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EXTRACTION AND FORMULATION OF A HERBAL PRODUCT: THE CRUDE FLAVONOID EXTRACT OF *GARCINIA KOLA HECKEL* (BITTER KOLA) SEEDS INTO LOZENGES

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ABSTRACT

Traditional medicine is the fore – runner of modern medicine. There is usually lack of standardization in the methods of extraction and formulation of traditional remedies into dosage forms. The seeds of *Garcinia kola Heckel* have been chewed and used for medicinal purposes, such as alleviation of mild throat infections. This claim was based on the fact that flavonoids can be extracted from the seeds. This study was set to obtain a crude flavonoid extract from the seeds of *Garcinia kola Heckel* and to formulate it into lozenges for the treatment of mild throat infections. The seeds of *Garcinia kola Heckel* were peeled, sun – dried to constant weight, and pulverised into coarse powder. Preliminary chemical tests were performed on the powder. Extraction of the crude flavonoid extract was done by maceration at room temperature (28 – 29^{0}C) and by soxhlet apparatus. The extracts were dried and weighed. The powdered extract was formulated into 100mg lozenges. The preliminary chemical tests performed on the powdered seeds of *Garcinia kola Heckel* revealed the presence of sterols and triterpenes, flavonoids, glycosides, saponins, resins, tannins, starch and proteins. Alkaloids were not detected. The percentage yield of the extract by maceration was higher (12.83%) than by soxhlet extraction (4.6%). The extracts have a characteristic chocolate colour, and were stable at room temperature. This work showed that crude flavonoid extracts can be obtained from *Garcinia kola Heckel* seeds by either

maceration or soxhlet extraction methods. The extract can also be conveniently formulated into lozenges.

KEY WORDS: Traditional medicine, *Garcinia kola Heckel*, Maceration, Soxhlet extraction, Lozenges.

INTRODUCTION

Natural products have remained and will still be a good source of therapeutic agents. Traditional medicine, is the fore – runner of modern medicine and it continuously nurtures it with its vast treasure of ancient remedies obtained from natural products. Traditional medicine can be described as the total combination of knowledge and practice, whether explicable or not, used in diagnosing, preventing, or eliminating a physical, mental or social disease which may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing [1].

Traditional medicine, like western or orthodox medicine, aims at healing or preventing disease. The African medicinal agents applied in traditional medicine fall into two groups: those used for rituals, sacrifices and other religious rites; and plants, herbs, seeds, roots, leaves and other substances that are supposed to have organic effect directly on the patient [2].

Despite the in – road remedies of plant origin have made into modern medicine, the preparation of these remedies by traditional method is beset with a lot of problems which are technological in nature. These include lack of adequate equipments and instruments, lack of standardization, harvesting methods, preservation, packaging and storage. In traditional method of preparation of remedies, there are no standard equipments and instruments. The available equipments and instruments include clay pots, bowls, wooden spoon, used bottles etc. Lack of standardization is another problem facing the traditional method. Standardizing a crude drug is not just that of specifying the amount of the decoction to be taken by the patient but it also includes all stages of preparation. The stages to be controlled include collection of the plant (harvesting), the extraction procedure, the final galenical (i.e. liquid extract, tincture etc) and the dosage form (i.e. tablet , capsule). At each of these stages, adequate control measures must be taken before a standardized finished product of the correct potency can be obtained. One of the major criticisms levelled against traditional medical practitioners is that their potions are neither standardized, nor are they dispensed to patients in specified doses or strictly regulated quantities. Another complaint is that the quantity specified is not related to

the age or weight of the patient. Quite often this is left to common – sense and can be dangerous if the preparation is toxic when taken by a child in too great a quantity. Also the method of standardization if employed at all, often varies from tribe to tribe.

Post harvest processing or preservation constitute another problem in traditional medical practice. Some plant constituents rapidly deteriorate soon after harvesting, either because they are attacked by enzymes (e.g. some glycosides) or they volatilize (e.g. volatile oils). Quick drying is indicated to stop enzyme action. Mould attack will also occur if the material is left damp during storage. Packaging and storage are other problems with traditional medicine. Traditional methods for processing plant products are grossly inadequate. There is, therefore, the need to introduce modern tecchnology into the manufacture of traditional remedies. This will include drying, comminution, extraction, evaporation and dosage form formulation.

***Garcinia kola Heckel* (Guttiferae)**

Garcinia kola Heckel is a cultivated small tropical tree which grows abundantly in different parts of Africa. The plant is cultivated in Southern Nigeria for its edible fruit and seed. The seed is known in commerce as " bitter kola" or "male kola" [3]. Across the places where it grows it is known by various names such as bitter kola, male kola (English name), orogbo (Yoruba), Aku ilu (Igbo) and Namijin goro(Hausa). It is also known as false kola mainly due to the absence of stimulants which characterizes the kola nut seeds. It is also known as male kola due to the reported aphrodisiac properties of *Garcinia kola*. When chewed, the seed has a bitter astrigent taste resembling that of raw coffee bean, followed by a slight sweetness. The bitter taste gained the seed its common name " bitter kola". The seeds of this plant are used in African traditional medicine for various therapeutic purposes based on pharmacological effects of the active components (flavonoids) in the seed and other parts of the plants [4].

Extracts of the seeds have been employed for the treatment of bronchitis and throat infections; for colics, colds and hoarseness of voice and for headache, dysentry and diarrhoea [5]. The seed is also said to be used in the treatment of post partum haemorrhage, urinary tract infections and emesis. It is also applied as an internal antidote to the poison of strophantus gratis [6]. Both the seeds which are believed to lenghten life span and the roots (used as favourable bitter chewing sticks) are used for oral hygiene presumably by exerting an antimicrobial action in the mouth [7]. *Garcinia kola* has been found to possess less toxicity

than hesperidine. A study has been made on the use of *Garcinia kola* as antihepatoxic agent [8]. The extracts have been used in place of codeine sulphate to prepare cough mixture [9].

Lozenges

Lozenges consist of medicaments incorporated in a flavoured basis and are intended to dissolve or disintegrate slowly in the mouth [9]. They are prepared either by moulding and cutting or compreession.

Moulded lozenges are usually prepared by mixing the medicaments in powder or solution, with the basis (sucrose and acacia or tragacanth), making the mixture into a uniform paste with water, acacia mucilage, and the other ingredients. The paste is then cut into uniform shapes. The lozenges are then dried in a hot – air chamber at a moderate temperature (50 – 60^0C).

Compressed lozenges are prepared by the same method as compressed tablets. Heavy compression is necessary in order to ensure slow disintegration in the mouth. Each lozenge is made up to the weight specified in the individual monograph with an inert excipient which usually contains not less than 50% of sucrose [9].

MATERIALS AND METHODS

MATERIALS:

Soxhlet Extraction Apparatus (Gallen Kamp, England), coarsely powdered seeds of *Garcinia kola Heckel,* petroleum ether B.P. 60^0 – 80^0 C (M & B Ltd., England), acetone (Hopkins and Williams Ltd., England), diethyl ether (Hopkins and Williams Ltd., England), aluminium pot with cover (Tower, England), cotton wool, n – hexane (M & B Ltd., England),ethyl acetate (M & B Ltd., England), sodium hydroxide solution, dilute ammonia solution (lab, reagent), rotary evaporator (Dauchi, W. Germany), separating funnel (Hopkins and Williams Ltd., England), mannitol, (BDH Chemicals Ltd. Poole England), sucrose, (BDH Chemicals Ltd. Poole England), Acacia powder (Merch, Darmstadt), Stearic acid (Merch, Darmstadt), Seives (sizes 16, 20), Oven (Manesty, England), F3 – tabletting machine (Manesty, England).

METHODS

Processing of primary material

The seeds of *Garcinia kola Heckel* were procurred from a local market in Nsukka Local Government Area of Enugu State of Nigeria. The seeds were identified and authenticated as

the seeds of *Garcinia kola Heckel* at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

The testa of the seeds were removed prior to sun – drying. The seeds were pulverised into coarse powder using cross – beater mill (DSKI, W. Germany), fitted with a sieve of 0.5 mm apperture. The yield in grams was recorded.

Extraction

Two methods were employed in the extraction of crude flavonoid constituents from the seeds of *Garcinia kola Heckel*.

Soxhlet Extraction Method

389.6g of the coarse powder of the pulverised seeds of *Garcinia kola Heckel* was defatted with petroleum ether. 1.5 litres of petroleum ether was used. A dark brown residue which deposited on standing was discarded. The defatted marc (372g) was air – dried and extracted with 1.5 litres of acetone. The acetone extract was concentrated under reduced pressure in a rotary evaporator for two hours, then the concentration was carried out using electric heater (ETA 038, CZECHOSLOVAKIA) until the extract was dry (47.0g). The dried acetone extract was digested with ethyl ether (550ml) for 48 hours. The diethyl ether soluble extract was evaporated to dryness under low heat (18g). Tests on this extract (diethyl ether soluble extract) showed and confirmed the presence of flavonoid.

Maceration Method

Extraction by maceration method was carried out in two steps:

800g of the pulverised seeds was macerated with a mixture of methanol and water in the ratio of 9:1. 1.8 litres of methanol and 200ml of water were used. The maceration time was ten hours. The resulting extract was decanted (1 litre).

The marc was again macerated for 8 hours with a mixture of methanol and water in the ratio of 1:1. One litre of each solvent was used. At each step, sufficient solvent was added to make a liquid slurry. Each liquid extract was separated from plant material by filtering rapidly with cotton wool plug in the neck of a filter funnel. The two extracts were then combined and concentrated to about one – third the original volume using electric heater adjusted to low heat. The resultant aqueous extract was cleared of contaminants by shaking with aliquots of n

– hexane (2.3 litres) in a separating funnel. Shaking with aliquots of n- hexane was carried out eight times.

The extracts were then combined and concentrated to a semi – solid consistency in a water – bath. The semi – solid extract was then air – dried, and weighed. The extract was also tested for flavonoid. Presence of flavonoid was indicated and confirmed, through chemical tests.

Formulation of the Crude Drug Extracts (1 & 11) into Lozenges

The following formula was used for the preparation of lozenges:

Drug Extract	100mg
Mannitol	500mg
Sucrose	50mg
Acacia mucilage	2%w/v
Stearic Acid	1%w/w
Aerosil	0.2%w/w
Starch paste	15%w/v.

Mannitol and sucrose were used as fillers, acacia mucilage as the binding agent, starch paste as the disintegrant, stearic acid and aerosil as the lubricants.

The powders were converted to granules by wet granulation method. The granules were dried in an oven. The granules were compressed into lozenges using the tabletting machine (Mansty, England). Average weight of tablets were determined using ten tablets randomly selected from each batch.

Preliminary chemical tests

The coarsely powdered seeds were subjected to some preliminary chemical tests. Plant constituents like starch, proteins, glycosides, saponins, alkoloids, flavonoids, tannins, sterols and triterpenoids were tested for. The tests were performed as enumerated below:

Test for Flavonoids

i) 2.0 g of the powdered seeds was dissolved in 5ml of water in a test tube. Few drops of sodium hydroxide solution (lab. Reagent) were added

ii) 2.0 g of the powdered seeds was boiled in water with 3ml ethyl acetate solution in a test tube, filtered, cooled and the filtrate shaken with dilute ammonia solution.

Test for Starch

1.5 g of the coarsely powdered seeds of *Garcinia kola Heckel* was shaken in 5 ml of water. Two drops of iodine solution were added to the test tube containing the solution of the crude drugs.

Test for Glycosides

1.0 g of the powdered seeds was boiled with 10 ml of water in a test tube. 2 ml of Fehlings solutions I & II was added to the solution and the solution was boiled in a water – bath for fifteen minutes.

1. The brick – red precipitate was filtered off with a filter paper. To 5 ml of the supernatant liquid obtained, 3 ml of dilute sulphuric acid was added. The solution was boiled for 15 minutes, cooled and neutralized with 3 ml potassium hydroxide solution (20%). 1ml Fehlings solutions I & II was added and the solution was boiled for 15 minutes in a water – bath.
2. To another 5 ml of the supernatant liquid obtained, 3 drops of ferric chloride solution was added. The solution was boiled, cooled and filtered. The filtrate was shaken with equal volume of carbon tetrachloride (CCl_4). The lower organic layer was separated and 5 ml dilute ammonia solution was added and shaken.
3. To 2.0 g of crude drug in a test tube were added 20 ml of ethanol (50%) and 10 ml lead acetate solution (15%). The solutions were boiled in a water – bath for 2 minutes, cooled and filtered into separators and extracted twice with 15 ml aliquots of chloroform. Two chloroformic extracts were thus obtained. The first chloroformic extract was partially evaporated in a water – bath and 2 ml aliquots of 3, 5 – dinitrobenzoic acid solution and 1 ml of sodium hydroxide solution were added.
4. The other chloroformic extract was completely evaporated to dryness in a water – bath and dissolved in 3 ml glacial acetic acid. To the solution, in a test tube, 2 drops of $FeCl_3$ solution were added and 2 ml concentrated sulphuric acid was carefully added by the side of the test tube.

Test for Proteins

Two drops of millions reagent were added to a solution of 2.0 g of the crude drug in a test tube.

Test for Alkaloids

2.0 g of the powdered seeds was boiled with 6 ml of dilute HCl in a test tube and filtered. The filtrate was divided into four portions.

To the first portion, two drops of meyer's solution were added.

To the second portion, were added two drops of Wagner's reagent.

To the third portion, were added two drops of Dragendorff's solution.

Two drops of picric acid were added to the fourth portion.

Test for Tannins

1.0 g of powdered seeds was weighed into a test tube. 5 ml water was added, and the solution was filtered. Two drops of $FeCl_3$ solution were added to the filtrate.

Test for Saponins

1.0 g of crude drug was added into a 5 ml of water in a test tube. The test tube was shaken vigorously. The above solution was filtered and 1 ml of the filtrate was introduced into a test tube containing 5 ml blood.

Test for Sterols and Triterpenoids

1.O g of the powdered seeds was dissolved in 5 ml chloroform in a test tube, poured into a mixture of a saturated solution of Antimony trichloride and chloroform and heated in a water – bath for 10 minutes.

RESULTS AND DISCUSSIONS

Extraction

The extraction methods used in the work were modelled by the researchers, after a review of methods used by previous workers on *Garcinia kola Heckel*.

Results obtained in table 1 showed that extraction by maceration gave a greater yield (12.83%) of flavonoid constituent than soxhlet extraction method which yielded 4.6%. Flavonoids possessing a number of unsubstituted hydroxy groups, or sugars, are polar compounds and are generally moderately soluble in polar solvents such as ethanol, methanol, butanol, acetone, dimethyl sulphoxide (DMSO), dimethyl formamide (DMF) and water. The presence of an attached sugar (commonly encountered) tends to render the flavonoid more water soluble and combinations of the above solvents with water are thus better solvents for glycosides [10] . In contrast, less polar aglycones such as flavanones, isoflavanones, and

highly methoxylated flavones and flavonols tend to be more soluble in solvents such as ether or chloroform [10]. Thus, in extraction by maceration, a mixture of methanol and water was used as eluent while only acetone was used in the case of soxhlet extraction. This, probably may explain why there was more yield of flavonoids with maceration.

Table 1: Yield from extraction

Method	Yield	% Yield
Soxhlet	18g	4.6%
Maceration	102.6g	12.83%

Preliminary Chemical Tests

The results of the preliminary chemical tests performed on the powdered seeds is shown on table 2 below. Starch, protein, glycosides (probably flavonoid glycosides), falvonoids, tannins, sterols and triterpenenes, and saponins were identified. It is a well known fact that in the treatment of burns and wounds, the protein of the exposed tissues are precipitated by tannins forming a mildly antiseptic protective coat under which the regeneration of new tissues may take place [11]. Medicinal plants which contain saponins (Quillaia) and triterpenoid saponins are known to have cough suppresant effects [11]. Flavonoids have been proved to possess antimicrobial properties. So *Garcinia kola* seeds have constituents of rich therapeutic values.

Table 2: Preliminary chemical tests

S/N	Chemical group Test	Observation	Inference
1	Starch: Iodine test	Blue – black colouration obtained	Starch present
2	Protein: Millions test	White precipitate obtained	Protein present
3	Alkaloid's : Meyer's reagent Wagner's reagent Dragendorff's reagent Picric acid	 No white precipitate No brown precipitate No orange precipitate No yellow precipitate	 Absence of alkaloids.
4	Glycosides: Fehling's Solution I & II	Brick – red precipitate obtained	Presence of glycosides
5	Flavonoids: Sodium hydroxide solution, Ethylacetate, Ammonium hydroxide	Yellow colouration	Presence of flavonoids
6	Tannins: $FeCl_3$	Green colouration	Tannin present
7.	Sterols and Triterpenes: Carry – price test	Yellow precipitate	Presence of sterols and triterpenoids
8	Saponins: Foam test Haemolysis test	Frothing occurred Translucent red colour formed	Saponins present

CONCLUSION

Natural products have been acclaimed to be a good source of therapeutic agents. The major criticisms levelled against traditional medical practitioners are lack of scientific proofs for their potions, crude method of preparation and poor product presentation and lack of knowledge of the active constituents. *Garcinia kola Heckel* seed is rich in medicinal constituents. Preliminary chemical tests revealed the presence of starch, proteins, glycosides, tannins, sterols and triterpenes, and saponins. Each of these constituents has established pharmaceutical and/ or clinical application [11, 3]. A lot of works have been done to provide scientific proof to claims on the medicinal use of *Garcinia kola Heckel* and this has been attributed mainly to the presence of flavonoids isolated from the seeds [8, 12, 13, 14, 15]. This work concentrated on the provision of a standardized method of isolation of the crude flavonoid extract and also its formmulation into a standard dosage form (lozenges) which can be used for treatment of mild throat infections.

It should be noted that this work on crude flavonoid extract of the seeds of *Garcinia kola Heckel* is the first of its kind. Previous researchers worked with pure constituents. Preliminary chemical tests showed that the *Garcinia kola Heckel* seeds extract is composed of mainly flavonoids which have antimicrobial activity. Thus the rigours and cost of separation and purification were avoided by using the crude extract. Also the maceration method used was quite simple and less time consuming and the yield was quite reasonable as the result indicated. The formulation of the crude extract into lozenges makes unit – dosing possible and easy. Also due to the palatability of lozenges, better adherence to dosage regimen is assured and this will lead to better therapeutic outcome.

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