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FORMULATION AND EVALUATION OF AQUEOUS EXTRACT OF GARLIC (*ALLIUM SATIVUM)* AS A PESSARY

J. Muazu * and H. Amos

Department of Pharmaceutics and Pharmaceutical Microbiology, University of Maiduguri, Nigeria.

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Correspondence to Author:
Jamilu Muazu

Department of Pharmaceutics and Pharmaceutical Microbiology, University of Maiduguri, Nigeria.

E-mail: jmuazu@unimaid.edu.ng

ABSTRACT: A pessary is a type of suppository that is meant to be inserted into the vagina for treatment of local or systemic diseases, in folk medicine garlic bulb is inserted into vagina for treatment of candidiasis. The aim of this work is to formulate and evaluate aqueous extract of garlic as a pessary. Aqueous garlic (*Allium sativum*) extract was obtained from the garlic bulbs. The pessary was formulated using the pouring method and cocoa butter as a base. The formulated pessaries were evaluated using physical appearance, crushing strength test, disintegration and dissolution times tests as well as content uniformity. The visual examination showed brilliant and smooth surfaced, consistently bullet shaped, light brown colour and mild garlic odour. The results of crushing strength (3.96 ± 0.92 KgF), disintegration time (5.17 ± 0.17 min) and content uniformity (96.03 ± 7.26 %) were all within the normal limits. The result obtained from the dissolution time test showed that the pessary dissolved within 30 min. The results indicated that aqueous garlic extract can be formulated into a pessary.

INTRODUCTION: Pessary is a solid dosage form intended for insertion into vagina where it melts, softens, or dissolves and exerts a local or systemic effect.

Allium sativum commonly known as garlic is a species in the onion genus, *Allium*. Its close relatives include the onion, shallot, leek, chive and rakkyo with history of human use of over 7, 000 years [1]. Garlic is native of central Asia and has long been a staple in the Mediterranean region, as well as a frequent seasoning in Asia, Africa, and Europe. It was known to ancient Egyptians and has been used for both culinary and medicinal purposes [2].

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Garlic has been used in treatment of infections such as cryptosporidium in HIV patients [3], fungal infection such as thrush [4].

Garlic is active against microorganisms that are resistant to antibiotics and the combination of garlic extracts with antibiotics leads to partial and total synergism [5].

Regular intake of garlic reduces the risk of esophageal, stomach, and colon cancer due to the antioxidant effect of allicin in reducing the formation of carcinogenic compounds in the gastrointestinal tract [6].

Ajoene is an active compound found in garlic which plays a role as antifungal agents [7]. Garlic has been shown to be as effective as ketoconazole, when tested on fungi *Malassezia furfur, Candida albicans, Aspergillus, Cryptococcus* and other *Candida species* [8], bulbs of garlic are inserted into the vagina for treatment of candidiasis [9].

The mode of action of aqueous garlic extract is not clear but it is believed to affect the structure and integrity of the outer surface of the yeast cells that leads to cell leakage [9, 10].

The aim of this study is to formulate and evaluate aqueous extract of garlic (*Allium sativum*) as a pessary to serve as a substitute to insertion of whole bulb into the vagina.

MATERIALS AND METHODS:

Allium sativum extracted from bulbs of *Allium sativum* (obtained from Gomboru Market Maiduguri Borno State), Cocoa Butter base (Obtained from Chemscience and Medilab. Industries, Kano Nigeria), Weighing balance (Sartorius AG Gottigen, Germany), Digital water bath (DK-8A, Shanghai, China), Disintegration tester (Erweka ZT-71, Germany), Dissolution tester (Erweka DT 700, Germany), Spectrophotometer (6405 UV/VIS Barlowond scientific, UK), Suppository mould (2 g), Blender (Philips. Japan) and Refrigerator (LG Japan). Hardness tester (Erweka, Germany)

Collection of *Allium sativa* bulbs:

Allium sativum bulbs were obtained from Gomboru market in Maiduguri, Borno state, Nigeria. It was transported by road to the University of Maiduguri. It was identified by a Taxonomist from the Department of Biological Sciences, University of Maiduguri and authenticated with a Herbarium number PCG/1314/009.

Extraction of *Allium sativum* bulb:

The method described by Muazu and Suleiman[11] was adopted. The fresh *Allium sativum* bulbs were peeled manually; the peeled bulbs were then washed with distilled water and blended using a blender (Philips, Japan). An aqueous extract was obtained using the rotary extractor thermostatically maintained at 55°C and was dried for 3 days at room temperature. The extract was weighed then size reduced using a porcelain mortar and pestle.

Determination of Microbial Load:

The method used by Emejuru *et al.* [12] was adopted with some modifications. Inoculation by pour plate method was carried out after 1 in 1000 serial dilutions of 1 g weight of the aqueous extract *Allium sativum* powder. One milliliter of the diluted sample was then aseptically aspirated into the media (Nutrient Agar). The media was poured aseptically into a sterile petri dish at 40-45^{o}C then swirled and allowed to solidify for incubation at 37°C for 24 hr.

Typical colonies of microbial growth on plates were counted at the end of incubation and the total numbers of counts were multiplied by dilution factor (1 x 10^3) to get the total viable count for all samples. The experiment was repeated twice and the mean value of three readings was taken as the microbial load.

Determination of Displacement Value:

The displacement value was determined as follows: 2 g suppository mould was used to prepare the suppository using pouring method.

Six (6) suppositories were formulated using base (cocoa butter) alone. The weight was taken. It is designated as " a"= 11.14 g

Six (6) suppositories were formulated containing 40 % of the garlic extract and the suppositories were weighed and designated as " b " = 12.14 g
The amount of cocoa butter present in the suppositories was determined. It is designated as "c".

$$c = \frac{60}{100}\ x\ 12.14 = 7.28\ g$$

The amount of medicament present was determined and designated as "d".

$$d = \frac{40}{100}\ x\ 12.14 = 4.86\ g$$

Amount of cocoa butter displaced by 4.86 g of the medicament; = a-c = 11.14 g – 7.28 g = 3.86 g.

$$Displacement\ value = \frac{d}{a-c} = \frac{4.86}{3.86} = 1.3$$

Formulation of the Pessary

TABLE 1 WORKING FORMULA FOR FORMULATION OF AQUEOUS GARLIC (*ALLIUM SATIVUM*) PESSARIES

S/No.	Ingredients	Quantity per Pessary	Quantity for 200 Pessaries
1.	Aqueous garlic extract (g)	0.12	24
2.	Tween 80 (ml)	0.25	50
3.	Cocoa butter (g) to	2.00	400

The Pessaries were formulated using the pouring method. A 2 g suppository mould was used. The cocoa butter (326 g) was weighed and placed in beaker on a digital water bath (DK-8A, Shanghai, China) and constantly stirred with a glass rod until all the cocoa butter have melted, aqueous *Allium sativum* extract was then poured into the melted cocoa butter and stirred with a glass rod. Fifty (50) ml of Tween 80 was added to make the mixture homogenous. The 2 g mould was cleaned, dried and placed on ice. The mixture was poured into the moulds to overflowing with constant stirring. The pessaries were allowed solidify by placing it in a refrigerator (LG, Japan). The solidified pessaries were then removed from the moulds and kept in the refrigerator at temperature (2– 8 °C) prior to quality control tests.

Quality Control on the Formulated Pessaries:

Physical examination and Visual characterization:

Twenty pessaries were randomly selected; shape, surface condition, colour and odour were observed and recorded. The twenty pessaries were longitudinally cut and examined through naked eyes for the assessment of physical characters like absence of fissuring, pitting, fat blooming, exudation and migration of active ingredients [13].

Length and width:

Twenty pessaries were selected randomly and, their length and width were measured using Vernier calipers.

Weight variation:

Twenty pessaries were randomly selected and weighed individually. The mean weight of the pessaries was then calculated and the standard deviation determined.

Melting point:

A pessary was randomly selected and placed in a test tube with phosphate buffer pH 7.2 maintained at temperature 37 ± 0.5 ℃. The time required by the whole pessary to melt or disperse in the media was noted.

Liquefaction time*:*

Liquefaction time was measured using a burette having a broad opening on one side and a narrow opening on the other; pessary was pushed inside from the broad end side to reach to the narrow end. Five (5) ml of phosphate buffer pH 7.2 was placed inside the burette, maintained at 37± 0.5 ℃. A thin glass rod was placed on the top of the pessary and the time at which the glass rod just inserts into the pessary was recorded as liquefaction time.

Hardness test:

Six pessaries were randomly selected and tested for hardness using the Erweka hardness tester (Erweka TBH 100, Germany). Each pessary was placed between the jaws of the tester and subjected to increasing pressure by turning the knurled knob until the pessary was crushed. The mean of the six determinations was taken as the crushing strength of the pessaries.

Disintegration test:

Six Pessaries were randomly selected and placed individually in the six tubes of the rack of the disintegrating machine (Erweka ZT – 71, Germany). The rack was then raised and lowered at constant rate in Phosphate buffer (pH 7.2) contained in a glass jar suspended in a water bath whose temperature was thermostatically maintained at 37 ± 1 °C. The time taken for the last pessary or its fragment to pass through the 2 mm mesh into the disintegrating medium was recorded as the disintegration time.

Dissolution Test:

Calibration Curve:

The calibration curve was constructed using the aqueous garlic extract and phosphate buffer pH 7.2 as the dissolving medium. A 50 mg weight of the extract was weighed and diluted 10 ml of phosphate buffer pH 7.2, 1.6 ml of the stock was then re-diluted in a 20 ml volumetric flask to give the following concentrations. 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 µg/ml. the absorbance of the different concentrations was spectrophotometrically determined at 254 nm wavelength using a spectrophotometer (Barlowond Scientific, 6405 UV/VIS, UK) and a graph of absorbance against concentration was plotted.

Procedure for Dissolution Time Test:

The study was carried out using dissolution apparatus (Erweka DT 700, Germany) USP Type II

(Paddle). The dissolution medium used was 900 ml Phosphate buffer, pH 7.2, thermostatically maintained at 37 ± 0.5 °C. The paddle was set to rotate at 50 rpm. One pessary was placed into the glass jar. Samples of the dissolution medium (10 ml) were then withdrawn at specified time interval 5, 15, 30, 45, 60 minutes respectively. The aliquots were filtered through Whatmann filter paper and analysed at 254 nm using UV spectrophotometer (Barlowond Scientific 6405, UK). After each withdrawal of the sample, same volume of fresh dissolution medium was replaced.

Content Uniformity:

The method of Manisha *et al* [14], was adopted with modification. Drug Content was determined spectrophotometrically. One pessary was placed in 200ml of phosphate buffer pH 7.2 maintained at 37 ± 0.5 ˚C till it melted. 1ml of sample was withdrawn and diluted to 100 ml with phosphate buffer pH 7.2. The drug content was determined by using UV-Vis spectrophotometer (Barlowond Scientific 6405, UK) by measuring absorbance of the filtered diluted sample at 254 nm.

RESULTS AND DISCUSSION:

FIG.1: SHOWING GARLIC PESSARIES

TABLE 2: RESULTS OF VISUAL EXAMINATION OF THE PESSARIES

S/No.	Features	Observations
1.	Shape	Consistently bullet shaped
2.	Surface condition	Brilliant, smooth
3.	Colour	Light brown
4.	Odour	Mild garlic odour
5.	Fissuring	No
6.	Pitting	No
7.	Fat blooming	No
8.	Exudation	No
9.	Migration of active Ingredient	No

TABLE 3: PHYSICOCHEMICAL PROPERTIES OF AQUEOUS GARLIC EXTRACT PESSARY

S/No.	Properties	Results
1.	Microbial Content (CFU/ml)	130.13 ± 12.87
2.	Length (mm) ± SD	29.64 ± 1.36
3.	Width (mm) ± SD	12.24 ± 0.81
4.	Uniformity of Weight (g) ± SD	1.86 ± 0.043
5.	Melting Time at 37 ± 0.5 °C (min)	31.28 ± 1.25
6.	Liquefaction Time at 37± 0.5 °C (min)	2.64 ± 0.53
7.	Crushing Strength (KgF)	3.96 ± 0.92
8.	Disintegration Time (min) ± SD	5.17 ± 0.17
9.	Content Uniformity (%) ± SD	96.06 ± 7.26

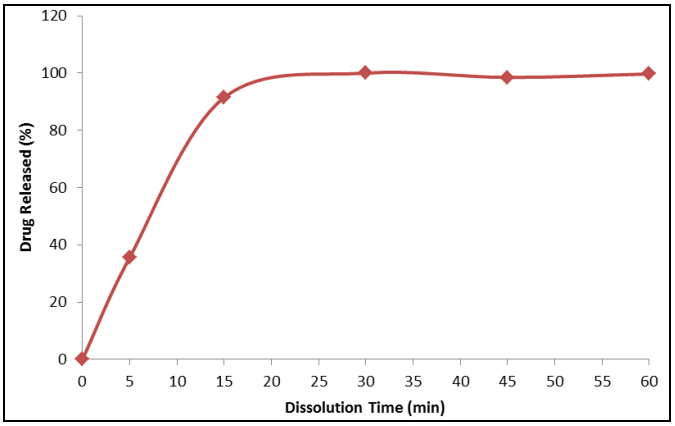

FIG. 2: SHOWING DISSOLUTION PROFILE OF *ALLIUM SATIVUM* PESSARY.

The results visual examination of the pessary indicated that the shape was consistently bullet shaped. The surface was generally smooth with light brown colour and a mild garlic odour (**Fig. 1** and **Table 2**). On examination of the cross section of the formulated pessary, there was no fissuring, pitting, fat blooming, exudation or migration of active ingredient,

Microbial load of the aqueous garlic extract was found to be 130.13 ± 12.87 CFU/g. The microbial count was in accordance to the standard specifications of ≤ 500 CFU/g [15]. This shows that the extract has fewer microbes and can be used as intended without it causing harm to the body.

The results of quality control tests are shown in **Table 3.** The length, width and weight of the aqueous *Allium sativum* pessaries were similar with little variation throughout the batch. These parameters are very important in packaging as well as patient's acceptance.

The results of melting and liquefaction times (Table 3) indicated that at body temperature the pessaries melted and liquefied at 31.28 ± 1.25 and 2.64 ± 0.53 min respectively. These measures the time necessary for pessaries to melt and liquefy under pressure similar to those found in the rectum in the presence of water at body temperature [13, 14]. The crushing strength of *Allium sativum* extract was found to be 3.96 ± 0.92 KgF showing good mechanical strength for handling and transportation and within specified limit of 3 – 6 KgF [16]. The disintegration time test measures the time required under a given conditions for a group of pessary to disintegrate into particles which mimics the time for a pessary to completely disintegrate when it enters the body. The result shows the pessaries disintegrated within 5 min, which was less than the 30 min required by official books [17].

Dissolution is the time it takes a pessary to go into solution. **Fig. 2** shows the percentage concentration of the drug released at different times. The highest concentration of the drug was 30 minutes. This means that at 30 minutes after administering the drug, the maximum concentration in the body is reached.

The pessary was stable when stored at constant temperature in the refrigerator but when kept in the open it dissolves. It shows that there is need for it to be refrigerated to prevent melting.

The drug content of the pessaries were within the permissible limits (95 – 105 %) indicating the uniform dispersion of drug in the cocoa butter base.

CONCLUSION: The aqueous garlic (*Allium sativum*) extract formulated into pessaries passed the all quality control tests carried out as specified in official books, therefore, it can be concluded that aqueous *Allium sativum* extract can be formulated as pessaries instead of the practice of insertion of the garlic bulb directly into vagina.

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