IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 6 Ver. IV (Nov - Dec. 2015), PP 83-89
www.iosrjournals.org

Encapsulation of the Ethanol Extract of *Garcina kola* and Evaluation of Its Physicochemical Properties

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Abstract: *This study seeks to formulate the seed extract of Garcina kola into capsule dosage form and to evaluate the physicochemical properties of the formulated capsules. The dry powdered seed, ethanol extract as well as the aqueous extract of the seeds were evaluated for their flavanoid content. The ethanol extract, lactose and maize starch as diluents, were wet granulated using maize starch BP mucilage and gelatin solution at (1 – 5%w/w) concentration. The resultant granules were sieved, dried and mixed with disintegrant and talc and the physicochemical characteristics of the granules and capsules were studied. These include: uniformity test, moisture content, flow rate, bulk density, tapped density, disintegration and dissolution rates. The result of the study shows that the dry powdered seed, the aqueous extract and the ethanol extract have flavonoid content of 0.483, 0.387 and 1.362 mg rutin equivalent per gram of dry sample respectively. The results also showed that the capsule had good disintegration time and dissolution profiles. Other characteristics were within acceptable values. This study has shown that Garcina kola seeds can be encapsulated as a solid pharmaceutical dosage form.*

Keywords: *mucilage, diluents, rutin, Garcinia kola, granules, capsule*

I. Introduction

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long term health when consumed by humans, and can be used to effectively treat human diseases. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total (Tapsell, *et al* 2006; Lai and Roy 2004).

In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethno medical" plant sources; 80% of these have had an ethno medical use identical or related to the current use of the active elements of the plant (Fabricant and Farnsworth 2001). The World Health Organization (WHO) estimates that 80% of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care. *Garcina kola* commonly referred to as Bitter kola is traditionally used by African who believes that it has purgative, anti-parasitic and anti-microbial properties (Maurice *et al*., 1999). Bitter kola was found helpful in ameliorating the pain and swelling experienced by people with knee osteoarthritis, and for the prevention of ulcer. The cold water extract of the root bark with salt is used in Southern Nigeria against cough and vomiting. In this same region of Nigeria, the seed is chewed to prevent nausea and vomiting in pregnancy and motion sickness.

In animal studies, *Garcina kola* increased the activities of the enzyme lactate dehydrogenase and glucose – 6 – phosphate dehydrogenase (Olajide and Adeniyi, 2011). *Garcinia kola* is also known to have activities against Ehrlich carcinoma and Human K562 leukaemia through its alkaloids and flavonoids (Andressa, *et al*, 2002). Studies have also been done on *Garcinia kola* extract on oestrus cycle, ovulation, implantation and pregnancy using adult female rats with the aim of its possible use as female contraceptive (Iranloye and Owokunle, 2008). Also used as an alternative to hops in the brewing industry (Ogu and Agu, 1995) and in the treatment of Cirrhosis and hepatitis in phytomedicine (Okwu, 2003; Nwankwo *et al*, 2000)

Garcina kola seed have been investigated for its ability to suppress colic disorders, cure head or chest cold. The anti- cancer and chemo - preventive properties of the plant have been studied (Farombi *et al*, 2005; Akintonwa and Essien, 1990). It has been reported (Adaramoye *et al*, 2005; Ibironke *et al*, 1997) that the flavonoids and phenolic compounds present in the plant are responsible for antioxidant, anti-inflammatory, anti-tumour, anti-hepatoxic, anti-ulcer and anti-microbial properties exhibited by the plant). Rutin, also called rutoside, quercetin-3-O-rutinoside and sophorin is the glycoside between the flavonol quercetin and the disaccharide rutinose (α-L-rhamnopyranosyl-(1,6)-β-D-glucopyranose). Rutin is a yellow coloured phenolic compound found in the invasive plant species *Carpobrotus edulis* and contributes to

the antibacterial and antioxidant properties of the plant. It is a solid powder and is soluble in water (12.5 g/100 ml) and with a melting point of 242^{O}C (Elmarie and Johan, 2001; Bouftira *et al*. 2012).

Objective of Study: To formulate the dosage form that will generally be acceptable and yet able to produce the therapeutic response desired when administered. It is also to have a dosage form that would attempt to overcome the disadvantages of most herbal preparations and at the same time deliver the active principle to the target site of action.

II. Materials And Methods:

Garcinia kola seeds were bought fresh dried from Uwa Market, Benin City, Edo State, Nigeria, on February 16th 2014. It was further air-dried, peeled and powdered. The powder was kept in a well tight container until ready for use.

Extraction: 1000g of the powdered seeds was extracted with ethanol using a Soxhlet extractor. The extract was concentrated to dryness by oven-drying at 50^{o}C for 6hours. The percentage yield was calculated according to the formula below:

$$\% \text{ yield of extract} = \frac{\text{final weight of extract}}{\text{Initial weight of powder}} \times 100$$

III. Determination Of The Rutin Equivalent Flavonoid Assay:

The assay procedure according to Onunkwo *et al*, 2004, was used. The dried powdered seed, the aqueous and ethanol extracts were used. These samples were used for the determination of rutin-like flavonoids present in the seed. One gramme of the seed powder was weighed out and placed in 100ml flask. Distilled water was added to obtain a 100ml mixture. This was agitated in a shaker for 1h and then filtered through a No1 Whatman filter paper. Five drops of freshly prepared Aluminium chloride solution was added to obtain a yellow coloration. The absorbance of the solution was measured at 405nm using a spectrometer (PG Instrument Ltd, Model T70UV/VIS Spectrometer). Calibration curve for Rutin was prepared using concentration ranging from 0.1 to 0.7µg/100ml. Five drops of Aluminium Chloride solution were added and the absorbance determined. The reference solution was treated similarly but without rutin, which contain 100ml of water and five drops of Aluminium chloride. A graph of absorbance against concentration of a pure sample of rutin was use to obtain the equivalent concentration of rutin in the *Garcina kola* from equation of the standard curve.

Preparation of granules and capsules: The granules were prepared using the wet granulation method with 1-5%w/v binder concentrations of gelatin solution and maize starch mucilage respectively in accordance with the method previously adopted by Alebiowu and Itiola, 2003. The granules were sieved, dried and stored in an air tight plastic container. 500mg granules were weighed carefully each time and filled into the capsule shells.

Table 1: Formulation of *Garcina kola* 300mg capsule using maize starch and gelatin as binders

Ingredient	Per capsule (mg)	Per 40 capsules (mg)
Garcina kola	300	12000
Lactose	150	6000
Maize Starch (bulking/disintegrant)	50	2000
Maize starch /Gelatin (as binders)	1%	q.s
	2%	q.s
	3%	q.s
	4%	q.s
	5%	q.s

Preparation of standard calibration curve of the rutin:

100mg of Rutin was dissolved in 100ml of water to give a stock of 1mg/ml. This was diluted to obtain solution of the following concentrations: 10, 20, 30, 40, 50 and 60µg/ml and analyzed using the UV spectrophotometer (T70 UV/VIS spectrometer, PG instrument LTD) at a wavelength of 405nm.

IV. Analysis Of Capsules

Uniformity of weight: 20 capsules were randomly selected from each batch. The weight of individual capsule was measured using an electronic weighing balance (Scout pro digital weighing machine). The average weight and standard deviation of the capsule (per batch) were calculated. Twenty (20) capsules in each batch were also weighed together and the average weight calculated (BP, 1998).

Determination of moisture content of extract: 5g of the different extract were weight into a porcelain crucible and placed in an oven maintained at 105 ± 0.5˚C. The crucible with the extract was weighed at intervals until there was no change in weight. The initial and final weight was calculated.

Determination of Bulk and Tapped density: 10g of the different batches of the granules were weighed into a measuring cylinder and their bulk densities were calculated. After a 100 taps of each batch in a measuring cylinder, the tapped densities were also calculated.

Disintegration time test: The capsule disintegration test unit (MK 4 Manesty machines Ltd, England) was used to obtain the disintegration time of the capsules. Six randomly selected capsules from each batch were used. The disintegrating medium was distilled water maintained at $37^{O}C \pm 0.5^{O}C$. Each capsule was placed in a tube closed at the lower end by a wire mesh. The tubes were moved up and down the disintegration medium by a motor to constantly agitate the capsules. Disintegration time was noted when all the particles from the capsules have passed through the mesh.

Dissolution rate test: The dissolution test was carried out using dissolution test apparatus. 900ml of distilled water was placed in a water bath maintained at $37^{o}C$ using a thermometer. This was fitted with cylindrical baskets rotated at 100 rpm using 900ml of distilled water as dissolution medium, maintained at $37 \pm 0.5^{\circ}C$. One capsule at a time from each batch was placed in the basket and lowered into the vessel containing the dissolution medium. A 5ml sample was withdrawn at various intervals and replaced with an equivalent volume maintained at same temperature $37\pm0.5^{\circ}C$ of the dissolution medium. The sample was filtered and the process continued for time intervals of 5, 10, 15, 30, 45, and 60 minutes. The absorbance of the withdrawn samples was measured at wavelength of 405nm using spectrophotometer.

V. Results And Discussion

Physiochemical properties of material

Garcinia Kola: It is a white, odourless seed with a bitter taste; when dried, milled and extracted, it yielded a dark brown crude extract that is soluble in water. It is also slightly hydroscopic.

Percentage yield of *Garcina kola* extract: Percentage yield of extract obtained was calculated using the formula shown below:

$$\% \text{ yield of extract} = \frac{\text{final weight of extract}}{\text{Initial weight of crude powder}} \times 100$$

Final weight after extraction and drying = 50 g
Initial weight before extraction = 1000g
Therefore the % yield is $50/1000 \times 100 = 5\%$

Table 2: Concentration and the absorbance values for the standard rutin

Standard Rutin Concentration (mg/ml)	Absorbance (nm)
0	0
0.1	0.217
0.2	0.268
0.3	0.375
0.4	0.497
0.5	0.575
0.6	0.748
0.7	0.780

Test samples

Samples	Absorbance
Water extract	0.473
Powder seed	0.529
Ethanol extract	1.536

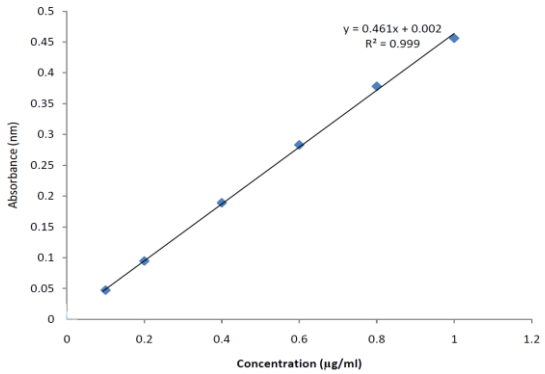


Figure 1: Calibration plot for Rutin

From the equation of calibration curve, the rutin equivalent of the samples was calculated. Where Y is the absorbance and X is the rutin equivalent

Table 3: Flavonoid content (Rutin equivalent per gram of the sample)

Samples	Absorbance	Concentration(mg/Rutin eqv/of sample)
Water extract	0.473	0.387
Powder seed	0.529	0.438
Ethanol extract	1.536	1.362

From the graph equation y = 1.090x + 0.050

$0.473 = 1.090x + 0.050$

$0.473 - 0.050 = 1.090x$

$0.423 = 1.090x$

$\frac{0.423}{1.0.90} = \frac{1.090x}{1.090}$

$X = 0.387$

The semisolid *Garcina kola* was completely soluble in ethanol while it was very slightly soluble in water leading to the very low concentration of the Rutin in the water extract.

Percentage concentration of Rutin: A chemical assay was conducted on the dry, powdered seeds as well as the crude aqueous extract of the seeds. The dry powdered seeds contain 0.044% of flavonoids while the crude extract contained 0.136% of flavonoids based on rutin used as the standard.

Table 1: Physicochemical properties of capsule each containing 300 mg of *Garcina kola* using maize starch mucilage as binder

Capsule parameter	1%w/v maize starch mucilage	2%w/v maize starch mucilage	3%w/v maize starch mucilage	4%w/v maize starch mucilage	5% w/v maize starch mucilage
Weight Uniformity (g)	0.59 ± 0.03	0.59 ± 0.03	0.59 ± 0.03	0.59 ± 0.03	0.59 ± 0.03
Moisture test (%)	26.6%	15.2%	13%	7%	6.6%
Flow time (sec)	2.8 ± 0.02	3.0 ± 0.03	2.6 ± 0.03	3 ± 0.03	2.8 ± 0.02
Bulk density(gm/ml)	0.48	0.49	0.53	0.51	0.53
Tapped density(gm/ml)	0.57	0.58	0.61	0.60	0.55
Disintegration time (minute)	7.35	7.40	8.15	10.71	10.84

Table 2: Physicochemical properties of capsule each containing 300 mg of *Garcina kola* using Gelatin as binder

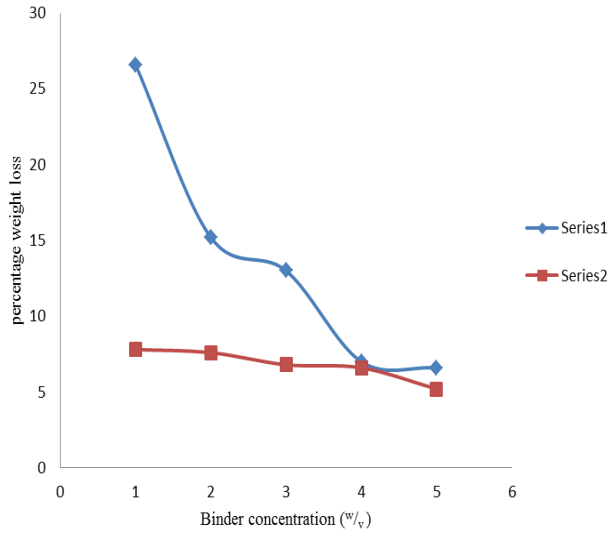
Capsule parameter	1%w/v Gelatin	2%w/v Gelatin	3%w/v Gelatin	4%w/v Gelatin	5%w/v Gelatin
Weight Uniformity (g)	0.58 ± 0.02	0.58 ± 0.02	0.58 ± 0.02	0.58 ± 0.02	0.58 ± 0.02
Moisture test (%)	7.8%	7.6%	6.8%	6.6%	5.2%
Flow time (sec)	2.8 ± 0.03	2.9 ± 0.02	3 ± 0.04	2.6 ± 0.03	3 ± 0.02
Bulk density(g/ml)	0.48	0.49	0.51	0.50	0.47
Tapped density(g/ml)	0.62	0.7	0.65	0.72	0.74
Disintegration time (minute)	5.47	6.56	6.73	6.99	7.95

Weight uniformity: The weight of capsules obtained using the binder types and concentrations vary slightly. The weight of the capsules obtained from the maize starch mucilage binder in Table 1, were higher compared to those obtained from Gelatin binder in Table 2, due to the fact that maize starch has a higher viscosity and hence higher moisture retention capacity and a stronger binding force.

Flow time: The flow time obtained from the granules was calculated as an average of three seconds for both binder types, which shows that the granules obtained had good flow properties.

Moisture content: The result obtained from the moisture test shows a decreasing percentage in the granules as the concentration of the binders increased as shown in Table 2. This could be due to the cohesive effect the binder has on the granules particles, thus decreasing the pore spaces in the granule matrix in the capsules. The stronger bond produced as the concentrations of the binder increased reduce the ability of the granules to absorb moisture from the surrounding environment.

There was a significant different between the moisture content obtained from both binders, maize starch mucilage was found to contain the highest percentage of moisture compare to gelatine. This is due to the ability of the binder to absorb moisture from the surrounding environment more than the gelatin.



Disintegration time: The disintegration time obtain from each capsule was less than 15 minutes. Capsules formulated with gelatin binders had the shortest time when compared to maize starch. Gelatin has a mean disintegration time of 5.47 minutes while maize starch has a mean disintegration time of 7.53 minutes. As the binder concentration increased, the disintegration time also increased. This is due to the stronger cohesive force the binder has on the granule particles as their concentrations increased. The bond between the particles becomes stronger as the binder concentration increases.

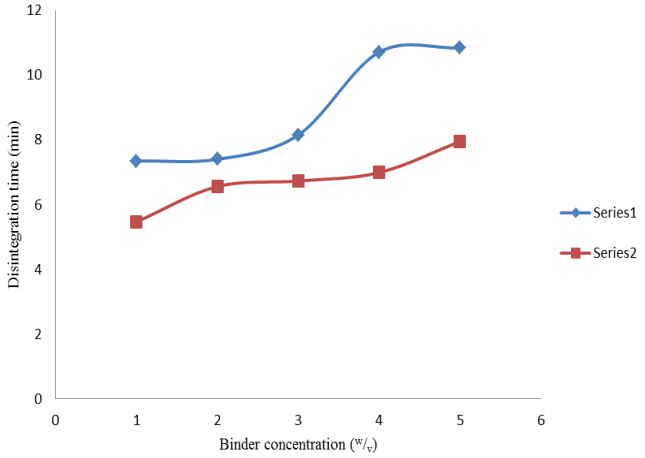


Figure 2: Effect of Binder concentration on the Disintegration Time of *Garcinia kola* capsules

Where series 1 is **maize starch** and series 2 is **gelatin**

VI. Dissolution Profile Studies

Figure 3 and 4: Percentage amount of Rutin released from *Garcina kola* capsule using gelatin and maize starch mucilage as binders

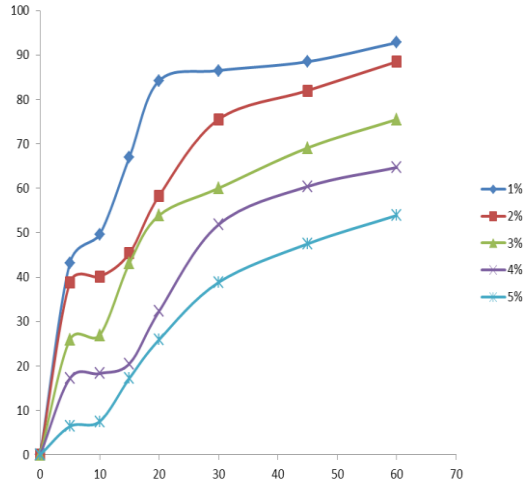


Fig 3: % of Rutin released using **gelatin**

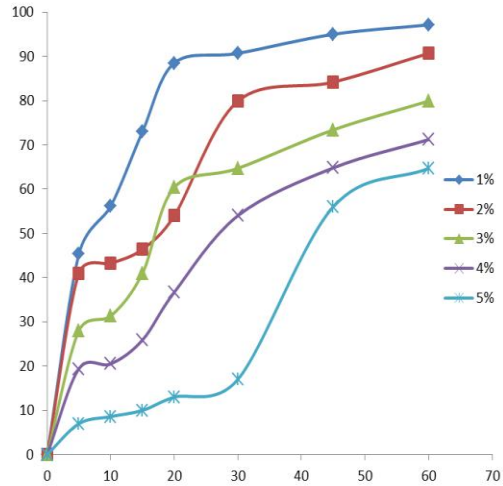


Fig 4: % of Rutin released using **maize starch mucilage**

The dissolution test reveals the rate of drug release at different time intervals. Figures 3 and 4 shows that the granules formulated with 3% maize starch mucilage release up to 60% in 30 minute, the same was also observed for gelatin. Concentration of the binder affects the rate of drug release from the capsule. The percentage drug release increases as the binder concentration decrease. This can be explained by the formation of stronger binding force between the granule particles as the concentration of the binder increases.

VII. Conclusion

The physicochemical properties of the capsules formulated with the binders were within specifications of the pharmacopoeia. Granules formulated with a lower binder concentration had the fastest rate of drug release, and those formulated with 3% gelatine and maize starch mucilage release 60% of the content within 30 minutes. Granules obtained from maize starch mucilage have a higher moisture content compared to that obtained from gelatine and hence adequate packaging and storage facilities must be ensured.

Therefore, extracts of *Garcina kola* seed can be formulated into capsules using maize starch and gelatin as binders within concentration range of 1 – 3% w/v.

Various researchers have reported several biological activities of *Garcina kola* ranging from its antimicrobial activities, hypolipidaemia effect, hypoglycaemia effect, anti cancer activities as well as its hepatoprotective effect. The encapsulation of *Garcina kola* will help in the standardization and optimization of its use.

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