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FORMULATIONS AND STANDARDIZATION OF THE AQUEOUS EXTRACT FROM THE LEAVES OF *MORINDA LUCIDA* BENTH, A HYPOGLYCEMATING PLANT

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Keywords:

Plant, *Morinda lucida* Benth, Hypoglycaemia, Aqueous extract, capsule

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ABSTRACT: With a view to enhancing endogenous knowledge about plants, the present study has been aimed at developing and standardizing dosage forms based on the aqueous extract of Morinda lucida Benth, a plant with hypoglycaemic anti-hyperglycaemic properties. After the aqueous extraction of the leaves of this plant, by maceration, phytochemical screening was carried out on a TLC plate by using the method of *Wagner* and *Bladt* (1996) then three capsule formulations were produced (F1, F2 and F3). Different pharmacotechnical tests were conducted according to the European Pharmacopoeia (Eur. Ph.). The presence of alkaloids, flavonoids and saponosids in the aqueous extract attests to its hypoglycaemic and anti-hyperglycaemic properties. Likewise, their presence in the capsules gives them these same properties and shows the neutrality of the used excipients on the phytochemical's compounds of the extract. The average capsule masses are 190.525 mg, 250.04 mg, and 381.54 mg, respectively, for the formulas F1, F2, and F3 capsules. The polyphenols that were used as a tracker in the aqueous extract did not show a good dissolution profile in milliQ water, which would be due to their complexation by gelatine or to their decomposition after dissolution in the medium, however, it was observed a dissolution in accordance with the specifications in 0.1 M hydrochloric acid. The formula F3 is that which complies with all the Eur. Phspecifications and could be recommended for preclinical testing in order to use as *Morinda lucida* Benth leaves substitute.

INTRODUCTION: Diabetes mellitus is a chronic disease that occurs when the pancreas does not make enough insulin or when the body does not use the produced insulin properly.

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Diabetes mellitus represents a major public health problem and is one of the four priority non-communicable diseases targeted by the world's governments for intervention.

In recent decades, there has been a steady rise in the number of diabetes mellitus cases and the prevalence of the disease [1]. The number of people affected by diabetes mellitus increased from 108 million in 1980 to 422 million in 2014. The global prevalence of diabetes in adults over 18 years of age has almost doubled since 1980, from 4.7% to

8.5% [2]. There are three types of diabetes mellitus: type 1, type 2 and gestational. Type 2 diabetes mellitus, also called insulin-resistant diabetes or diabetes of maturity, results from the body's misuse of insulin. Type 2 diabetes mellitus covers the majority of cases of diabetes mellitus. It is largely the result of being overweight and lacking physical activity [1]. Type 2 diabetes mellitus can cause complications in many parts of the body and can increase the overall risk of premature death. Possible complications include myocardial infarction, stroke, kidney failure, amputation of the legs, loss of vision, and nerve damage [1]. In response to this global health challenge, the world health organization (WHO) Expert Committee on Diabetes Mellitus called for further evaluation of popular methods of managing the disease due to the mortality and morbidity resulting from the related complications and inconveniences associated with the use of ordinary anti-diabetic drugs [3]. To achieve this goal, several medicinal plants have been inventoried for their hypoglycaemic efficacy. Among native plants used in the local treatment of diabetes mellitus is found *Morinda lucida* Benth. *Morinda lucida* Benth, belonging to the Rubiaceae family, is a medium-sized tree used as a medicinal plant in West Africa for the treatment of malaria, diabetes, hypertension, stroke, dysentery, stomach aches, ulcers, leprosy, gonorrhea, and other restless conditions [4, 5]. *Morinda lucida* Benth is still called Sulfur Tree in French; xwèsin, xwèswè, xwèso, wètin, gwèsi or kwema in Fon; oruwo or oju ologbo in Yoruba; acike mashi, xwaso or wuli in adja; zaklaoun, zazaklaoun, zanklaoun or xwayiso in minan and kasi làga in Bariba. Studies have shown that the leaves and bark of *Morinda lucida* Benth exhibit anticancer [6], hepato-protective [7], hypoglycaemic and antidiabetic properties [8]. The methanolic extract from the leaves of *Morinda lucida* Benth has been shown to be hypoglycaemic in diabetic rats and antihyperglycemic in normal rats [9, 10]. The same is true for the ethanolic extract of these leaves in diabetic rats treated with alloxan [4]. This study aims to develop and standardize dosage forms based on the aqueous extract of the leaves of *Morinda lucida* Benth, a plant with hypoglycaemic and anti-hyperglycaemic properties.

MATERIALS AND METHODS:

Plant Material: The fresh leaves of *Morinda lucida* Benth were harvested in April 2021 in Dassa-Zoumé, a central region of Benin Republic. The authentication of the plant was carried out at the National Herbarium of Benin Republic at the University of Abomey-Calavi under number YH 546 / HNB.

Preparation of the Plant Extract: The fresh leaves of *Morinda lucida* Benth were cut, washed with water to remove all contaminants, and dried in the laboratory at room temperature. The dry leaves were then ground. 50 g of the powdered plant material obtained were macerated in 500 mL of Milli-Q water for 48 h then filtered. The filtrate was concentrated at 40 ° C in an oven. The extract obtained was stored at 4 ° C to prevent degradation.

Phytochemical Screening: The screening was carried out by the method of Wagner and Bladt (1996)[11] using Thin Layer Chromatography (TLC). The extract and the capsules were dissolves in Milli-Q water and in 0.1 M hydrochloric acid before the screening.

Galenic Formulation:

Hard Shell Capsules Based on Aqueous Extract of *Morinda lucida* Benth: It was made capsules using the three formulations given in **Table 1** produced by adding different proportions of excipient to the extract.

TABLE 1: AMOUNT IN PERCENTAGE OF INGREDIENTS PER CAPSULE

Formulas	Active extract (%)	Flow regulator (%)	Binder (%)	Lubricant (%)
FormulaF1	62.5	37.5	-	-
FormulaF2	62.5	28.57	8.92	-
FormulaF3	59.52	27.21	8.5	4.76

Assessment of Capsules Quality:

Uniformity of Mass: The difference in mass between each empty capsule and its corresponding full was calculated, the average mass of powder contained in the capsules was then determined. The deviations around the mean were calculated and compared to the standards of the Eur. Ph[12].

Disintegration test: The test was performed using two disintegration media: Milli-Q water and 0.1M

hydrochloric acid at 37±0.5 ° C using the HANSON disintegration apparatus.

Dissolution test: The dissolution test was performed with SOTAX dissolution apparatus with palette speed at 75 rpm in Milli-Q water and 0.1M hydrochloric acid at 37±0.5 ° C.

Determination of Total Polyphenols in the Crude Extract: The total polyphenols were quantified in crude extracts, capsules and dissolution media samples. The method of *Singleton and Rossi* (1965) [13] is used to determine polyphenols with some modifications.

The assay was carried out using the Folin-Ciocalteu reagent. 625 µL of the Folin-Ciocalteu reagent was added to125 µL of the sample. After five (5) minutes of incubation for, 500 µL of sodium carbonate (Na_2CO_3) at 75 mg / mL was added. The mixtures were then incubated for 2 h. Their absorbance was read on a VWR UV-6300 PC UV-Visible spectrophotometer at a wavelength of 760 nm.

Statistical Analyses: Statistical analysis was carried out using EXCEL 2019 software and GraphPad Prism 8.0.

RESULTS: Plants with hypoglycaemic and antihyperglycemic potential play an important role in controlling the blood glucose level, thus preventing humans from hyperglycaemia.

Extraction Yield: The aqueous extract of *Morinda lucida* Benth leaves was obtained with a percentage yield of 15.31±0.76% (w / w), corresponding to a total mass of 76.55 g of extract harvested.

Phytochemistry of the Aqueous Extract of *Morinda lucida* Benth: The phytochemical screening carried out on the crude aqueous extract, dissolved in Milli-Q water and in hydrochloric acid, reveals the presence of alkaloids, coumarins, flavonoids, and saponosids. The results are seen in **Table 2**.

TABLE 2: PHYTOCHEMICAL COMPOSITION OF THE AQUEOUS EXTRACT OF *MORINDA LUCIDA BENTH* LEAVES DISSOLVED IN MILLI Q WATER AND IN 0.1M HYDROCHLORIC ACID

Group of compounds	Class	*M. lucida* Benth milliQ water	*M. lucida* Benth HCl
Nitrogen compounds	Alkaloids	+	+
Terpene compounds	Triterpenes	-	-
Heterosides	Saponosids	+	+
	Glycosides	-	-
	Anthracene derivatives	-	-
Phenolic compounds	Tanins	-	-
	Flavonoids	+	+
	Glycosylated coumarins	+	+
	Coumarin genins	+	+

+ : Présent; - : non detected.

Assessment of Capsules Quality: Following the various formulations, pharmacotechnical tests were carried out on the obtained capsules in order to verify their stability and to approve their compliance with the requirements of the Eur. Ph.

Phytochemistry of the Capsules: The results presented in **Table 3** show that the alkaloids, coumarins, flavonoids, and saponosids initially present in the raw aqueous extract of *Morinda lucida* Benth are found in the capsules produced from the extract.

TABLE 3: PHYTOCHEMICAL COMPOSITION OF THE CAPSULES PRODUCED

Group of compounds	Class	Formula 1		Formula2		Formula3	
		H_2O	HCl	H_2O	HCl	H_2O	HCl
Nitrogen compounds	Alkaloids	+	+	+	+	+	+
Terpene compounds	Triterpenes	*	*	*	*	*	*
Heterosides	Saponosids	+	+	+	+	+	+
	Glycosides	*	*	*	*	*	*
	Anthracene derivatives	*	*	*	*	*	*
Phenolic compounds	Tanins	*	*	*	*	*	*
	Flavonoids	+	+	+	+	+	+
	Glycosylated coumarins	+	+	+	+	+	+
	Coumarin genins	+	+	+	+	+	+

+: Présent; *: non searched.

Uniformity of Mass: The average masses of the obtained capsules are 190.52 ± 4.95 mg, 250.04 ± 29.06 mg, and 381.54 ± 5.08 mg, respectively for the formulas F1, F2, and F3. The mass of the contents of 20 capsules weighed separately is shown in **Table 4**. The percentages of each mass compared to the mean were calculated in each case and compared to the specifications of the Eur. Ph.

TABLE 4: UNIFORMITY TEST OF DIFFERENT CAPSULES FORMULATED

N°	Masses F1 (mg)	Percentage (%)	Masse F2 (mg)	Percentage (%)	Masse F3 (mg)	Percentage (%)
1	187.5	98.41	235.9	94.35	382.2	100.17
2	179.9	94.42	254.7	101.86	387.3	101.50
3	208.8	109.59	281	112.38	375.8	98.49
4	184.3	96.73	261.5	104.58	376.6	98.70
5	211.3	110.90	238.8	95.50	375	98.28
6	168.3	88.34	250.3	100.10	376.2	98.60
7	186.3	97.78	231.4	92.54	388.6	101.85
8	196.1	102.93	240.1	96.02	382.2	100.17
9	186	97.62	265	105.98	381.9	100.09
10	199.2	104.55	257.4	102.94	376.8	98.75
11	193.8	101.72	243.5	97.38	386.5	101.29
12	178.1	93.48	267.3	106.90	375.8	98.49
13	192.1	100.83	236.4	94.54	372.4	97.60
14	196	102.87	252.1	100.82	387.2	101.48
15	202.6	106.34	257.7	103.06	385.3	100.98
16	188.4	98.88	213.2	85.26	383	100.38
17	177.2	93.01	240.9	96.34	382.5	100.25
18	198.5	104.18	269.4	107.74	381.1	99.88
19	195.6	102.66	227.2	90.86	386.5	101.29
20	180.5	94.74	277	110.78	387.9	101.66
Sum	3810.5	2000	5000.8	2000	7630.8	2000
Average	190.52	100	250.04	100	381.54	100

Active Ingredient Content: Taking into account the percentage of active ingredients in each formulation, the average active compound content of the capsules was determined and presented in **Table 5**.

TABLE 5: AVERAGE WEIGHT OF ACTIVE COMPOUND IN THE CAPSULES

	FormulaF1	FormulaF2	FormulaF3
Active compound content (mg)	119.08	156.8	227.09

Disintegration test: The results are shown in **Table 6.** The mean disintegration time in water of the three formulas is around 19 min while in HCl it is faster and is about 10 min.

TABLE 6: CAPSULE DISINTEGRATION TIME (MINUTE (MIN): SECOND (S)

N°	Milli-Q water			HCl		
	Formula 1 min : S	Formula 2 min : S	Formula 3 min : S	Formula 1 min : S	Formula 2 min : S	Formula 3 min : S
1	17 : 09	16 : 47	18 : 28	9 : 22	9 : 48	9 : 45
2	18 : 19	17 : 20	18 : 42	9 : 34	10 : 08	9 : 54
3	19 : 16	18 : 08	18 : 57	9 : 45	10 : 17	10 : 05
4	19 : 53	19 : 12	19 : 14	9 : 58	10 : 23	10 : 11
5	20 : 02	20 : 28	19 : 46	10 : 07	10 : 32	10 : 20
6	20 : 30	20 : 45	20 : 07	10 : 21	10 : 44	10 : 33
Average	19 : 18	19 : 17	19 : 20	9 : 25	10 : 31	10 : 13

Calibration Plots of Gallic Acid and Determination of Polyphenols in the Extract: Two calibration plots were established from the regression lines y = 0.0021x + 0.1092 and y = 0.0022x + 0, 0797 respectively for gallic acid dissolved in milliQ water **Fig. 1** and in 0.1M hydrochloric acid **Fig. 2** with r^2= 0.9985 and r^2= 0.9992 respectively.

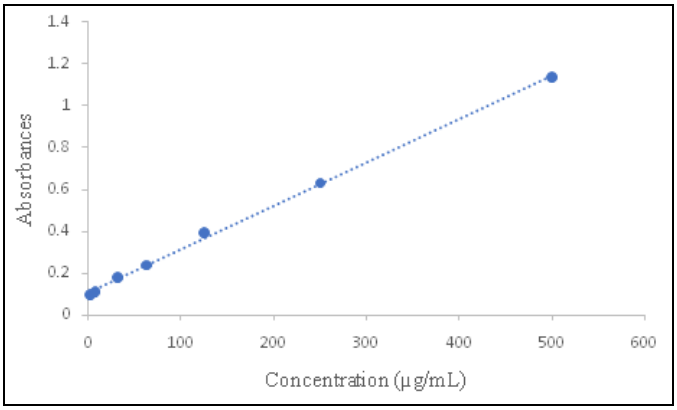

FIG. 1: CALIBRATION CURVE FOR GALLIC ACID DISSOLVED IN MILLIQ WATER

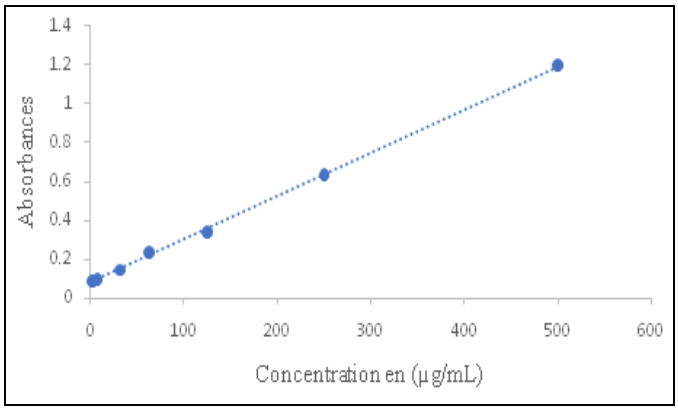

FIG. 2: CALIBRATION CURVE FOR GALLIC ACID DISSOLVED IN 0.1 M HYDROCHLORIC ACID

Determination of Total Polyphenols in the Crude Extract: It was obtained in the aqueous extract concentrations of 116.5714 µg Eq AG / mg and 109.303 µg Eq AG / mg, respectively for dissolution of the extract in Milli-Q water and in 0.1 M hydrochloric acid.

Dissolution test: The capsules were dissolved in Milli-Q water and in 0.1M hydrochloric acid following the recommendations of the Eur. Ph. 6. The samples were subjected to an assay to quantify the extract dissolution during the time.

A good dissolution profile was observed in hydrochloric acid media with the dissolution of more than 75% after 15 min **Fig. 4** compared to the dissolution profiles in Milli-Q water **Fig. 3**.

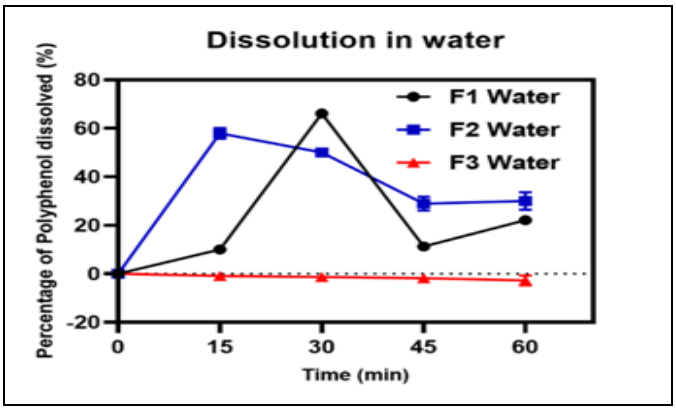

FIG. 3: DISSOLUTION RATE IN MILLIQ WATER FOR DIFFERENT FORMULA

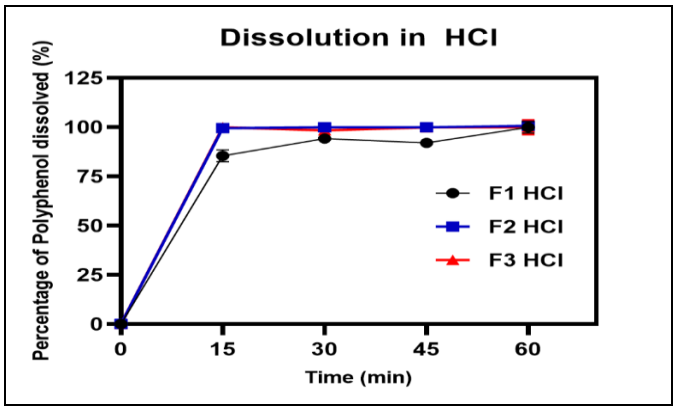

FIG. 4: DISSOLUTION RATE IN 0.1 M HCL FOR DIFFERENT FORMULA

Table 7 establishes the dissolution percentage of the capsules of each formulation at 45 min. It appears that in Milli-Q water medium, the dissolution is11.30% and 28.99% for formulation F1 and F2 respectively and zero for formulation F3. While in 0.1 M hydrochloric acid medium, the dissolution was high with 92.03% for F1 and 100% for F2 and F3.

TABLE 7: DISSOLUTION RATE OF DIFFERENT FORMULA AT 45 MIN IN MILLIQ WATER AND IN 0.1M HYDROCHLORIC ACID MEDIUM

	MilliQ water	HCl 0,1 M medium
Formula F1	11.30%	92.03%
Formula F2	28.99%	100%
Formula F3	0%	100%

DISCUSSION: Plants contain a multitude of phytochemicals compounds with diverse properties, the study of which allows each plant to be associated with therapeutic potential for the good of humans and animals. The aqueous extract of the leaves of *Morinda lucida* Benth was obtained with a yield of 15.31 ± 0.76% (W/ W), and the phytochemical screening carried out reveals the presence of alkaloids, coumarins, flavonoids, and saponosidsin agreement with the observations of Chokki M *et al.*, (2020) [14]. The extraction yield of 15.31±0.76% obtained is higher than that of 9.99% found by Ogboye R. M. *et al.*, (2021) [15,] and an absence of tannins should be noted.

These differences could be due to the difference in the place of harvest and the methods of extraction. Previous studies have established the hypoglycaemic activity of alkaloids and flavonoids [16, 17] and the antihyperglycemic activity of flavonoids and saponosids [14]. The presence of these three compounds in the aqueous extract of *Morinda lucida* Benth, in accordance with the findings of Adeleye O. O. *et al*., (2018) [18], confirms the endogenous beliefs of anti-diabetic activity of *Morinda lucida Benth*. The non-variability of the results of compounds after dissolving the aqueous extract in a 0.1 M hydrochloric acid solution compared to that dissolved in MilliQ water showed that the gastric acidity has no destructive influence on the said chemical groups.

The tests carried out on the formed capsules established a variability of the results in relation to the composition of each formula. According to the different formulas obtained, it was noted a progressive and proportional increase between the average mass of the capsules and the quantity of extract. This mass growth is due to the optimization of the formulations by adding other excipients. From formulation F1 to formulation F2, the addition of binder allowed the extract to be concentrated in the mixture since the binder is denser than the flow regulator; and the addition of lubricant to the F2 formulation to obtain the F3 formulation resulted in better fluidity of the powder, favourable to the good filling of the capsules.

The non-variability of the results, following the phytochemical screening on the capsules, shows that the active compounds present in the extract are preserved in the finished products. Therefore, the excipients have no altering effect on them. Likewise, the stomach's acidity does not affect the chemical composition of the finished products obtained. The assay revealed, by the results of Osuntokun O. T. *et al.*, (2016) [19], a significant amount of polyphenols in the aqueous extract of *Morinda lucida* Bent leaves, which justifies the choice of this group of compounds as a tracer for the dissolution test. In addition, the amount of polyphenols in the aqueous extract dissolved in water and in hydrochloric acid confirms the previous results of the neutral action of stomach acid on the phytochemical constituents of the extract. For the mass uniformity test, comparing the results obtained to the specifications of the Eur. Ph., Only Formulation F1 and Formulation F3 were conformed to the specifications. The average mass of the capsules of the formulation F2 was less than 300 mg; the deviations between the masses and their average were not within specification (more than two of the twenty units deviated from the average mass by a percentage of 10 and the mass of some units deviated from twice that percentage). As for the formula F1 and F3, the averages masses of the capsules were respectively less and greater than 300 mg, and the differences between the masses and their averages were in accordance with the specifications

The formulated capsules passed the Eur. Ph., disintegration test with an average disintegration time of 19 min 18 S, 19 min 17 S and 19 min 20 S respectively for formula F1, F2, and F3 in Milli-Q water and 9 min 25 S, 10 min 31 Sand 10 min 13 S respectively for formula F1, F2, and F3 in 0.1 M hydrochloric acid. These results comply with the Eur. Ph., standards. According to which hard shell capsules should have a disintegration time of 45min at most. The stomach acidity reproduced here in vitro by the concentration of 0.1 M hydrochloric acid shows this time its action on the disintegration time of the capsules by promoting faster disintegration.

The dissolution test is an important parameter in order to assess the ability of the capsules to release the active ingredient they contain into the medium. To monitor the percentage of dissolution of the capsules, polyphenolic compounds were used as tracers. However, capsules content did not show a good dissolution profile in Milli-Q water. The low percentage of dissolution observed is thought to be attributable to the instability of the polyphenols in water. On the other hand, they have a good dissolution profile with 75% in 0.1 M hydrochloric acid medium 45 min, which is in accordance with the specifications of the Eur. Ph. These revealed the favourable action of gastric acidity on the dissolution of the capsules.

CONCLUSION: In total, *Morinda lucida* Benth contains flavonoids, alkaloids, saponosids which would be responsible for its anti-diabetic activity. From the three capsules formulations obtained, the

formula F3 with the highest average mass (381.54 mg) passed all the pharmacotechnical tests with none of its units deviating from the average mass by a percentage more 7.5%, a disintegration time of 19 min 20 S and 10 min 13 S respectively in Milli-Q water and in hydrochloric acid and the total dissolution of 100% at 45 min in 0.1 M hydrochloric acid medium. It can therefore be used as *Morinda lucida* Benth leaves to substitute for its hypoglycaemic and anti-hyperglycaemic properties.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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