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THE ORAL CAPSULE- THE MOST APPROPRIATE DOSAGE FORM FOR *CROTON MEMBRANACEUS*

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ABSTRACT

Recent studies have provided relevant scientific support for the use of *C. membranaceus* root in the treatment of prostate cancers. In the current study, we formulated both liquid and solid oral dosage forms from the ethanol extract of *C. membranaceus* root and investigated their physicochemical properties, release effects and suitability in comparison with the currently used aqueous decoctions and directly powdered plant material, in order to determine the most appropriate and suitable dosage form that will maximize the benefits of therapy with this species. The prepared solution from the ethanol extract had characteristics similar to the aqueous decoction, but unlike the later, it maintained its specifications and stability throughout the period of study. However, the use of large volumes, convenience and suitability for long term use, will limit their application. The assessed properties of the prepared tablets and capsules met all the specifications required for good quality and they can be used in simple and convenient doses of one tablet or capsule, three times a day, in place of unstable decoctions and large volume solutions. The *in vitro* release profile of the capsules was however, better (98.8±1.3 % after 45 minutes) and significantly higher ($P<0.01$) than that of the tablets (85.2±1.6 %), and with the protection of the extract from light and masking of unwanted color and taste as added advantages, the oral capsules should be the preferred choice of solid dosage form for *C. membranaceus*. The capsules of the direct plant material had active content (2.8±1.4 mg) and *in vitro* cumulative release after 45 minutes (42.0±2.6 %) lower and significantly different ($P<0.01$) from those of the tablets and capsules prepared with the extract. The results strongly support the assertions that the formulation of suitable and appropriate herbal remedies from plant extracts may be more desirable, advantageous and therapeutically more beneficial than incorporating the direct plant materials, and that the oral capsule of *C. membranaceus* root extract will ensure compliance and maximize therapeutic outcomes.

INTRODUCTION: The use of the root of *Croton membranaceus* (Euphorbiaceae) in the treatment of prostatic hypertrophy is well known in Ghana [1]. It is used at the Centre for Scientific Research into Plant Medicine (CSRPM), Mampong-Akwapem, for the treatment and management of prostate enlargement and related cancers. In earlier studies, we revealed significant cytotoxic activity of the extract on human cancer cells [2], and of compounds isolated from this species, against human prostate cancer (PC-3) cells [3], which provided relevant scientific support for the use of *C. membranaceus* in the treatment of cancers.

The dosage forms currently employed are; the aqueous root decoctions, which are required in large volumes and are bitter and unstable, and the oral capsule of the directly powdered plant material, with uncertain drug release properties. In the current study, we investigated various formulations of the root extract of this species against the current dosage forms in order to determine a suitable and standard formulation that will maximize the benefits from the use of the plant in treating cancers.

MATERIALS AND METHODS:

Plant material: The roots of *C. membranaceus* were collected in November 2009, at Mampong-Akwapem, Ghana, and authenticated at CSRPM and a voucher specimen (CSRPM/09/CM/02) was placed in their herbarium. The plant material was oven dried at 50^{o}C and comminuted into coarse powder for extraction.

Extraction: The powdered *C. membranaceus* root was extracted in batches of 500 g each with 2.5 L of ethanol (96 %) using a Soxhlet extractor. The extracts obtained were pooled and concentrated to a syrupy mass under reduced pressure before drying completely over silica gel in a vacuum chamber at room temperature. This produced a dark brown extract (extractive value 3 %w/w) which was stored in refrigerator until used further.

Preparation of the formulations:

Liquid dosage forms: Locally and at the CSRPM, 3 %w/v aqueous decoctions of powdered *C. membranaceus* root are prepared and used in doses of a tumblerful (100 ml), every eight hours for the treatment of prostate hypertrophy.

Decoction: Based on the traditional preparation and use, 30 g of powdered *C. membranaceus* root was boiled in 1 L of distilled water for 2 hours, cooled, decanted, the marc pressed and the pooled extract filtered through a 0.45 µm Whatmann filter paper. The product was then made up to 1 L with more water, bottled, labeled, stored in a cool dry place away from light and studied for six months.

Modified decoction: After a careful study of the physicochemical properties of the decoction as prepared above, a similar product was again prepared, but this time sweetened with aspartame (0.01 %w/v) and preserved with a combination of methyl and propyl Paraben (0.1 %w/v), stored in a cool dry place and studied for six months.

Liquid formulation from the ethanol extract: In accordance with the stated dosage of the decoction as; a tumblerful (100 ml), three times a day, and with the extractive value of *C. membranaceus* in water as 0.56 %w/w, a similar but optimized liquid product (**Table 1**) was prepared to contain 16.8 mg of the ethanol extract per dose (100 ml). Due to its partial water solubility, the required amount of extract was predissolved in ethanol, diluted with the other ingredients and then made up to volume with water. The total amount of ethanol was 10 %v/v. This formulation was stored in a cool dry place and studied for six months.

TABLE 1: FORMULATION OF A LIQUID PRODUCT FROM THE *C. MEMBRANACEUS* ROOTS EXTRACT

Ingredient	Quantity
C. membranaceus root extract	16.8 mg
Ethanol	10 ml
Aspartame	10 mg
Methyl Paraben	0.08 g
Propyl Paraben	0.02 g
Water upto	100 ml

Solid dosage forms: A dose of 500 mg of powdered *C. membranaceus* root is taken three times a day for prostate hypertrophy and cancers. Therefore, with an extractive value of 3 %w/w in ethanol, 15 mg of extract will be required per dose. As such the tablets and capsules were formulated to contain 15 mg of *C. membranaceus* root extract per unit.

Tablets: Using a die volume corresponding to a tablet weight of 320 mg, granules for compressing 200 tablets (**Table 2**) were prepared by the wet method and assessed. After addition of the talc and uniform mixing, the granules were compressed on a single stage Manesty tablet press and the tablets analyzed.

Capsules: For an expected capsule content weight of 250 mg, a granulation for 200 capsules (Table 2) was prepared by the wet method and analyzed. After addition of the glidant (talc), the granules were filled into size 2 hard gelatin capsule shells using a semi-automated capsule filling machine (Model TMP Mini T-50) and the resulting capsules evaluated.

TABLE 2: FORMULATION OF 200 TABLETS (DIE VOLUME, 320 MG) AND 200 CAPSULES (FILL WEIGHT, 250 MG) OF *C. MEMBRANACEUS* ROOT EXTRACT

Ingredients	Quantity (g)	
	Tablets	Capsules
C. membranaceus root extract	3.0	3.0
Lactose	53.96	41.5
Maize Starch	3.2	2.5
Acacia gum (for Mucilage)	3.2	2.5
Talc	0.64	0.5

Commercial capsules: A random sample of 200 capsules of a commercial product of *C. membranaceus* root (coded CC_1) was also obtained from the Kumasi market for analysis, evaluation and comparison. They were 250 mg capsules with a dosage of two capsules to be taken three times a day.

Analysis and evaluation of the formulations:

Liquid formulations: The organoleptic characteristics (color, odor and taste), solubility, viscosity, pH and stability in terms of active content and microbial growth or load of the formulations were determined and monitored throughout the study period of six months.

Granules:

Angle of repose: The fixed height cone method was used in the determination of the angle of repose. A quantity of each set of granules was used to carefully build a cone through a funnel clamped at a height of 2 cm onto a horizontal surface until the tip of the pile just touched the lower tip of the funnel. The diameter of the resulting cone was measured and the angle of repose calculated from the relationship; $\theta = \tan^{-1} (h/r)$, where h and r, are the height and radius of the base of the cone, respectively [4]. The determinations were done in triplicate.

Compressibility index and Hausner's ratio: 50 g of granules were each poured gently into a 200 ml measuring cylinder and the initial volume (V_o) of the granules measured. The cylinder was then tapped from a height of 3 cm until there was no further change in volume and the final volume (V_f) determined. The bulk density (ρ_{bulk}) and tapped density (ρ_{tapped}) were calculated and used to determine the compressibility (Carr's) index [$(\rho_{tapped} - \rho_{bulk}) / \rho_{tapped} \times 100$] and Hausner's ratio ($\rho_{tapped} / \rho_{bulk}$) for each set of granules, after triplicate determinations [4].

Tablets and Capsules:

Weight uniformity: A random sample of twenty tablets was individually weighed and the average mass determined. The percentage deviations of the individual masses from the average mass were then calculated. Also, twenty capsules of the prepared and commercial batches were each selected at random. Each capsule was weighed on an analytical balance, carefully emptied of its content, the shells reweighed and the weight of content determined. The collective weight of content, average weight of content per capsule and the deviations (%) of individual content weights from the mean were calculated [5].

Resistance to crushing (Hardness): Ten tablets were randomly selected and each tablet was placed between the jaws of a Monsanto hardness tester with its diameter along the direction of applied force and the force (N) required to just crush the tablets measured [4, 5]. The mean crushing force for the tablets, was then determined.

Friability: A random sample of twenty tablets was carefully dedusted and accurately weighed on analytical balance. The sample was then placed in the drum of the Roche's Friabilator and rotated 100 times, after which the tablets were removed, carefully dedusted and accurately reweighed, and the percentage weight loss determined [5].

Disintegration time: The disintegration time was determined in distilled water at 37±0.5^{o}C. For each test, a tablet/capsule was placed into each of the six tubes of the Erweka disintegration apparatus with discs and operated for 15 minutes [4]. Triplicate determinations were conducted for both tablets and capsules.

Content uniformity: Ten tablets or capsules were randomly selected. Each tablet or the content of each capsule was dissolved in 30 ml of 10 %v/v ethanol, filtered through a 0.45 µm Whatmann filter paper, diluted appropriately and assayed spectrophotometrically at 248 nm. The assayed contents were expressed as a percentage of the average content per dosage unit (15 mg).

***In vitro* dissolution study:** The *in vitro* release of the *C. membranaceus* root extract from the tablets and capsules was conducted using an Erweka six vessel rotating paddle USP type II apparatus [4, 5, 6]. The dissolution medium was 900 ml of 0.1M Hydrochloric acid (pH 1.0), maintained at 37±0.5^{o}C and with a paddle speed of 100 rpm. A dosage unit was placed in each vessel and samples of 20 ml solution were withdrawn at predetermined time intervals (between 5-60 minutes) and replaced with fresh dissolution medium at 37^{o}C. With the commercial product two capsules were placed in each vessel. The samples were filtered through 0.45 µm Whatmann filter paper and diluted appropriately to obtain 0.05 %w/v solutions. With the absorbance measured on a UV/VIS spectro photometer at 248 nm, the concentration of the extract in these solutions was determined using the equation of the calibration curve. The mean cumulative percent release of the extract from the tablets and capsules were then calculated from five replicate determinations.

Statistical analysis: The results were expressed as mean±SEM of at least three replicate determinations and the data subjected to a one-way ANOVA, with levels of significance established at $P<0.01$, using GraphPad Prism 5 software.

RESULTS AND DISCUSSION: A 3 %w/v aqueous decoction of *C. membranaceus* root is used locally in doses of a tumblerful (100ml), three times a day in the treatment of prostate hypertrophy and related cancers [1]. The preparation of fresh decoctions as and when needed is ideal but practically difficult to achieve. As such non-

compliance and inaccurate dosing may arise, leading to poor treatment outcomes. Multiple-dose or large volume preparations, especially in commerce, will require preservation and modification of color and taste. Due to the low extractive value of *C. membranaceus* root in water (0.56 %w/w) compared to that in ethanol (3 %w/w), the possible formulation of a liquid product from the ethanolic extract of the plant (Table 1) makes economic and commercial sense. However, due to the incomplete aqueous solubility of the extract, predissolution in ethanol was required (with the total amount of ethanol within 10%v/v).

Although, the freshly prepared decoction had an acceptable color, odor and taste for adults (the target group), it developed a bad odor and had a high level of microbial counts (6.0 x 10^7 cfu per ml) exceeding the USP (2007) and JP (2001) limits for herbal drugs, after 24 hours [5, 7, 8]. Preservation maintained the integrity of the decoction, but for only 2 months. The prepared solution of the ethanol extract had characteristics similar to the preserved decoction, but maintained its microbial load for up to 6 months (**Table 3**) [9].

TABLE 3: ANALYSIS OF THE LIQUID FORMULATIONS

Physicochemical parameters	Decoction	Modified decoction	Liquid formulation of the ethanol extract (solution)
Color	Greenish yellow	Light greenish yellow	Light yellow
Odor	Characteristic	Characteristic	Characteristic
Taste	Characteristic bitter taste	Characteristic bitter taste	Characteristic biting taste
Solubility	No residue	Clear	No residue
Viscosity	Very fluid	Fluid	Readily pourable
pH	6.0 – 6.4	6.2 – 6.8	6.6 – 6.7
Stability			
• Active content [initial – final (mg/ml)]	1.58 – 1.10	1.62 – 1.40	1.66 – 1.65
• Microbial load [initial – final (cfu/ml)]			
- *Total aerobic bacteria*	3 x 10^3 – 6 x 10^7	2 x 10^2 – 4 x 10^6	5 x 10^1 – 6 x 10^2
- *E. coli*	------	------	------
- *Salmonella spp.*	------	------	------
- *Mould and Yeast*	1 x 10^0 – 3 x 10^5	1 x 10^0 – 4 x 10^2	------
- *Enterobacteria and other gram –ve bacteria*	------	------	------

Key: ------ = No growth; cfu/ml = colony forming units per ml

Overall, it is practically possible to formulate a liquid oral dosage form for *C. membranaceus* for use within a couple of months, but issues of the use of large volumes or bulk, additives, acceptability, convenience and suitability for long term usage, makes considerations of solid dosage forms worthwhile. The formulation of the *C. membranaceus* root extract into tablets and capsules were investigated (Table 2). The flow properties of the granulations for the tablets and capsules (**Table 4**) were assessed before tabletting and encapsulation, respectively. The angle of repose, compressibility (Carr's) index and Hausner's ratio were used to determine the suitability of the granules [6]. Whilst angle of repose is a characteristic related to interparticulate friction or resistance to movement between particles, compressibility index and Hausner's ratio are indirect measure of bulk density, particle size and shape, surface area and cohesiveness [4, 5].

Measurement of these parameters give qualitative and quantitative assessment of the internal cohesiveness and frictional forces under low levels of external loading, as encountered in mixing, tabletting and capsule filling [10]. Angle of repose less than 30°, compressibility (Carr's) index between 1-15 %, and Hausner's ratios within 1-1.8, are accepted indicators or characteristics of good to excellent flow properties [4]. The tablet and capsule granules had values of angle of repose (21±2.0°; 23±1.0°), Carr's index (14.2±0.2 %; 13.9±1.3 %) and Hausner's ratios (1.14±0.01; 1.12±0.02), resp., (Table 4). Therefore, both granulations possessed good flow properties and were suitably appropriate for tabletting and capsule filling.

TABLE 4: FLOW PROPERTIES OF THE GRANULES

Parameters	Tablet granules	Capsule granules
Angle of repose (°)	21 ± 2.0	23 ± 1.0
Compressibility index (%)	14.2 ± 0.2	13.9 ± 1.3
Hausner's ratio	1.14 ± 0.01	1.12 ± 0.02

Results are Mean ± SEM of n = 3

The tablets obtained (**Fig. 1**) had an average mass of 316±8.2 mg which varied uniformly within 1.2-4.3 %. The mean content of active extract per tablet was 14.5±1.0 mg and no individual tablet varied in active content by less than 85% or more than 115% of this value (**Table 5**).

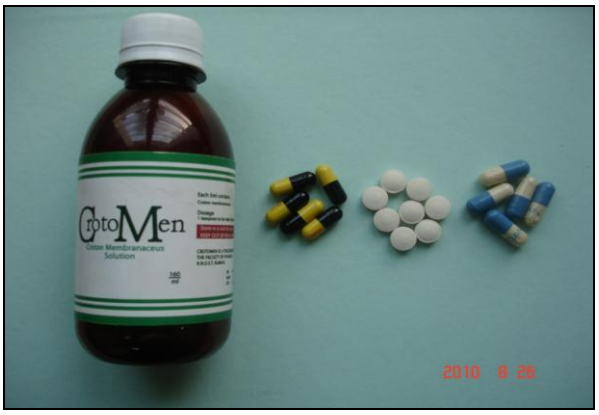

FIG. 1: ORAL FORMULATIONS OF *C. MEMBRANACEUS* ROOT AND ROOT EXTRACT: FROM LEFT TO RIGHT; LIQUID FORMULATION OF THE ETHANOL EXTRACT; COMMERCIAL CAPSULES; PREPARED TABLETS AND PREPARED CAPSULES

These results indicated the prepared tablets were uniform in weight and in active content, which will ensure accurate dosing. With a mean crushing force of 5.5 ± 0.5 Kg and a friability of less than 1%, the tablets will not easily break up and can withstand passage through machines and handling in packaging, storage and transport. The tablets also showed an average disintegration time of less than 5 minutes and a 85.2±1.6 % *in vitro* cumulative release of extract in 45 minutes (**Table 5, Fig. 2**), all of which are within acceptable limits [4, 11]. Therefore, the prepared tablets have met the required specifications and are of good quality, which can be used in simple and convenient doses of one tablet, three times daily, in place of the large volume unstable decoctions.

TABLE 5: ANALYSIS OF THE TABLETS AND CAPSULES

Parameters	Prepared tablets	Prepared capsules	Commercial capsules (CC_1)
Weight uniformity			
- *Average mass (mg)*	316 ± 8.2	248 ± 5.6	214 ± 2.3
- *Mass variations (%)*	1.2 – 4.3	2.5 – 6.8	2.8 – 7.6
Crushing force (N)	53.9 ± 0.5		
Friability (% weight loss)	0.45 ± 0.16		
Disintegration time (minutes)	4.15 ± 0.06	5.24 ± 0.34	5. 56 ± 0.35
Content uniformity			
- *Average content (mg)*	14.5 ± 1.0	13.8 ± 1.4	2.8 ± 1.4
- *Content variations (%)*	99.2 – 103.4	95.6 – 97.8	85.8 – 88.2
In vitro dissolution after 45 minutes (%)	85.2 ± 1.6	98.8 ± 1.2	42.0 ± 2.6

Results are Mean ± SEM, n ≥ 3

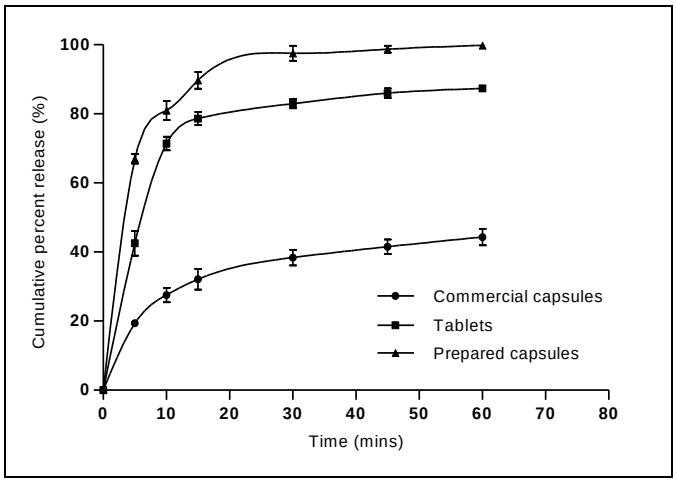

FIG. 2: THE *IN VITRO* RELEASE OF *C. MEMBRANACEUS* ROOT EXTRACT FROM ITS TABLET AND CAPSULE FORMULATIONS

The prepared capsules (Fig. 1) had a mean content mass of 248±5.6 mg with no individual weight deviations up to 10 %. The average active content per capsule was 13.8±1.4 mg, with all individual contents within the limits of 85-115 % of this average (Table 5). The results indicated that the prepared capsules were also uniform in weight and in active content that will ensure dose accuracy and uniformity.

Their disintegration time of less than 6 minutes and *in vitro* release of 98.8±1.2 % at 45 minutes were also within acceptable limits [4, 5]. The release profile of the prepared capsules was however, better and significantly higher (P<0.01) than that of the tablets. As such, *in vivo* studies are currently underway to correlate *in vitro* release data. Therefore, with the protection of the extract from light and masking of undesirable color and taste as added advantages, the capsules should be preferable to uncoated tablets as a solid oral dosage form for *C. membranaceus*.

The commercial capsules (Fig. 2) on the other hand, showed a mean content mass of 214±2.3 mg and individual mass deviations within 2.8- 7.6 %. The average active content per capsule was 2.8±1.4 mg, and although, the individual content of actives were within acceptable limits (85-115 %) of this average, the active content shown was very low and significantly different (P<0.01) from the expected content of 7.5 mg per capsule. Therefore, the doses of such capsules may be uniform but then will be highly inaccurate, and will lead to under dosing, ineffective therapy and drug resistance [12]. Also, the commercial capsules disintegrated within acceptable limits (<6 minutes) but exhibited a very low and poor release profile (Table 5; Fig. 2). Their *in vitro* cumulative release after 45 minutes was only 42.0±2.6%, which is lower and significantly different (P<0.01) from the release profiles of the prepared tablets and capsules.

The commercial capsules appear to be a direct encapsulation of the powdered plant material. As such poor, incomplete dissolution and extraction from the material within the capsules might be amongst the reasons for the low active content and poor release characteristics, compared to the formulations of its extract as in the tablets and capsules prepared in this study. The results therefore, strongly support the assertions that the formulation of suitable and appropriate herbal remedies from plant extracts may be more advantageous, desirable and therapeutically more beneficial than incorporating the direct plant materials.

CONCLUSION: In all, the study demonstrated that the physicochemical parameters and properties of tablets and capsules prepared from the *C. membranaceus* root extracts (and not the powdered plant material) were all within acceptable limits, hence are appropriate and suitable for use to offer stable and convenient doses of one tablet or capsule, three times daily, instead of large volume unstable decoctions of *C. membranaceus* used in the treatment of prostatic

hypertrophy and related cancers. However, the capsules of the *C. membranaceus* root extract exhibited the best and most significant ($P<0.01$) release effects and characteristics and should be the solid oral dosage form of choice for *C. membranaceus*. In addition to protection of the extract from light and masking of undesirable color, odor and taste, the oral capsule of *C. membranaceus* root extract, will ensure compliance and maximize therapeutic outcomes.

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