World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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FORMULATION OF CAPSULES WITH HEPATOPROTECTIVE PROPERTIES BASED ON THE LEAVES OF *DESMODIUM ADSCENDENS* (FABACEAE)

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World Journal of Advanced Research and Reviews, 2026, 31(03), 180–190

Publication history: Received on 24 July 2026; revised on 01 September 2026; accepted on 03 September 2026

Article DOI: https://doi.org/10.30574/wjarr.2026.31.3.2269

ABSTRACT

Liver health is of paramount importance, given its key role in detoxification and metabolism. Liver diseases, particularly viral hepatitis, represent a major cause of mortality, with current therapeutic approaches limited by their high cost. The aim of this study was to develop capsules based on *Desmodium adscendens* extract to improve the management of liver problems, by developing an improved traditional medicine (ITM). To this end, an aqueous extract of *Desmodium adscendens* leaves was prepared and its physicochemical properties were determined. Phytochemical screening was carried out to characterise the extract. The capsules were formulated and manufactured, followed by rigorous pharmacotechnical and microbiological testing.

Phytochemical screening revealed the presence of numerous bioactive secondary metabolites, including phenolic compounds, saponins, tannins and flavonoids, confirming their role in the plant's hepatoprotective activity. The addition of 2% anhydrous colloidal silica stabilised the hygroscopic extract and improved its characteristics to facilitate capsule filling. 'Size 0' capsules, each containing 486 mg of extract, were produced, with two capsules per day required to achieve the effective dose.

These results validate the potential of this plant for the development of improved traditional medicines, offering a new, accessible therapeutic option for the management of liver conditions.

Keywords: *Desmodium adscendens*, Capsules, Hepatoprotective, Formulation, Phytotherapy, Improved traditional medicine

1 INTRODUCTION

The liver plays a central role in many vital functions, including digestion, metabolism, detoxification and hormonal regulation [1]. Owing to its many functions, any impairment of the liver can lead to life-threatening conditions [2]. In April 2024, a WHO report identified viral hepatitis as the second leading cause of death from infectious disease worldwide, accounting for 1.3 million deaths per year – the same as tuberculosis. In Cameroon, the Ministry of Public Health reported in 2025 that hepatitis B affects 11.2 per cent of the general population, compared with 1.3 per cent for hepatitis C. The prevalence of hepatitis D among people infected with hepatitis B is 10.5 per cent. These infections can lead to severe complications, including liver failure, which may require a liver transplant [3]. Viral hepatitis currently accounts for 23 per cent of indications for liver transplants in France [4]. Despite global progress in the prevention of hepatitis, the number of deaths is rising because very few people receive treatment. Current therapeutic approaches are hampered by their cost and variable efficacy. These concerns have spurred the search for other safe and effective medicinal alternatives, particularly those of natural origin [2]. In this context, medicinal plants and their bioactive secondary metabolites have received considerable attention due to their enormous potential for the management and treatment of various forms of liver disease [2].

Certain medicinal plants, such as *Desmodium adscendens*, play a crucial role in protecting the liver. *Desmodium adscendens* is a perennial herb used in traditional medicine in the form of a decoction to treat liver diseases. It is native to West Africa and South America [5]. Several studies have demonstrated and confirmed its efficacy [6–7]. The empirical use of medicinal plants has certain drawbacks, such as the enormous quantities of liquid that need to be consumed and the approximate dosing [8, 9]. The formulation of improved traditional medicines (ITMs) makes it possible to produce new, low-cost medicines that combine scientifically proven efficacy with accessibility [8]. This is why, in the present study, we set out to formulate capsules based on *Desmodium adscendens* leaf extract to improve the utilisation of this local resource in the management of liver problems.

2 MATERIALS

The plant material consisted of leaves of *Desmodium adscendens*, which were collected in the village of Sa'a (Central Region, Cameroon) in February 2025.

The main laboratory equipment used included: a grinder, an electronic balance, a rotary evaporator, a drying oven, a water bath, graduated cylinders, a mortar, a semi-automatic gelatin mould, a disintegrator, a sieve column, a standard funnel and a stopwatch. The laboratory reagents required were mainly: distilled water, 0.1N HCl solution, magnesium shavings, 10% iron (III) oxide solution and Stiasny's reagent.

3 METHOD

3.1 Preparation of the aqueous extract

Fresh leaves of *Desmodium adscendens* were washed, rinsed with distilled water, dried at room temperature and then ground. A decoction was prepared using the leaf powder and distilled water and left to stand for 24 hours. After cooling, the mixture was filtered through Whatman No. 1 filter paper and evaporated at 45 °C to obtain a powder; the extraction yield was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{Mass of the aqueous extract}}{\text{Mass of the dry powder}} x\ 100$$

3.2 Physico-chemical properties of the extract

3.2.1 Determination of residual moisture

A sample of extract powder weighing approximately 2 g (Mi) was placed in an oven at 102 °C; after 2 hours, the sample was weighed every 30 minutes until the difference between two consecutive weighings was < 5 mg. The mass obtained was denoted Mf, and the residual moisture content H was determined using the formula:

$$H = \frac{Mi - Mf}{Mi}\ x\ 100$$

3.2.2 Determination of pH

A 10% aqueous dispersion of the extract was prepared and filtered using Whatman filter paper. The pH of the filtrate was measured using a pH meter after calibration with acid and base standard solutions.

3.2.3 Determination of solubility

Solubility was determined by gradually adding increasing volumes of water to approximately 100 mg of dry extract of *Desmodium adscendens* at room temperature, in a beaker containing 10 ml of deionised water. The mixture was stirred continuously for 10 minutes after each addition of water. The solubility of the powder was checked visually. The test was repeated three times.

3.2.4 Phytochemical screening of the extract

Standard precipitation and colouration tests using standard reagents were carried out to determine the phytochemical groups present in the extract; [11].

3.2.5 Determination of the total phenolic content

Phenolic compounds are among the active metabolites responsible for the hepatoprotective activity of *Desmodium adscendens* [11]. The total phenol content of the extract was determined using the Folin-Ciocalteu method (comprising phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$)) [12]. To 100 ml of the extract solution, 500 ml of Folin-Ciocalteu reagent (diluted 10-fold) was added. Then, 2 minutes later, 2 ml of 20% sodium carbonate was added and the contents were thoroughly mixed. After incubation for 30 minutes at room temperature in the dark, the absorbance of the mixture was measured using a spectrophotometer (Spectroquant Pharo 300) at 760 nm. The polyphenols were quantified using a linear calibration curve (y = ax + b) generated from a 'tannic acid' standard at various concentrations ranging from 25 to 125 mg/L under the same conditions as the sample. The results were expressed in milligrams of tannic acid equivalent (TAE) per gram of dry extract.

3.2.6 Formulation and manufacture of capsules

3.2.6.1 Determination of the dose to be administered

The conversion table for animal dose to human equivalent dose (HED) published by the FDA (table 1) was used as a guide for calculating the daily human dose. The effective dose established in mice had already been determined by Yongoua in 2024 as 100 mg/kg [11].

Table 1: Table for calculating the human equivalent dose [13]

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

3.2.6.2 Preparation of the powder

The physicochemical properties of the extract were used to guide the selection of excipients for the manufacture of the capsules [14]. These excipients were added to improve the profile of the aqueous extract during capsule filling and to maintain the stability of the capsules [14]. The quantity of extract required for 100 capsules was placed in a mortar with varying doses of excipients. The mixtures were used to identify the optimal formulation

3.2.6.3 . Particle size analysis of the powder mixture

Particle size analysis of the optimal powder formulation was carried out using a series of sieves with mesh sizes of 710 µm, 500 µm, 355 µm, 250 µm, 180 µm and 125 µm, with agitation for 5 minutes. The residue from the various sieves was weighed using a precision balance, and histograms of the simple and cumulative particle size frequencies were produced in order to graphically determine the median particle size (d50), corresponding to the size of 50 per cent of the particles [15], and to assess the homogeneity of the powder.

3.2.6.3.1 Flow

The flow time taken for 100 g of powder to pass through the standardised funnel specified in the pharmacopoeia was measured. To do this, the outlet was sealed and the powder was placed into the dry funnel. After opening the outlet, the time taken for the entire sample to flow out was timed.

3.2.6.3.2 Settlement behaviour

A 100 g sample of the powder mixture was placed in a graduated cylinder and the volume (V0) recorded. Subsequently, 10, 500 and 1,250 settlement tests were carried out, corresponding to V10, V500 and V1250 respectively. If the difference between V500 and V1250 was less than 2 ml, then V1250 was taken as the final settled volume. Otherwise, further settling was 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 (CI) and the Hausner index (HI) according to the following formulae:

$$CI = \frac{V_0 - V_n}{V_0} \times 100 \qquad \text{and} \qquad HI = \frac{V_0}{V_n}$$

The test was repeated three times and the mean values were taken into account [16]. The flow fluidity was interpreted using the scale in table 2.

Table 2: Fluidity Scale

Carr Index	Flow	Hausner Index
1-10	Excellent	1-1,11
11-15	good	1,12-1,18
16-20	Fairly good	1,19-1,25
21-25	Average	1,26-1,34
26-31	Average	1,35-1,45
32-37	Poor	1,46-1,59
>38	Extremely poor	> 1,60

3.2.7 Filling and inspection of capsules:

Given the characteristics of the powder, it was possible to fill the capsules using the level-off method on a semi-automatic 100-unit capsule filler. The batches were subjected to the standard pharmacopoeia tests [16]:

- Organoleptic test: colour, appearance, taste and the presence or absence of cracks were recorded.
- Mass uniformity: determination of the maximum acceptable deviation from the average mass of 20 randomly selected capsules.
- If the average mass m is < 300 mg, the tolerable deviation is 10 % of m.
- If the average mass m is ≥ 300 mg, the permissible deviation is 7.5% of m.
- Disintegration time: none of the capsules placed in the 6 tubes of the disintegration apparatus must show any solid residue after 30 minutes of operation at 37° ± 2 °C.

Microbiological quality: the microbiological quality test was carried out in accordance with the techniques recommended by the European Pharmacopoeia, 9th edition. A stock solution corresponding to 1 g/ml was prepared by homogenising the capsule powder in physiological saline. Two dilutions, one at 1:10 and the other at 1:100, were then placed in 90 mm Petri dishes. As shown in table 3, the culture medium and incubation time depended on the type of micro-organism being tested for.

Table 3: Conditions to be observed for microbiological testing.

Culture medium	Incubation time	incubation temperature	Observation at the end of incubation
Plate Count Agar (PCA)	24 H	33°C	Count Colonies
Sabouraud Dextrose Agar (SDA)	24 H	33°C	Count Colonies
MacConkey	48 H	44°C	No red colonies surrounded by a halo
Chapman	72 H	33°C	No yellow/white colonies surrounded by a yellow zone
Bile Esculine Azide (BEA)	72 H	33°C	No translucent colonies surrounded by a black halo

4 RESULTS

4.1 Physicochemical Properties of the Extract

The leaves of *Desmodium adscendens* collected in Sa'a (Central Cameroon region) on 14 February 2025 were identified and confirmed at the National Herbarium of Cameroon by comparison with the original specimen held there. The aqueous extract was obtained with a yield of 13.4 per cent and was in the form of a dark brown powder with a pungent odour and a bitter taste. The powder was hygroscopic and had a solubility of 10–50 g/l, a pH of 6.02 and a residual moisture content of 6 per cent. As shown in table 4, the powder mainly contained alkaloids, polyphenols, flavonoids, terpenoids, glycosides and anthocyanins.

Table 4: Phytochemical composition of the extract

Compound families		Reagents / test	Result
Alcaloïds		Draggendorf / Meyer	(+)
Phenolic compounds		$FeCl_3$ 10%	(+)
Flavonoïds		Magnésium / HCl	(+)
Terpénoïds		CH_2Cl_2 / H_2SO_4	(+)
Anthraquinones		Borntrâger :NaOH	(-)
Tanins	Catechinic	Réactif de Stiasny / FeO_3	(+)
	Gallic	Réactif de Stiasny / FeO_3	(-)
Glucosides		Acide Acetique,$FeCl_3$,H_2SO_4	(+)
Anthraquinones		NH_4OH 10%	(-)
Saponins		foam >1cm	(-)

The phenolic compound content was determined using the tannic acid calibration curve shown in Figure 1. This curve was a straight line exhibiting good linearity and a high correlation coefficient (0.9996) between absorbance and tannic acid concentration. The *Desmodium* leaf extract was found to contain 68.60 ± 0.76 mg tannic acid equivalents (TAE) per gram of powder

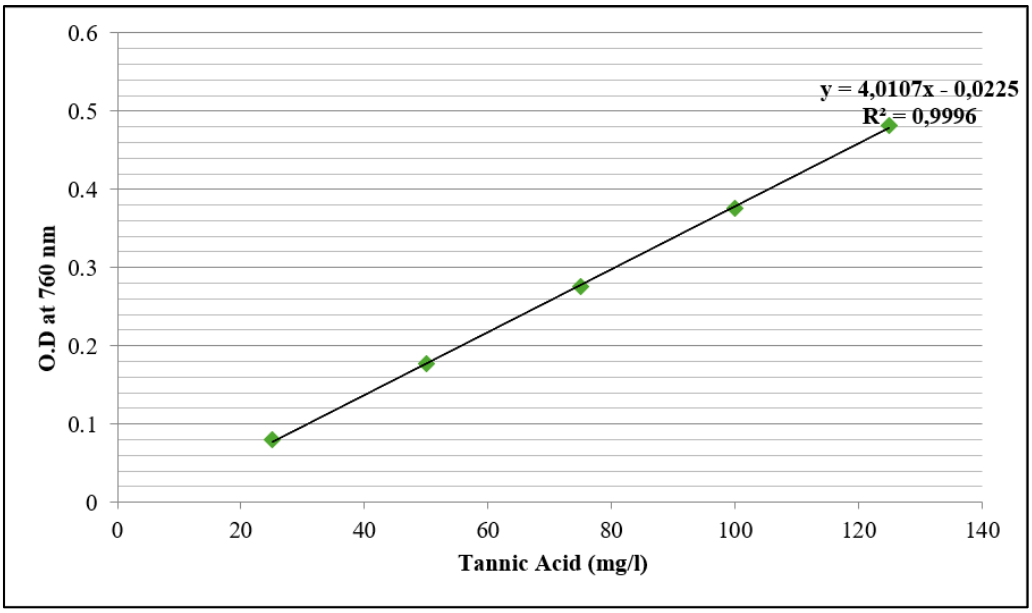

Figure 1: Calibration curve for tannic acid

4.2 Formulation of the capsules.

4.2.1 Determination of the human dose

The effective dose of 100 mg/kg determined in mice in 2014 by Yongoua [11] corresponds to an Equivalent Day Dose of 100 × 0.081 mg/kg in humans, i.e. 100 × 0.081 × 60 mg per 24 hours for a 60 kg adult, or 486 mg of extract per day.

4.2.2 Stabilisation of the dry extract

To counteract the hygroscopic nature of the extract, anhydrous colloidal silica had to be added. As shown in table 5, a range of silica concentrations was tested, leading to the adoption of a 2 per cent colloidal silica content to achieve optimal conditions.

Table 5: Stabilisation tests on *Desmodium ascendens* extract powder using anhydrous colloidal silica

	F1	**F2**	**F3**	**F4**
Description	Formula containing 0.5% silica	Formula containing 1% silica	Formula containing 2% silica	Formula containing 3% silica
Mass of extract	4g	4g	4g	4g
Silica	0,02	0,04	0,08	0,06
Observation	Stable for one day	Stable for 5 days	Stable for 4 weeks	Stable for 3 days

4.2.3 Pharmaceutic properties of the stabilised powder

4.2.3.1 Particle size analysis of the stabilised powder

Particle size analysis of the stabilised powder (F3) determined that the d50 corresponded to a 250 µm sieve mesh size. Furthermore, Figure 2 shows that 54 per cent of the F3 powder was retained on the 300 µm sieve. This powder could therefore be considered homogeneous and suitable for encapsulation

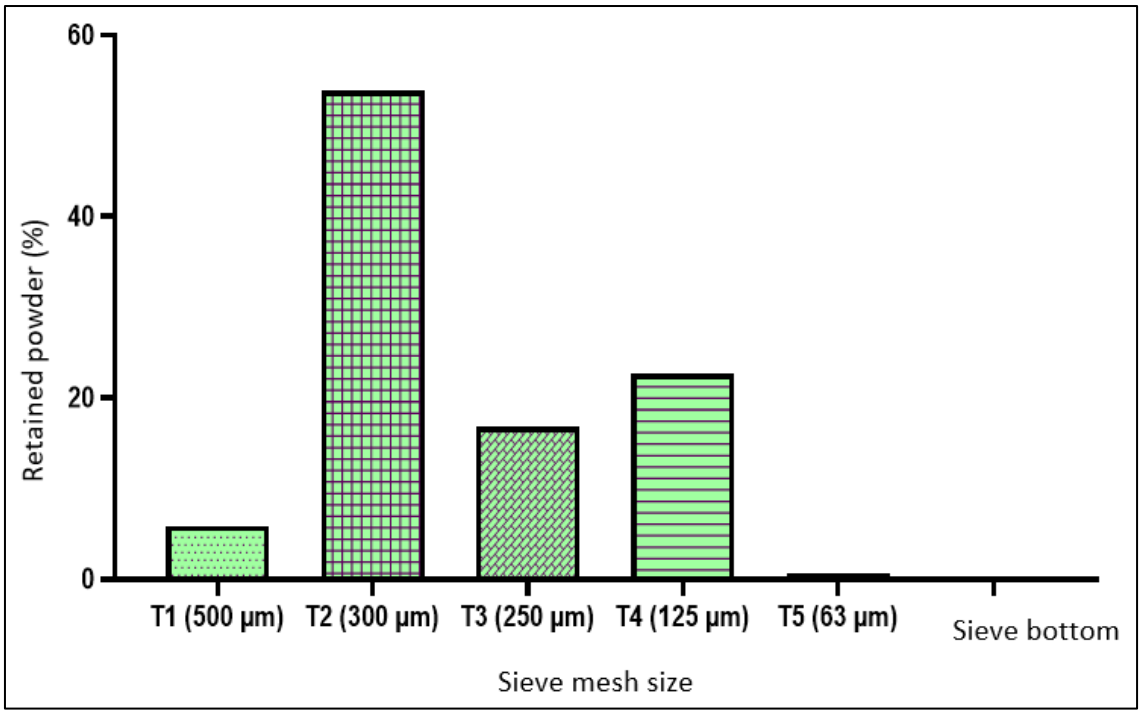

Figure 2: Percentage of powder retained as a function of sieve mesh size

4.2.3.2 Settling and flow properties

A mass of 17.14 g of stabilised powder (F3), corresponding to 25 ml, was placed in a graduated cylinder without being compacted. Settling tests were carried out using a settling apparatus to determine the Carr index and the Hausner index using the preceding formulae. The results are presented in table 6.

A flow test was also carried out on the stabilised powder. It took 5 seconds for the 100 g of powder to flow completely.

Table 6: Compaction suitability tests on the stabilised powder (F3)

Initial volume Volume initial	**25 ml**
Final volume Volume final	21,5 ml
Carr's index Indice de Carr	14 %
Hausner's index Indice de Hausner	1,16

4.2.4 Capsule formulation

Table 7 details the final capsule formulation. A portion of the powder was stabilized under these conditions; calculations based on the capsule filling chart indicated that, using size No. 0 capsules, no diluent was required to achieve proper filling.

Table 7: Final formulation for the preparation of *Desmodium adscendens* capsules

Constituent	**Role**	**Composition per capsule**	**Composition for 100 capsules**
Dry extract	Active ingredient	486 mg	48,600 mg
Anhydrous colloidal silica	Lubricant and stabilizer	9.72 mg	972 mg
Total		495.72 mg	49,572 mg

4.2.5 Pharmaceutic Tests

4.2.5.1 Mass Uniformity

The masses of the contents of the 20 capsules randomly selected for this test averaged 588.35 mg, which corresponds to a maximum permissible deviation (E%) of 7.5 per cent, or 44.13 mg. The limits (588.35 ± 44.13 mg) were therefore 544.22 mg and 632.48 mg. None of the values for the contents of the 20 capsules sampled fell outside these limits.

4.2.5.2 Disintegration test

All six capsules randomly selected for this test disintegrated after 3 minutes 45 seconds.

4.2.5.3 Microbiological tests

Table 8 presents the results of the microbiological testing of the capsules. This table demonstrates the good microbiological quality of the preparation: very low contamination.

Table 8: Microbiological testing of capsules

Tests	Result (CFU/g)	Standard (CFU/g)
Total mesophilic aerobic count	267	10^3
Total yeast and mould count	0	10^2
Test for Enterobacteriaceae	0	Not detected
Test for Enterococci	0	Not detected
Test for Staphylococcus aureus	0	Not detected

4.2.6 Packaging and labelling

As shown in Figure 3, the capsules produced were packaged and labelled under the name 'DESPATITE'. The polyethylene terephthalate bottles bear information relating to the product's identification and traceability. The secondary packaging is a cardboard box containing the same informations as those on the label.

Figure 3: DESPATITE capsules

5 DISCUSSION

The aqueous extract, obtained with a yield of 13.4 per cent, was dark brown. This result corroborates that of Chuisseu et al., who reported a yield of over 10 per cent [17]. Phytochemical analysis of the aqueous extract of *Desmodium adscendens* leaves showed that it was rich in bioactive secondary metabolites, notably phenols, flavonoids, terpenes,

saponins and alkaloids. This result is similar to those reported by Yongoua et al. in 2024 and Magielse et al. in 2013 [7,11]. These compounds have been shown to play a role in the plant's hepatoprotective activity; saponins bind to bile acids and help to eliminate them from the body [6]; Flavonoids serve to inhibit the pro-inflammatory activities of enzymes involved in the production of free radicals, such as cyclooxygenase, lipoxygenase or inducible nitric oxide synthase. Antioxidant metabolites such as polyphenols have the ability to maintain cellular integrity by slowing down or halting the chain of oxidative reactions within the cell [18].

The extract's solubility in water is rather low: 0–50 g/l. This could explain the great quantity of solvent used to extract the bioactive compounds.

Colloidal silica, chosen as an excipient, has the advantage of modulating hygroscopicity and making the powder more suitable for encapsulation; it has lubricating and absorbent properties [14]. A 2 per cent content of anhydrous colloidal silica yielded the best results. This is in line with Allen's recommendations, which suggested this concentration for solid oral formulations [19].

The effective human dose of 972 mg/day for a 60 kg adult is well below the toxic dose (5,452.92 mg/kg) derived from the findings of Quaye et al [20].

Traditional healers in the harvesting area recommended consuming the decoction twice a day. It is on the basis of this information that the effective human dose of the capsules can be administered in two doses.

Particle size analysis revealed that more than 50 per cent of the particles in the dry extract powder of *Desmodium adscendens* were retained by the 300 µm sieve, thereby classifying the powder as homogeneous according to the criteria of the European Pharmacopoeia. This profile is particularly well-suited to capsule formulation, as powder that is too fine is generally cohesive and prone to agglomeration, whilst powder that is too coarse may compact poorly. Powders with an intermediate particle size distribution offer a good compromise for acceptable flow and compressibility.

The measured flow rate of 5 seconds for 100 g indicates excellent flowability of the mixture. According to industry standards, a powder with a flow rate of ≥ 10 g/s is considered highly free-flowing [21]. This performance is confirmed by a Hausner index between 1.11 and 1.25. The Carr index ranged between 11 and 15 per cent and the Hausner index between 1.11 and 1.18, indicating a free-flowing powder. These results demonstrate good flowability, which is essential for the uniform filling of capsules. These values comply with the manufacturing standards described in *Pharmaceutical Powder Compaction Technology* [22]. The excellent flowability is partly attributable to the 2 per cent anhydrous colloidal silica, which acts here as an anti-caking agent by reducing inter-particle interactions—a role recognised in the literature as effective in improving the flowability of hygroscopic powders [22, 23].

The mass uniformity test is a fundamental regulatory requirement for solid unit dosages. All the capsules tested remained within ±7.5 per cent of the mean mass, which complies with the requirements of the 11th edition of the European Pharmacopoeia for capsules weighing > 300 mg [24]. This is favourable for therapeutic safety and inter-dose bioequivalence.

The mean disintegration time measured was 3 minutes and 45 seconds, which is well below the maximum limit permitted by the European Pharmacopoeia for capsules [24]. This result indicates that the gelatine coating has a good capacity to release the powder mixture under physiological conditions. The non-detection or low levels of microorganisms ensure compliance with international regulatory criteria, thereby limiting the risks of contamination that could interfere with the efficacy of the active ingredient or cause infections in patients.

6 CONCLUSION

The aim of this study was to develop a stable and cost-effective galenic formulation based on dry aqueous extract of *Desmodium adscendens* (Fabaceae), a plant traditionally used for the management of liver disorders. The phytochemical composition of the powder revealed the presence of various secondary metabolites likely to possess hepatoprotective activity.

The hygroscopic nature of the dry extract justified the addition of anhydrous colloidal silica to improve the powder's suitability for encapsulation. The selected daily dose (972 mg of dry extract divided between two size No. 0 capsules) ensures a consistent content of phenolic compounds (88.56 mg of EAT/g), which are recognised for their beneficial effects in the management of liver conditions.

Compliance with both the pharmaceutical and microbiological recommendations for the capsules demonstrates their effective ability to release their contents in a physiological environment and minimises the risk of contamination. This

study contributes to the scientific validation of Desmodium adscendens and forms part of an initiative to develop the African Pharmacopoeia through traditional medicines that meet international scientific standards.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

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