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Formulation of an Improved Traditional Medicine (ITM) with antidiabetic and antihypertensive properties in capsules based on the leaves of *Bidens pilosa* L. (Asteraceae)

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Abstract
Diabetes and hypertension are major public health problems worldwide, particularly in developing countries, where populations are increasingly turning to herbal medicine. However, the traditional use of plants is largely unsystematic and involves poor preservation practices. The objective of this study was to formulate an improved traditional medicine in capsule form based on a dry extract of Bidens pilosa leaves. The capsule form was chosen for its stability, ease of administration, and ability to mask the taste. The extract obtained by decoction followed by controlled drying was a hygroscopic powder. The extraction yield was 17.5%. The total polyphenol content, determined by the Folin-Ciocalteu method, was 88.56 mg tannic acid equivalent per gram of extract. The powder was stabilized by the addition of 2% anhydrous colloidal silica. From a pharmacotechnical standpoint, the flow rate, Carr's index, and Haussner index were favorable. The recommended daily dose was 2.43 g of dry extract for a 60 kg individual, divided into six size 0 capsules for two administrations. These capsules met the requirements of the European Pharmacopoeia, 11th edition. This study demonstrates the feasibility of a reproducible pharmaceutical formulation of a plant extract while preserving its polyphenol content.

Keywords: Bidens pilosa, ITM, colloidal silica, capsules, antidiabetic, antihypertensive, formulation

Introduction

Chronic non communicable diseases such as type 2 diabetes and hypertension represent major public health challenges in low- and middle-income countries. They account for approximately 41 million deaths each year, or 71% of global mortality, with a faster rate of increase observed in sub-Saharan Africa. In Cameroon, the prevalence of hypertension is estimated at 32.1% and that of diabetes at 5.8% [1–4]. Available treatments are often costly and difficult to access due to a lack of social coverage and inadequate health insurance systems. Furthermore, treatment non-adherence remains high, particularly when treatments are not adapted to patients' sociocultural realities [1].

Given these limitations, traditional medicine continues to play an important role in the management of chronic conditions. The World Health Organization, recommends the rational integration of traditional medicines into national health systems. Among the plants used in sub-Saharan Africa is *Bidens pilosa* L. (Asteraceae), a herbaceous specie widely distributed in tropical and subtropical regions. It is traditionally used for its anti-inflammatory, antidiabetic, antihypertensive, and antimicrobial properties [5-7]. Pharmacological studies have confirmed several of these activities, notably the ability of leaf extracts to reduce blood glucose levels and blood pressure in rats [7 –10].

In practice, traditional practitioners generally use a decoction of *Bidens pilosa* leaves, but this liquid form has several drawbacks: instability, the need to administer a large volume, unpleasant taste, and a limited shelf life. With the aim of effectively utilizing this local resource, this study set out to test the formulation of an improved traditional Medicine (ITM) in capsule form based on an aqueous extract of *Bidens pilosa*, with a therapeutic focus on treating diabetes and hypertension.

Material and Methods

1. Materials

The plant material consisted of *Bidens pilosa* leaves. They were dried and then coarsely ground.

The other equipment consisted mainly of a grinder, an electronic scale, a drying oven, a water bath, a mortar, a 100-capsule semi-automatic capsule filler, culture media, numerous laboratory reagents, and a glassware kit.

2. Methods

2.1 Preparation of the aqueous extract

A known mass of leaves was placed in distilled water and the mixture was brought to a boil for 20 minutes. After cooling, the supernatant was collected, and the residue was then soaked again in 1.5 L of distilled water; another decoction was performed following the same procedure as before until a new filtrate was obtained; The filtrates were mixed, filtered through No. 2 Whatman paper, and evaporated in an oven at 45 ± 2°C until a dry mass was obtained. The resulting dry extract was weighed, and the extraction yield (R) was calculated using the formula:

$$R = \frac{mass\ of\ dry\ matter}{mass\ of\ leaf\ powder} \times 100$$

2.2 Physicochemical properties of the extract

2.2.1 Organoleptic properties.

The determination of the organoleptic properties involved observing, touching, smelling, and tasting the dry extract.

2.2.2 Solubility

Solubility was determined by gradually adding increasing volumes of distilled water to 100 mg of *Bidens pilosa* extract at room temperature. The mixture was stirred

continuously using a magnetic stirrer for 10 minutes after each addition of water. The solubility of the powder was checked visually and estimated in grams per liter according to Table I.

Table 1: Solubility in grams per liter [11]

Approximate solubility							
Volume of water (ml)	**0,1**	**0,5**	**1**	**2**	**10**	**100**	**>100**
Solubility (g/l)	>1000	1000 à 200	200 à 100	100 à 50	50 à 10	10 à 1	< 1

2.2.3 Residual moisture.

A 2g sample (mass m) of the dry extract of *Bidens pilosa* was placed in an oven at 102°C and weighed every hour until the difference in mass was less than 0.5 mg. The last mass is the final mass (m'). The residual moisture content (RM) was calculated using the formula

$$RM\% = \frac{m - m'}{m} \times 100$$

The test was repeated three times, and the average value was adopted.

2.2.4 pH determination

A 10% aqueous dispersion of the dry extract of *Bidens pilosa* was prepared and filtered using Whatman paper. The pH of the filtrate was measured using a Thermo Scientific pH meter after calibration with acid and base standard solutions. The test was repeated three times [12] and the average value was used.

2.2.5 Phytochemical screening

The determination of phytochemical groups present in the extract was performed according to the standard methods described by Harbone in 1973 [13].

2.2.6 Determination of total phenols in the extract.

The total phenol content was determined using the method described by Mbopi *et al* [14]. The reagent consists of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). During the oxidation of phenols, it is reduced to a mixture of blue oxides of tungsten and molybdenum. These blue pigments have a maximum absorption that varies depending on the qualitative and/or quantitative composition of the phenolic compounds and the pH of the solutions, which is generally achieved by adding sodium carbonate. The reaction mixture in this test consisted of 100 µL of extract solution, 500 µL of Folin-Ciocalteu reagent diluted 1:10 with water, and 2 mL of a 20% sodium carbonate solution added two minutes later. The mixture was stirred and incubated at room temperature in the dark for 30 min, then the absorbance was measured using a Spectroquant® Pharo 300 spectrophotometer at 760 nm. Each test was performed three times for each sample concentration. Polyphenol quantification was performed using the linear calibration curve (y = ax + b) generated by a "tannic acid" standard at various concentrations ranging from 25 to 125 mg/L under the same conditions as the sample.

2.3 Capsule formulation

2.3.1 Powder Stabilization

The dry extract was hygroscopic and unsuitable for encapsulation. The "Handbook of Pharmaceutical Excipients" was consulted to select anhydrous colloidal silica to address this issue. The impact of anhydrous colloidal silica on the stability of the dry extract was evaluated by preparing several mixtures with increasing concentrations: 0.5%, 1%, 2%, 2.5%, and 3%. These tests aimed to determine the optimal concentration to improve the powder's stability while maintaining good encapsulation suitability. Small quantities of the active ingredient and excipient were weighed and mixed in a mortar. After pulverizing the extract in the mortar, the other powders were added in ascending order of quantity, and the texture of the various mixtures was evaluated over several weeks.

2.3.2 Particle size analysis

The particle size analysis of the stabilized extract was performed using a sieve stack (600 µm, 300 µm, 200 µm, 100 µm, 50 µm, bottom) with agitation for 5 minutes. The residues from the various sieves were weighed using a precision balance, and histograms of the single and cumulative particle size frequencies were plotted to graphically determine the median particle size (d50), corresponding to the size of 50% of the particles (Table II), and the fineness of the powder. The test was repeated three times [12].

Table 2: Classification of powders accrding to their fineness [12]

Descriptive term	D50 (µm)	Cumulative volume distribution Q(x)
Coarse	> 355	$Q^{[355]} < 0,50$
Moderately fine	180 – 355	$Q^{[180]} < 0,50$ et $Q^{[355]} \geq 0,50$
Fine	125 – 180	$Q^{[125]} < 0,50$ et $Q^{[180]} \geq 0,50$
Very fine	≤ 125	$Q^{[125]} \geq 0,50$

Q(x) = Cumulative distribution of particles with a size less than or equal to x; D50 = Median particle size (50% of particles are smaller and 50% are larger).

2.3.3 Dose determination.

The therapeutic dose of *Bidens pilosa* extract in adult humans was determined using Table III [15]. Calculations were based on the animal dose, which is 150–350 mg/kg of extract for the antihypertensive effect in rats [10] and 0.5 g/kg for the antidiabetic effect in mice [7, 16]

Table 3: Conversion factors for determining the Human Equivalent Dose) [15].

Species	EHD (mg/kg) = animal dose divided by	EHD (mg/kg) = animal dose multiplied by
Mouse	12,3	0,081
Hamster	7,4	0,135
Rat	6,2	0,162
Guinea pig	4,6	0,216
Rabbit	3,1	0,324
Dog	1,8	0,541
Marmoset	6,2	0,162
Baboon	1,8	0,541

2.3.4 Pharmaceutic properties of the stabilized extract

Flowability: A 100 g sample of the stabilized extract of *Bidens pilosa* was placed in a standard funnel whose opening had been previously sealed. The flow time of the powder was then measured after opening the funnel's opening. Flow was considered satisfactory when the flow time was less than 10 seconds for 100 g. The test was repeated three times, and the average was recorded [12]. If

the difference between V500 and V1250 was less van 2ml, then V1250 represents the final compacted volume. Other wise, the further compactions were carried out until the difference between V500 and the final volume (Vn) was less than 2 ml.The values obtained were used to calculate the Carr Index (C I) and Hausner Index (HI) according to the formulas $HI = \frac{V0}{Vf}$ and $CI(\%) = \frac{V0-Vf}{V0} \times 100$

Settling Capacity: A 100-gram sample of the stabilized extract was placed in a graduated cylinder, and the volume (V0) was recorded. Subsequently, 10, 500, and 1250 settling steps were performed, with their volumes (V10, V500, and V1250) recorded.
The test was repeated three times, and the average was classified in accordance with the standards of the pharmacopoeia [12] (Table II).

Table 4: Flowability scale [12]

Compressibility index (%)	Flowability Index	Hausner Index IH
1-10	Excellent	1,00-1,11
11-15	Good	1,12-1,18
16-20	Fair enough	1,19-1,25
21-26	Passable	1,26-1,34
26-31	Mediocre	1,35-1,45
32-37	Very mediocre	1,46-1,59
> 38	Extremely mediocre	> 1,60

A V10-V500 value of less than 20 indicates good powder reorganization

2.3.5 Filling, inspection, and packaging of capsules
The amount of powder corresponding to 5 daily doses was placed in a graduated cylinder; the volume was then transferred to the capsule filling table to determine the capsule size to be used [17] for the batches.

2.4 Pharmaceutical testing of the capsules produced.
2.4.1 Organoleptic characteristics
The macroscopic characteristics of the capsules were described (appearance, color, and sealing of the capsules) [12].

2.4.2 Mass uniformity
20 capsules were randomly selected and weighed individually using a precision balance. The mean (X) and the tolerance limit (e) as a percentage of the mean weight were determined in accordance with the 11th edition of the European Pharmacopoeia, which requires that no more than 2 units deviate by e% from the value X, and that no value reaches a deviation of 2e%. Thus, if X is ≤ 300 mg, then e = 10; and if X > 300 mg, e = 7.5. The test was repeated three times [12] and the average was taken into account.

2.4.3 Disintegration time
This test was performed on 6 capsules selected at random and placed individually into the tubes of the disintegration apparatus. A disc was placed in each compartment; the disintegration medium used was water at 37°C. The time required for complete disintegration was recorded.

2.4.4 Microbiological quality
The presence of certain microorganisms in the preparation may reduce or even negate the product's therapeutic activity. Therefore, to ensure the preparation's low microbial load, a test was conducted as recommended by Good Manufacturing Practices.

Sample preparation: 1 g of powder was mixed with 10 mL of sodium chloride peptone water at pH 7.0, then an agent to inactivate the extract's antibacterial activity (sodium thiosulfate) was added; this serves to temporarily neutralize the antibacterial activity to allow for a realistic enumeration of the microbial flora initially present in the finished product. Several dilutions to one-hundredth and one-thousandth were prepared from the sample.

Enumeration of total mesophilic aerobic flora, molds, and yeasts: Inoculation was performed by scraping. For each dilution, inoculations were performed in a single batch and for 3 Petri dishes per medium. In each Petri dish, 0.1 mL of the prepared sample was added. The dishes were incubated at 30–35°C for 24 hours.

Screening for specified microorganisms: Testing for the absence of Staphylococcus aureus: The subculture was performed on "Chapman" agar medium and incubated at 35–37°C for 24 hours. The absence of black colonies of Gram-positive cocci surrounded by a clear zone indicated the absence of Staphylococcus aureus;

Verification of the absence of coliforms: 0.1 mL of each sample was spread on "Hecktoen" culture medium in Petri dishes. The plates were incubated at 35–37°C for 24 hours. The possible presence of Escherichia coli is indicated by the growth of red colonies surrounded by a halo; those of Salmonella are blue, and Shigella colonies remain green;
Verification of the absence of enterococci: 0.1 mL of each sample was added and spread onto "Bile Esculin Azide" (BEA) culture medium. Incubation was performed at 35–37°C for 24 hours.

Microbiological quality acceptance criteria
TAC (total aerobic count) in CFU/g: 10 [3];
TML (total mold and yeast count) in CFU/g: 10 [2];
Screening for specified microorganisms: absence of Staphylococcus aureus, Enterobacteriaceae, and Enterococci in 1 g or 1 mL of sample.

Results
1. Plant Material
Fresh leaves of Bidens pilosa were collected in December 2024 in Dshang, in the West Region of Cameroon, in the Menoua Department, specifically in its lowlands, while the plant was not in bloom, between 6:00 and 9:00 a.m, following the recommendations of the traditional healer. The plant was identified as Bidens pilosa L. (Asteraceae) by comparison with the original specimen from the National Herbarium (No. J871/SRFCam), registered by Leeuwenberg under No. 3417.

2. Physicochemical characteristics of the dry aqueous extract of *Bidens pilosa leaves*
2.1 Physical characteristics of the extract
Organoleptic characteristics and extraction yield: The extraction of 200 g of powder with 2 L of water yielded a dry extract with a yield of 17.5%; it was a compact, hard powder, very bitter, with the odor of burnt plant leaves, and dark brown in color. The extract was highly hygroscopic:

after 10 minutes of exposure to open air, it became pasty and very sticky.
The extract had a solubility of 100 to 200, a pH of 5.55 ± 0.01, and a moisture content of 5.5%

2.2 Phytochemistry of the aqueous extract of *Bidens pilosa leaves*

The results of the phytochemical screening of the extract are presented in Table III. The following compounds were identified in the extract: flavonoids, phenols, alkaloids, glycosides, triterpenoids, sterols, tannins, saponins, and anthraquinones.

Table 5: Phytochemical composition of the extract

Secondary metabolites	Reagents/ Test	Results
Alkaloids	Draggendorf	+
Anthraquinones	NH_4OH 10%	+
Flavonoids	Magnésium / HCl	+
Glucosides	Acetic Acid, $FeCl_3$, H_2SO_4	+
Phénols	$FeCl_3$	+
Saponins	Foam index	+
Sterols	CH_2Cl_2 / H_2SO_4	+
Tannins	Stiasny Reagent / FeO_3	+
Triterpenoids	CH_2Cl_2 / H_2SO_4	+

(+): presence of secodary metabolite (-): absence of secondary metabolite

2.3 Total Phenolic Content of the Aqueous Extract of *Bidens pilosa leaves*

Determining the total phenolic content of the dry extract of *Bidens pilosa* required the preparation of a tannic acid calibration curve. Standard tannic acid solutions with concentrations ranging from 25 mg/L to 125 mg/mL were prepared. The absorbance of each solution was measured at 760 nm using a spectrophotometer, following reaction with the Folin-Ciocalteu reagent and incubation for 30 minutes. The results were expressed in milligrams of tannic acid equivalent (TAE) per gram of dry extract (Figure 1)

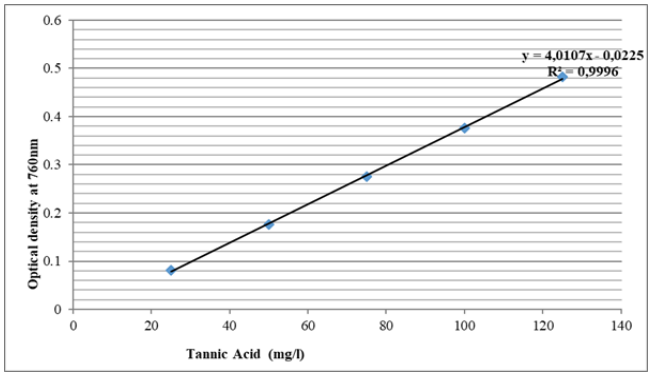

Fig 1: Calibration curve for tannic acid

The resulting curve showed excellent linearity with a regression line equation of y = 4.0107x - 0.0225 and a correlation coefficient $R^{[2]}$ = 0.9996, indicating a strong correlation between tannic acid concentration and measured absorbance. This curve served as a reference for calculating the tannic acid equivalent (TAE) concentration in the tested extracts. One gram of extract contained 88.56 ± 0.76 mg TAE.

3. Capsules formulation

3.1 Stabilization of the extract:

The extract was hygroscopic and unsuitable for encapsulation. To improve its rheological properties, increasing amounts of anhydrous silica were added to reduce or eliminate its hygroscopicity. The mixtures were stored at 25°C and a relative humidity of 75 ± 5%. The behavior of the mixtures after 4 weeks is summarized in Table IV

Table 6: Testing of formulations tested with anhydrous colloidal silica

Formulas tested (Colloidal Silica)	Initial appearance	Final Observation finale 4th week	Conclusion
0,5%	Pasty	Sticky difficult to handle	Directly eliminated
1%	Powdery appearance	Compact powder	Eliminated
2%	Homogeneous powder	Compact powder	Good Stability
2,5%	Homogeneous powder	Slight tendency to clump	Very good stability
3%	Homogeneous powder	Loss of fluidity and onset of agglutination	Less stable than 2 and 2.5%

The formulation containing 2% anhydrous colloidal silica was the most stable

3.2 Particle size analysis

The *Bidens pilosa* extract containing 2% anhydrous colloidal silica showed that the median particle diameter (D50) of 50% of its particles ranges between 125 and 180 µm. The powder is therefore fine. Furthermore, the 180 µm sieve retained more than 50% of the powder. This powder can be considered homogeneous

3.3 Recommended extract dose

The work of Ubillas RP *et al* [12] indicated a daily antidiabetic dose of 500 mg/kg for mice. Taking into account the corresponding FDA factor, the Equivalent Human Dose is 500 × 0.081 × 60 = 2,340 mg of extract per day for a 60-kg adult. Meanwhile, Dimo *et al* [10]. had determined the antihypertensive dose in rats to be between 150 and 350 mg per kg per day. An antihypertensive dose of 250 mg/kg/day was adopted for mice so that the equivalent extract amount corresponds to that of the antidiabetic effect, since 250 × 0.162 × 60 = 2,430 mg of extract per day for a 60-kg human. Since the difference between 2,340 mg and 2,430 mg of daily extract is not significant, it is reasonable to assume that this dose will have both antidiabetic and antihypertensive effects.

3.4 Pharmaceutical characteristics of the powder.

The powder, containing 98% extract and 2% anhydrous colloidal silica, had a flow time of 6.38 seconds, a Carr index of 17.5%, a Hausner index of 1.21; so, it was suitable for encapsulation.

3.5 Capsule filling, unit formulation and dosage.

A quantity of powder corresponding to 5 days doses (2430 × 5 = 12,150 mg), placed loosely into a 25-mL graduated cylinder, yielded an apparent volume of 22 mL. This volume, when referenced in the capsule filling table, was found to be sufficient for 30 size No. 0 capsules. Thus, the unit formula for the capsules is as specified in Table V.

Table 7: Unit formula for the contents of the capsules

Constituents	Proportions(%)	Mass (mg)
Aqueous extract of *Bidens pilosa* leaves (12150mg)	98	12150:30 x 0,98= 396.9
Anhydrous colloidal silica	2	12150:30 x 0.02= 8.1
Total	100	405

Each capsule contains 396.9 mg of extract, which corresponds to 88.56 / 396.9 g EAT of total phenols and 8.10 mg of colloidal silica, for a total of 405 mg of mixture in a No. 0 capsule; the daily dose of extract is 2.43 g. This corresponds to 2430: 98 x 100 = 2479 mg of total powder, or 2479: 405 = 6 No. 0 capsules per day. Based on this, batches of 100 capsules were filled using a semi-automatic 100-capsule filling machine.

4. Testing of the capsules

4.1 Macroscopic appearance of the capsules. The capsules obtained show no physical defects; as shown in Figure 2, they are uniform in size and color

Fig 2: Capsules obtained

4.2 Mass uniformity. Table VI presents the results of the mass uniformity test for the capsule contents. The results show an average mass of 412.25 mg, a lower limit of 382.33 mg, and an upper limit of 443.16 mg. No values fell outside these limits.

Table 8: Results of the mass uniformity tests for Bidens pilosa capsules

N°	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Mn	430	410	420	410	410	410	420	400	410	430	400	405	420	400	420	410	420	420	400	400
Average mass of capsule contents = 412.25mg																				
Tolerable limit deviation = 7,5 % soit 30,91 mg																				
Upper limit = 443,16 mg Lower limit = 381,33 mg																				

Mn = Individual masses in mg of the contents of the 20 capsules sampled

4.3 Disintegration time: The six capsules selected at random and used for this test disintegrated in 5 minutes and 27 seconds. This time is less than the 30-minute limit specified in the European Pharmacopoeia.

4.4 Microbiological quality of the capsules. Table VII presents the results of the microbiological quality control of the capsules. It shows that the extract contains no yeasts, molds, coliforms, staphylococci, or enterobacteria. However, 240 CFU/g were counted on the "Plate Count Agar" (PCA) medium.

Table 9: Microbiological testing of the capsules

Tests	Results	Standards
Enumeration of total mesophilic aerobic bacteria	240 UFC/g	10 [3] UFC/g
Enumeration of total yeasts and molds	00	10 [2] UFC/g
Detection of *enterobacteria*	00	Absent
Detection of *enterococci*	00	Absent
Detection of *Staphylococcus aureus*	00	Absent

The results are expressed in colony-forming units per gram of capsule powder (CFU/g)

5. Packaging and labeling

The manufactured capsules were called GLUCOTENSIA. They were packaged in opaque polyethylene terephthalate bottles labeled with the standard information regarding product identification and traceability. The secondary packaging (Figure 3) is a cardboard box on which the same information is repeated, along with the composition.

Figure 3: Processing

Discussion

The extraction yield of 17.5% is in the same order of magnitude as the results reported by Pérez *et al* [18], who observed yields ranging from 10% to 20% depending on extraction conditions and the plant parts used. Fernandes *et al* [19]. also report an aqueous extraction yield from leaves between 12% and 18%; this confirms the validity of the method used here.

The hygroscopicity observed with the extract is a common phenomenon for extracts rich in polar compounds such as flavonoids, tannins, saponins, and phenolic acids [18, 19]. These compounds readily form hydrogen bonds with water vapor in the environment [18]. Their presence was confirmed by phytochemical screening, corroborating the results of previous studies [20, 21].

The phenolic compound content (88.56 mg EAT/g) is consistent with the findings of Singh *et al* [22]. and Falowo *et al* [23]. The high polyphenol content may explain the antioxidant, hypoglycemic, and antihypertensive effects [7, 24].

The anhydrous colloidal silica chosen as an excipient combats hygroscopicity while improving the powder's flowability. Regarding the 3% mixture, the compaction observed at the end of the third week is consistent with data from the "Handbook of Pharmaceutical Excipients," which notes that silica, at concentrations above 3%, significantly increases the specific surface area of the mixture (more sites for interaction with moisture) and thus increases electrostatic charges, which destabilizes the homogeneity of the mixture or promotes agglomeration [25].

The powder formulated with 2% colloidal silica exhibited a Carr index of 17.50%, a Hausner index of 1.21, and a flow time of 6.38 seconds, indicating excellent flowability according to the criteria of the European Pharmacopoeia [12]. Furthermore, the fine particle size and the mean diameter (D50) ranging from 125 to 180 µm confirmed the powder's homogeneity, an essential parameter for the uniform filling of capsules.

The dosage of 6 capsules (3 capsules in the morning and 3 capsules in the evening) is acceptable in practice given the drawbacks of the traditional practitioner's solution.

Conclusion

This study led to the development of a stable and reproducible formula based on dry aqueous extract of *Bidens pilosa* (Asteraceae), a plant traditionally used in the management of diabetes and hypertension. The chosen approach, centered on the capsule form, demonstrated several advantages, particularly in terms of active ingredient stability, dosage accuracy, and ease of administration. The selection of anhydrous colloidal silica as the sole excipient stabilized the hygroscopic extract while meeting the requirements for simplicity and biopharmaceutical compatibility specific to improved traditional medicines.

The selected daily dose (2.43 g of dry extract distributed across six size 0 capsules) ensures a consistent content of phenolic compounds (88.56 mg of EAT/g), recognized for their beneficial effects in preventing metabolic complications. This approach is part of a strategy to scientifically validate local phytotherapeutic resources with a view to integrating them into public health policies.

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