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FORMULATION AND *IN-VITRO* EVALUATION OF ORAL CAPSULES CONTAINING AN ETHANOL EXTRACT OF *XIMENIA AMERICANA*: A MEDICINAL PLANTS USED FOR BREAST CANCER MANAGEMENT

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ABSTRACT

Ximenia americana stem (XA) bark extract is widely used by several Africa traditional medical practitioners for the treatment of breast cancer and other neoplastic diseases due to its effectiveness, safety and availability. This study was aimed at formulating the extract as a solid pharmaceutical product. The stem bark extract of *Ximenia americana* was obtained by maceration technique and thereafter screened for its phytochemical contents and *in-vitro* cytotoxicity. Five different batches of XA granules were prepared by wet granulation technique and their flow properties investigated via standard pharmaceutical methods. Subsequently the granules batches were formulated as capsules. Uniformity of capsule weight, the capsule crushing strength as well as the drug release profiles of each batch were determined. The crude extract yield was 23% w/w and revealed the presence of cardiac glycosides, flavonoids, saponins, and tannin. All the batches of XA granules were fairly free flowing with ranking order of flowability: $XA_2 \leq XA_4 \leq XA_5 \leq XA_1 \leq XA_3$. The capsule weight was uniform as none of the batch deviated from the mean by 5%. All the batches disintegrated to release the active medicament with the pharmacopeia stipulated time of 15 minutes and the capsules crushing strength were well above the official limit of 4.5KgF. The cumulative amount of XA in solution at 45 minutes were above 75%, indicating compliance with the United States Pharmacopeia (USP) standards. There is however the need for the optimization these XA formulations before commercial scale up.

Keywords: *Ximenia americana,* Herbal medicine, pharmaceutical formulations.

INTRODUCTION

Worldwide, herbal medicines are becoming attractive choice for treatment of various types of diseases especially neoplastic diseases of various etiology, (Esmaeili *et al.,* 2021). And as such crud plant extract formulations are equally gaining attentions by both pharmaceutical researchers and pharmaceutical manufacturing companies given rise to a corresponding increase in the number of commercially available herbaceuticals.

Pycnogenol® is the registered trade name for a French formula of a specific blend of procyanidins extracted from the bark of the *Pinus maritime* or *Pinus pinaster* available as herbal dietary supplement in tablet or capsule dosage form, (Sarvmeili *et al.,* 2016; Simpson *et al.,* 2019). Crude extract of African bitter leaves is today formulated as pharmaceutical capsules

which are available in various strength and sold for the management and cure of different physiological disorder such as diabetes mellitus, chemo preventives and powerful antioxidant, Okwuzu *et al.,* 2017; Farombi and Owoeye 2011).

Ximenia americana is a bush-forming shrub/small tree that belongs to the Ximenia genus in the Olacaceae family. It is also known by the common names hog plum, tallow wood reaching a height of 7 metres and is typically found in tropical woodlands in Africa, Asia, America, and Australia (23 feet). Its leaves range in shape from spear-like to oval and are produced on spur branches. *X. americana* produces small, fragrant flowers and fruit. Although most flowering takes place during the dry season, flowers and fruits mature and ripen throughout the year without being influenced by the weather, Kefelegn *et al.,* 2021).

Different parts of the plant *Ximenia americana* are useful in traditional medical practice, the fruits for example are edible and are boiled in water for the treatment common flu and cold. Aqueous and tincture of *Ximenia americana* leaves are use in the treat of diabetic mellitus, while the stem bark and root bark are traditionally used in the treatment sever pains and tumor (Mwangi *et al.,* 1994; De Menezes *et al.,* 2019).

Phytochemical screening of *X. americana* had revealed that the plant is rich in bioactive polyphenols as well as showed the presence of saponins, alkaloids, tannins, flavonoids, terpenes, triterpenes sterols, and coumarins in all extracts and fractions, (Le *et al.,* 2012; Nguyen *et al.,* 2016).

The antineoplastic activity as well as the safety profile of the stem bark extract are well demonstrated and documented with LD_{50} well above 5,000 mg/kg, (Taha *et al.,* 2022; Togbossi *et al.,* 2021; Voss *et al.,* 2006). The stem bark extract is equally rich in riproximin, a secondary plant protein shown to be responsible for its anti-neoplastic activity, (Bayer *et al.,* 2012; Adwan *et al.,* 2014). There is therefore the need to formulate and develop useful pharmaceutical products from the stem bark extract of *X. americana* for the benefit of the populace that depend on the plant material for their therapeutic agent.

METHODS

Extraction

The adopted extraction method was a modified version described by Kunle *et al.* (2002). The fresh stem bark of XA was obtained from Zuru town in Zuru local government area of Kebbi state Nigeria. This was identified and voucher number assigned by the department of Biological Sciences, Faculty of Science Usmanu Danfodiyo University Sokoto. The stem bark was thereafter air dried and milled using laboratory mortar and pestle into fine powder from which about 500 g were weighed and suspended in 2.7 L of ethanol (50 ^{O}C) maintained under constant agitation with the aid of a magnetic stirrer for about 18 h and thereafter filtered and evaporated into a dried mass using a hot air oven (Gallenkamp, ModelOV-335, Vindon Scientific Ltd, UK), maintained at 60 ^{0}C.

Phytochemical screening

Aliquot amount of the extract was screened for the presence or absence of some plant secondary metabolites through the standard protocols, (Kakou *et al.,* 2020; Vedamurthy *et al.,* 2018).

Formulation and Production of XA granules

Batch wet granulation technique, (Zhang *et al.,* 2021) was employed to produce granules containing XA as the active pharmaceutical ingredients according to table 1. Starch and microcrystalline cellulose were use as binders while lactose was employed as the bulking agent with each capsule formulated to contain 290 mg of granules

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Table 1.0 Batch Formulation of XA Capsule (290mg)

Material (mg)	XA_1	XA_2	XA_3	XA_4	XA_5
XA	68.9%	68.9%	68.9%	68.9%	68.9%
Lactose	QS	QS	QS	QS	31.1%
Starch	10%	20%	____	____	____
M.C.C	____	____	10%	20%	____
Total	100%	100%	100%	100%	100%

Micrometrics properties of HS granules

The obtained dried XA granules were subjected to the following tests;

a) Particle size analysis

The mean granule sizes (MGS) of each of the formulation were determined with the aid of laboratory sieves of various pore sizes (1000 µm to < 75 µm (Pan) arranged in descending order. A 100 g of sample was placed in the topmost sieve, belt of the shaker properly engaged and the Endecott sieves shaker operated for 10 minutes. Fraction of sample retained on each sieve was collected, weighed and the MGS values were calculated using Eq.1 (United States Pharmacopoeia 2012).

$Mean\ granule\ size$ = [Σ (% $retained$) × ($sieve\ size$)]/100…. Eq.1

Where MSG is mean granule sizes.

b) Moisture absorption content analysis

The moisture content was determined using protocol described by Crouter and Brien (2014). Briefly a 50g from each of the sample was placed in a humidity chamber for 48 hrs for moisture uptake. Each of sample was reweighed after 48 hours and the weight loss was determined using Eq. 2.

MC= [50g-W2/50g] ×100 .... Eq2

Where MC= Moisture content, 1W and W2 are initial weight and final weight of the granules

c) Angle of repose (AR)

This was determined using a powder flowability tester (HMKFLOW 329, Dandong, HMKTEST Instrument Co. Ltd,China.). A 100 g of sample from each of the batch was allowed to flow through the

orifice of the instrument funnel fixed at an angle and height of about 45 ° and 8 cm respectively. The dimensions of height and radius of conical heap of powder formed were noted. The angle of repose was computed using Eq. 3 (United States Pharmacopoeia 2012).

$Tan\ \theta$ = height of cone/ 0.5 ×diameter……Eq. 3.

d) True, Bulk and Tapped densities

The loose volume (*Vb*) of a known weight (30g) of sample was noted in a measuring cylinder, while *Vt* was the volume of the same sample after 100 tapping allowing rearrangement and realignment of sample particles. The bulk (*BD*) and tapped *TD*) densities as well as other flow characteristics such as compressibility index (*CI*) and Hausner ratio (HR) of the samples were obtained using the expression in Eq 4-7.

$BD = {30g}/{Vb}$ ……Eq4.

$TD = {30g}/{Vt}$ ……Eq5.

$HR = {TD}/{BD}$ …… Eq6.

$CI = 100 \times \frac{TD-BD}{TD}$ ……Eq7.

Encapsulation of XA granules

The encapsulation process was conducted using a semi-automated capsule filling machine (Zonesun ZS-DTC, China) operated according manufacturer instructions programmed to produce 100 capsules per batch.

Evaluation of XA Capsules

The quality assessment of all the batches of capsule were conducted according to the United States pharmacopeia convention 2020 described below;

i. Capsule weight Uniformity

To ensure the accuracy of unit dose and consistency of the quality of formulated capsules, samples from each batch were subjected to weight uniformity test according to USP 2020 protocol. In brief twenty capsules were randomly selected and weighed separately using a digital weighing balance (BP-2C, BIoevopeak, China). All the experiment were carried out in triplicates and the results were averaged.

ii. Capsule Friability Test (CFT)

The weight of twenty capsules (*IW*) randomly selected per batch was obtained with the aid of a digital weight balance (BP-2C, BIoevopeak, China) and there after the sample were transfer into a Roche friabilator (Erweka TAR120, GmbH, Heusenstamm, Germany) which subjected samples to 100 revolutions within 4 minutes. The final weight (*FW*) of the samples were noted after carefully removing the samples from the Friabilator and cleaning (Osei-Yeboah and Sun, 2015). The weight lost (friability) for each batch due to abrasion were computed and expressed as percentage of the initial weight of tablets (Eq 8)

$$CFT = {IW - FW}/{IW} \times 100$$ ……Eq8

iii. Capsule Crushing Strength Test (CCST)

The force required to diametrically break ten capsules from each batch were noted using a digital hardness tester (porTAB-01, ToroTech, Canada). The mean of three determinations was recorded and taken as

crushing strength for the batch (Oyeniyi and Mojiminiyi 2022).

iv. Capsule Disintegration Time Test

Conducted using a disintegrating apparatus as described by USP 2022 (Model BJ-3, Ningbo Hinotek Instrument Co., Ltd, China). The mean time for six capsules placed in disintegrating basket to break into pieces and passed through the screen mesh at the bottom of the basket was noted for each formulation. The disintegrating medium is 1000 mL deionized maintained at 37 ^{O}C and all other experimental conditions were as prescribed by United States Pharmacopoeia 2022.

v. Dissolution studies

Applying the beer Lambert theory, freshly prepared solution of the extract (1mcg/mL) was scanned in a UV-visible spectrophotometer at different wave length to obtain the λ_{max} and thereafter different concentrations of the extract were scanned in the UV-visible spectrophotometer operating at obtained λ_{max}. Values obtained were then use to construct a calibration curve of the extract.

The dissolution test was undertaken using USP apparatus I (basket method) in 6 replicates for each batch. The dissolution medium was 1000 ml 0.1N HCl which was maintained at 37 ± 0.5°C. In all the experiments, 5 ml of dissolution sample was withdrawn at 0, 10, 20, 30, 40, 50, and 60 minutes and replaced with equal volume to maintain sink condition. Samples were filtered and assayed by ultraviolet spectrophotometry at 365nm, (Santana *et al.,* 2018; USP 2022)

STATISTICAL ANALYSIS

The data obtained were subjected to statistical analysis (ANOVA) using the student's test as a statistical tool. A 95% confidence interval ($p \leq 0.05$) was considered significant.

RESULTS AND DISCUSSION

The extraction technique (cold maceration in ethanol for 5 days) was successful with a yield of 10.05 % w/w. The yield is high enough to encourage commercial cultivation of the plant for various medical purposes, (Aragão *et al.,* 2018). The extract equally tested positive for the presence of array of plant secondary metabolites (table 2.).

XA granules

The flow of granules during filling of capsules is particularly important to ensure uniform capsule weight and by extension uniform dosing. Also, the flow property investigation is necessary to avoid problems of irregular flow and flow obstruction during encapsulation, (Aulton and Taylor 2017). The XA granules properties is as presented in Table 3. There are observable significant differences ($p \leq 0.05$) in all the flow parameters values such as angle of repose, Hausner ratio as well as the flow rate. The differences may be due to the nature and different proportions of excipients utilized in the formulation (Bo *et al.,* 2022; Thapa *et al.,* 2019).

Table 2: Phytochemical Constituents of *Ximenia americana L* stem bark ethanol extracts

Secondary Metabolites	Revealing reagent	Intensity
Alkaloids	Dragendorff	+
Flavonoids	PEG	+++
Lignans	Vanillin phosphoric	+++
Mono, sesqui and diterpenes	Vanillin sulphuric	+++
Naphthoquinones	10% Ethanolic KOH	+++
Saponins	Sulfuric vanillin	+
Hydrolysable tannins	Ferric chloride 2%	++
Triterpenes and steroids	Sulfuric vanillin	+++

Where PEG represent polyethylene glycol reagent

Base on the classification system developed by the United State Pharmacopeia (USP 2022) the produced XA granules are best classified as moderated flowing granules since the AR and HR values were above 25^0 and 1.15 respectively. They may also not require mechanical vibration to flow when during the production process. Granules and powder with CI values below 10 %, AR values below 1.11, and AR values below 25 are classified as free flowing and will ensure uniform dosing and uniformity of capsule/tablet weight, (USP 2022).

Table 3: *Ximenia americana* (XA) Granule Properties

Granule Properties	XA_1	XA_2	XA_3	XA_4	XA_5
AR (^{0})	35.82±0.13	29.9±0.02	38.6±0.03	30.8±0.06	31.8±0.06
Bulk density (g/mL)	1.48±0.09	0.80±0.15	0.72±0.11	0.82±0.08	0.75±0.07
Tapped density (g/mL)	1.92±0.11	0.96±0.09	0.98±0.15	0.99±0.05	0.95±0.10
MGS (µM)	377.5±1.29	328±0.27	380.6±0.29	325±0.77	481±1.87
MC (%)	1.34	1.40	1.34	1.42	1.32
HR	1.30±0.01	1.20±0.09	1.36±0.16	1.21±0.05	1.27±0.05
CI (%)	22.92±0.14	16.7±0.06	26.5±0.16	17.2±0.11	21.1±0.24
Flow Rate (g/s)	10.2±0.88	15.8±0.64	11.8±0.44	15.6±0.34	11.0±1.24

Key: AR = Angle of repose, MC = Moisture content, MGS = Mean granule size, HR = Hausner ratio and CI = Compressibility index

Granule sizes have remarkable effects on the characteristics of the granule and quality of the capsules/tablets. And it's one of the critical parameters that require adequate attention during pharmaceutical product formulation and development. Increase in mean granules sizes may result in corresponding increase in capsule/tablet

weight variation, non-uniform dosing and a complete failure to comply with the pharmacopeia requirement as it relate to content uniformity, (Rajani, *et al.,* 2017; Hlinak *et al,* 2006). Granules with MGS values between 200 – 400 µm, are accepted as ideal by most pharmaceutical industries, (Shanmugam, 2015). The MGS values for all the batches of XA formulations were all within the industrial standard and are expected to give excellent pharmaceutical capsules.

Table 4. Properties of XA capsules

Parameters	XA_1	XA_2	XA_3	XA_4	XA_5
ACW (g) ± (%DM)	0.5±0.97	0.5±0.87	0.49±0.82	0.49±0.82	0.49±0.88
CDR $_{45}$ (%)	76.71	85.20	86.62	90.69	75.49
CCS (KgF) ± (SD)	6.9±2.05	7.5±0.32	6.4±0.32	6.5±2.05	6.5±2.05
DTT (Min) ±(SD)	9.4±2.35	6.0±1.32	6.0±1.32	6.6±1.32	6.6±1.32

Keys: AWC= Average capsule weight; CCS = Capsule crushing strength; DTT = Disintegration time test; CDR = Cumulative Drug Released in 45 minutes

The mean weight for each batch of capsules ranged from 490 mg to about 500mg with percentage deviation from the mean less than 1%. The batches of XA capsules complied with the requirements of the United States pharmacopeia as regards weight uniformity. Precisely the maximum deviation from the mean acceptable is 5% for capsules weighing above 300 mg, while 10 % weight variation is allowed for capsules weigh below 300 mg, (USP 2022). Also capsules and tablets are expected to posse enough strength (≥ 4.5KgF) to with stand breakage during normal handling and transportation. The CCS values for the batches are all above the minimum limit and the leakage of the capsule content as result of capsule breakage is not expected through the shelf life if the products are properly stored and handle, (Owusu *et al*., 2021). The British pharmacopeia expects all oral capsules to disintegrate within 15 minutes to release the active medicament for dissolution of administration, (BP 2020; Donauer and Löbenberg, 2007). All the batches of XA capsules met this requirement as they all disintegrate before 15 minutes. The observed significant ($p \leq 0.05$) batch to batch variations in the disintegration time may also be due to the nature and concentration of diluent used (Oyeniyi and Yusuf 2020).

Table 4. Absorbance of various concentrations of XA

Concentration (µg/mL)	Absorbance
50	0.166
100	0.190
200	0.217
300	0.277
400	0.325
500	0.352

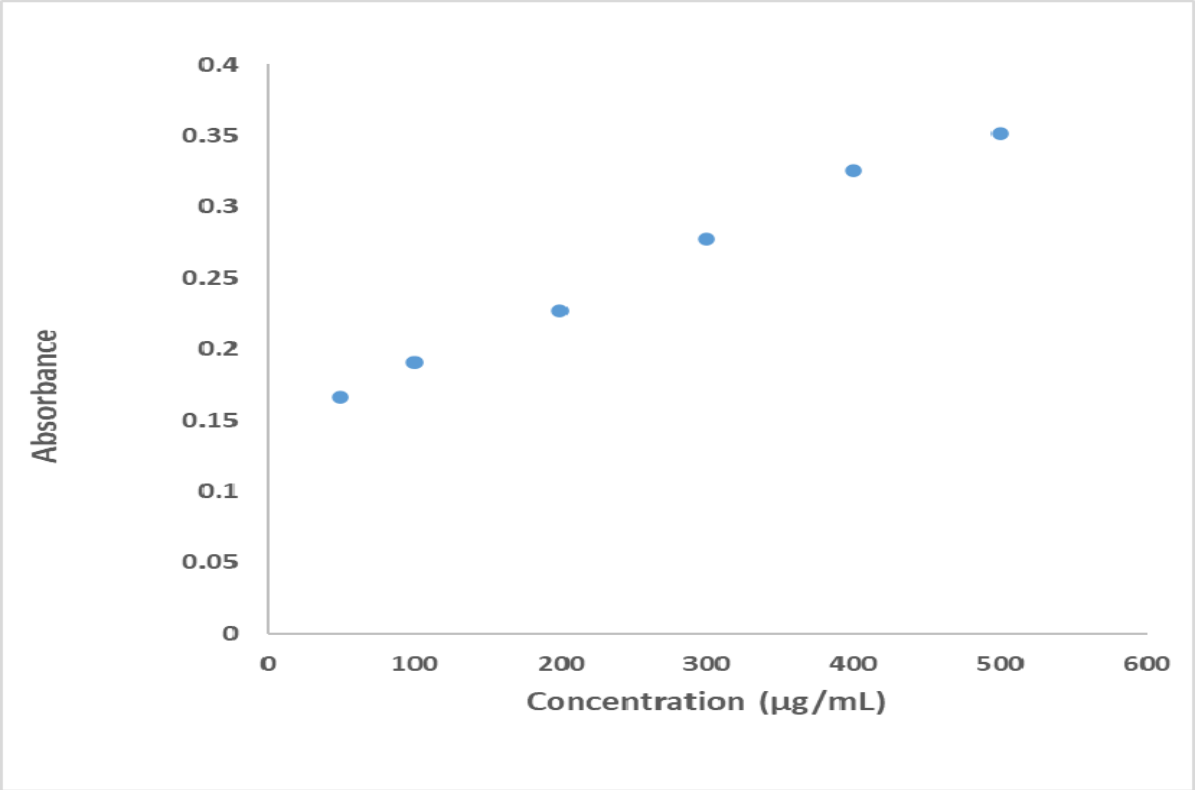

Figure 2: Calibration curve for XA extract

Figure 2 shows the calibration curve obtained for XA at various concertation. A near linear calibration plot (R^2 of 0.989) was obtained which shows a good relationship between the concentration and absorbance. This shows that absorbance values of *XA* solutions at λmax 365 nm can be used to evaluate the active principles present in the formulation, (Esmaeili, *et al* 2021).

The important of dissolution investigation can never be over emphasized in production and development of oral dosage forms. It is also the basic quality control tool in assessing the suitability of a formulation in the drug development process (Archer *et al.,* 2022). *The in-vitro* dissolution profile of formulated capsules is as presented in Figure 3. According to BP 2022, all non-modified release solid dosage forms, the minimum of 70% of the active ingredient should go into solution with 45th minutes. All the batches of XA capsules passed the dissolution test since the cumulative percentages of drug in solution are all above 75 %. And they will all expected to dissolve excellently in physiological solution (*in−vivo*) making the active ingredient (XA extract) available for absorption and the desired therapeutic activity (Salunkhe *et al.,* 2021).

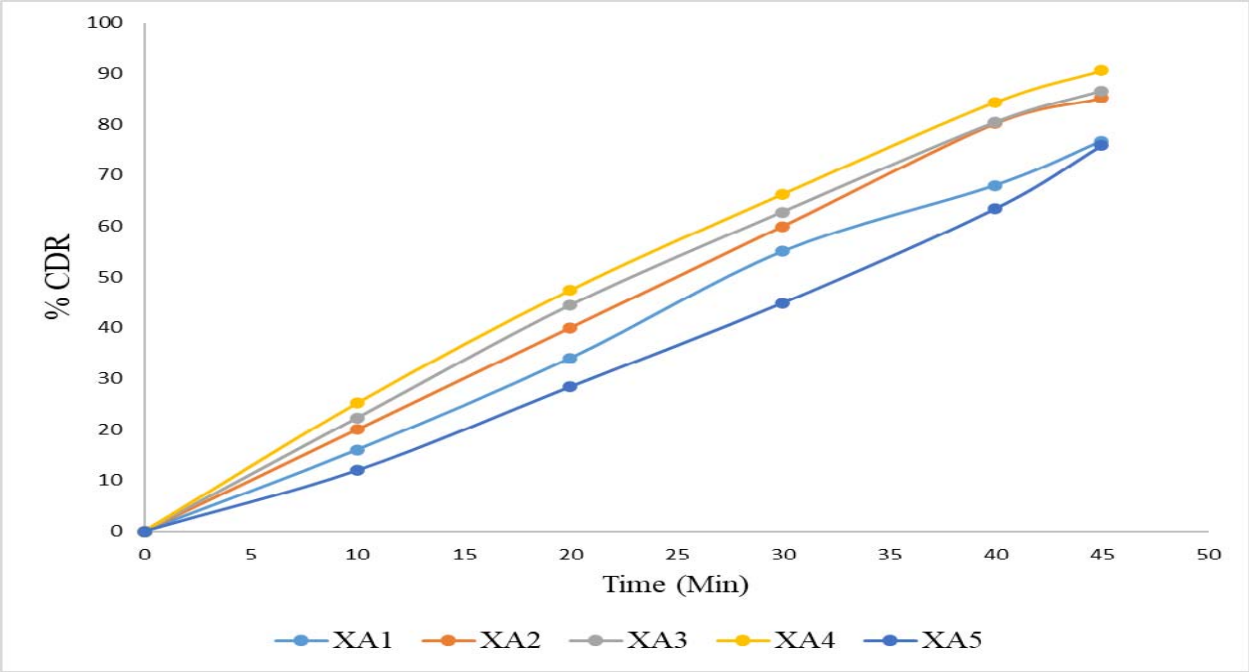

Figure 3: *In-vitro* Cumulative Drug Release Profile for the Formulated XA Capsules

SUMMARY AND CONCLUSION

All the batches of *Ximenia americana* capsules prepared met the pharmacopeia standards with regards to the uniformity of capsule weight, the crushing strength and the release profiles. The observed significant differences in among the batches indicate a need for further formulation studies that will lead to the optimization of the product.

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