20 mg of QE/100 g) and ethanolic extracts (15.27±0.04 mg of QE/100 g) of *B. pilosa* compared to *K. pinnata* (Figure 2). Our results are consistent with previous studies conducted on *B. pilosa* that reported presence of flavonoids, alkaloids, tannins, steroids, terpenoids, cardiac glycosides and saponins [41]. Previous studies have demonstrated presence of phytochemicals such as flavonoids and phenolic acids among others [42,43,44] and radical scavenging activity of *K. pinnata* extracts *in vitro* [42] thereby reinforcing their potential for management of type 2 diabetes. Hyperglycemia in chronic diabetes accelerates development of associated complications due to formation and accumulation of oxidative products that subsequently cause irreversible tissue damage. Different *in vitro* studies have demonstrated that flavonoids, alkaloids, phenolic acids, terpenoids, tannins and saponins that have been identified in the current study play a crucial role in management of diabetes and prevent development of associated complications through varied mechanisms. Flavonoids restores normal glucose metabolism and fatty acid storage by up-regulating both PPAR-α and PPAR-γ, reduces formation of ROS thus lowering oxidative stress responsible for permanent tissue damage in chronic hyperglycemia. Phenolic acids have been reported to destroy free radicals besides inhibiting their formation thereby preventing their accumulation in tissues and subsequent development of complications that ensue due to persistent hyperglycemia [45]. Some phytochemicals like alkaloids and terpenoids potentiate secretion of insulin by pancreatic β-cells resulting in regulation of blood glucose and restore antioxidant status which is crucial in prevention of diabetic complications [46].

This study demonstrates that extracts from the two plants; *K. pinnata* and *B. pilosa* are potential agents that can be used for management of diabetes and prevention of development of associated complications. We plan to introduce food supplements and herbal preparations based on the two plants under the trade names; Diabetone pilosa and Diabetone pinnata. We shall also encourage Kenyans to consume *B. pilosa* leaves as vegetables and *K. pinnata* as herbal supplement due to their medical benefits.

## 5. Conclusion and Recommendations

Both *K. pinnata* and *B. pilosa* are rich sources of flavonoids, phenolic acids, alkaloids, tannins, steroids, terpenoids, saponins and cardiac glycosides all of which possess antihyperglycemic activity. These phytochemicals also play a role in prevention of development of diabetic complications. However there is need to separate and determine the different pure compounds present in each of the two plants then proceed to test them using animal models that are phylogenetically closer to humans.

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## Statement of Competing Interest

The authors do not have any competing interests.

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