QUALITY CONTROL OF TANZANIAN *Curcuma longa* L. RHIZOME: AN ANTI-INFLAMMATORY DRUG

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Abstract

Background: People in developing countries have been using medicinal plants for their health care for a long time. Most of the plants they use have been officially recognized in many Pharmacopoeias world wide. Medicinal plants have also provided templates that have lead to discovery of currently clinically useful drugs. *C. longa* L. is clinically recommended for the treatment of hyper acidity, flatulent or atonic dyspepsia, peptic ulcers and inflammation due to rheumatoid arthritis.

Broad objective: To do a quality control on the medicinal plant *Curcuma longa* L. growing in Tanzania according to WHO specifications.

Study settings: School of Pharmacy, MUCHS, Dar-es-Salaam, Tanzania.

Methodology: *C. longa* rhizomes collected from Tanga were purchased at Kisutu market. WHO set standards for widely used medicinal plants, were used. The macroscopic and microscopic characters of the rhizome were recorded. Powdered plant material were then extracted in methanol and the extract used for carrying out other identity tests including thin layer chromatography and high performance liquid chromatography, curcumin as a standard. Quality tests like total ash, acid insoluble, water and alcohol soluble extractives, curcumin and volatile oil contents, were determined according to WHO monographs.

Results: As the amount of active ingredients can differ in plants of the same species growing in different climates, soil and time of collection, quality control results on *C. longa* rhizome growing in Tanzania show that it meets the WHO set standards. The results of this study, when compared with specifications set by WHO, (in brackets), for *C. longa* rhizome show that the amount of total ash is 6.8 (not more than 8%), acid insoluble ash 0.98% (not more than 1%), water soluble extractive 13.2 % (not less than 9%), alcohol soluble extractive 17% (not less than 10%), percentage volatile oil 5.4% (not less than 4%) and curcuminoids 7.1% (not less than 5%).

Conclusion and Recommendations: *C. longa* growing in Tanzania meets WHO quality specifications as a medicinal plant. It can therefore be used as a raw material in the manufacture of different drug formulations. The rhizome can be used in powder form, extract or the volatile oil obtained from it.

Key words: Medicinal plants, Curcuma longa, Tanzania

Introduction:

Medicinal herbs have been in use for thousands of years in one form or another. In many developing countries, a large proportion of the population relies heavily on medicinal plants to meet their primary health care needs. In addition to herbal drugs used for their pharmacological action there are many herbs which are natural sources of food supplements, taste enhancers, colors and cosmetics. Synthetic drugs are becoming more and more expensive thus becoming unaffordable by many people in developing countries leave alone their side effects.

The areas of concern for a regulatory body while assessing or approving the quality of plant material and herbal remedies are therapeutic claims that the product makes compared with its actual efficacy. Others are safety of the products due to the complex compounds extracted from herbs, contamination from heavy metals like lead and arsenic, microbiological load and mycotoxins that may be produced due to fungal contamination.[1] Assurance of safety, quality, and efficacy of medicinal plants and herbal products is therefore a key issue. WHO emphasizes the use of modern technique and application of suitable standards to ensure quality control of medicinal plant products. Simple tests like foreign matter, macroscopy, microscopy, ash content, extractive values in various solvents, volatile components, bitterness value, tannins, swelling and foaming indexes and thin layer chromatography especially, can reveal a lot of information.

WHO has compiled a list of medicinal plants and established international specifications for the most widely used medicinal plants and simple preparations. In response to the need for providing safe and effective herbal medicines for use in national health-care systems, WHO has in its first volume, published monographs on 28 selected medicinal plants that are widely used in its regions.[2] The plants were selected because there is sufficient scientific information to substantiate safety and efficacy.[3] The monographs contain detailed botanical descriptions of the plant and drug material of interest, general identity tests, purity tests, chemical assays, dosage forms and medicinal uses that are well established in many countries and are in official pharmacopoeias or national monographs.

Curcuma longa L. (Zingiberaceae) (Syn. *Curcuma domestica* Val. Tumeric) is among the selected plants. Its rhizome is an important drug clinically proven for treatment of various diseases. Among uses supported by clinical data are treatment of acid, flatulent, or atonic dyspepsia.[4] Other uses described in pharmacopoeias include treatment of peptic ulcers, pain and inflammation due to rheumatoid arthritis.[5- 9] The anti-inflammatory activity of the rhizome

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was demonstrated in animal models and appears to be mediated through the inhibition of trypsin and hyaluronidase enzymes.[9,10] Curcumin and its derivatives are responsible for the anti-inflammatory activity of the drug.[10,11]

Curcuma longa known in Kiswahili as *binzari, manjano*, grows very well in most parts of Tanzania particularly in Lushoto, Morogoro and Kigoma. It is locally used as a condiment.

It is a well established fact that active ingredients in plants can vary due to soil, climatic conditions and time of collection. It is therefore necessary to carry out a quality control of raw plant materials intended for manufacture of medicines. Rhizomes of *Curcuma longa* growing in Tanzania can be used for manufacture of the much needed drugs at a minimal cost provided it has the specification required by WHO monographs. In an effort to achieve that, rhizomes from the plant were taken and a quality control carried out according to the WHO specifications.

Materials and Methods

Fresh *C. longa* rhizomes were purchased from Kisutu market where the vendors said to have obtained the rhizomes from Lushoto in Tanga region. Once in the laboratory the sample was washed to remove the soil. Curcumin reference sample was purchased from Sigma-Aldrich CHEMIE, GmbH. P.O. Box 1120, 89552, Steinhim, GERMANY.

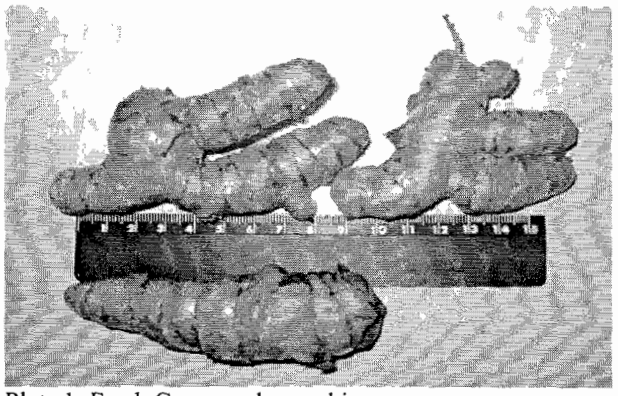


Plate 1: Fresh Curcuma longa rhizome

General appearance:

The rhizome is oblong and some spear shaped and round 5 – 10 cm long and 1.8 – 2 cm thick. Externally, it is yellowish – brown with roots or root scars and pieces of cork and annulated due to leaf base scars. Fracture is short and orange-yellow inside, showing a cortex separated from a central cylinder by a distinct endodermis.

Organoleptic properties

Odor aromatic; taste aromatic, slightly bitter. When chewed it colors saliva yellow.

Microscopic characters

Transverse section of the rhizome is characterized by presence of thin walled rounded parenchyma cells and scattered vascular bundles. Under the epidermis are layers of cork with brick shaped parenchymatous cells and oleoresin cells with brownish content. The cortical vascular bundles are of collateral type.

Powdered plant material

The powder is colored deep yellow. Fragments of parenchyma cells are seen containing numerous starch grains colored yellow by curcumin. Scattered numerous vessels and fragments of cork cells, unicellular trichomes and oil droplets are seen.

Quality control of powdered *C. longa* rhizome

The dried plant material was powdered manually and passed through a sieve size No. 60. The resulting powder was then used for analysis.

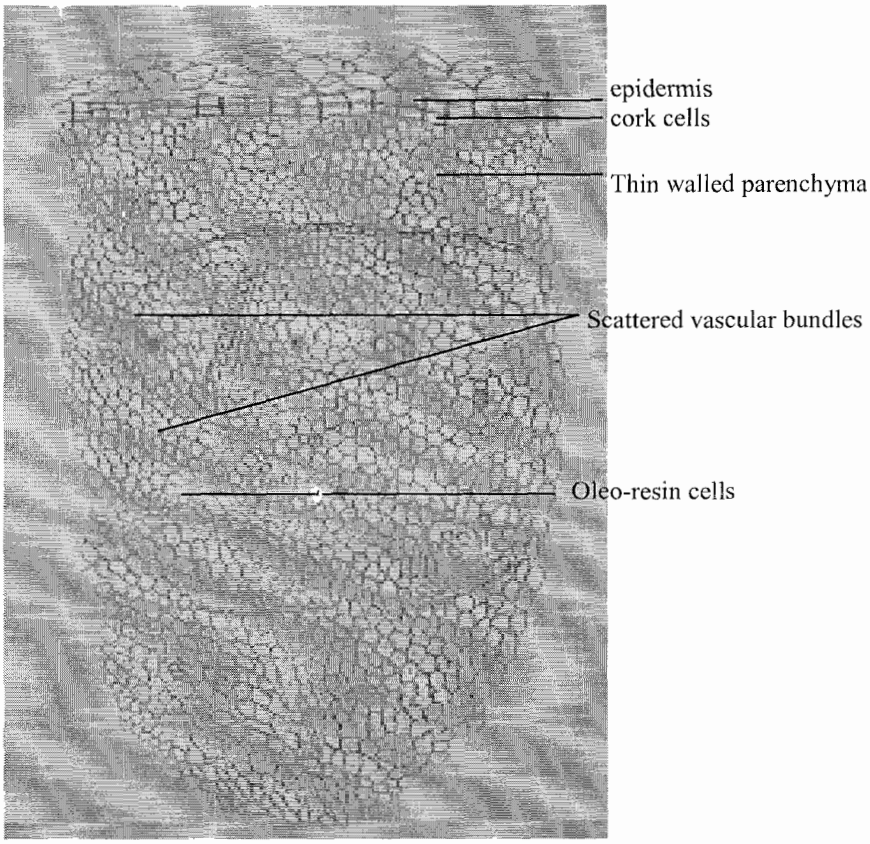
General identity tests:

A. *Macroscopic and microscopic examination:* Plates 1, 2 and 3

B. *Thin-layer chromatography(TLC):* An amount of 10g of the sample, in powder form, was extracted with 2ml of acetic anhydride then added with a few drops of sulfuric acid and observed under ultraviolet light (366 nm). The solution shows blood-red color.

The TLC sample solution (A) was prepared by placing 1g of the powder in a stopered test-tube then added with 3ml of methanol, shaking for a while and set aside for one hour. The standard solution (B) was prepared by dissolving 1mg of curcumin in 1 ml of methanol. Both solutions were spotted (5µl) separately on a silica gel G coated plate and run into a solvent system containing benzene:chloroform:ethanol (49:49:2) as a mobile phase and allowing the solvent front to ascend 17 cm. The plate was then dried in air and examined under UV light (366 nm), locating the spots (Plate 4). The plate was then sprayed with 10% w/v solution of phosphomolybdic acid in ethanol and heated at 105°C for 5 minutes. Plate 5 shows the TLC profile of the constituents.

C. *Identification by HPLC.* 10 mg of reference curcumin was taken and dissolved in 10 ml of HPLC solvent system, methanol:water (80:20). An amount of 1g powdered sample was added with 10ml of methanol in a conical flask and left in contact for 3 days before filtering. The filtrate was then dried and re-dissolved in 10 ml HPLC solvent above. An amount of 10µl was injected and retention time for curcumin noted, then 10µl of the sample solution. The two retention times were compared for identity (fig 3).



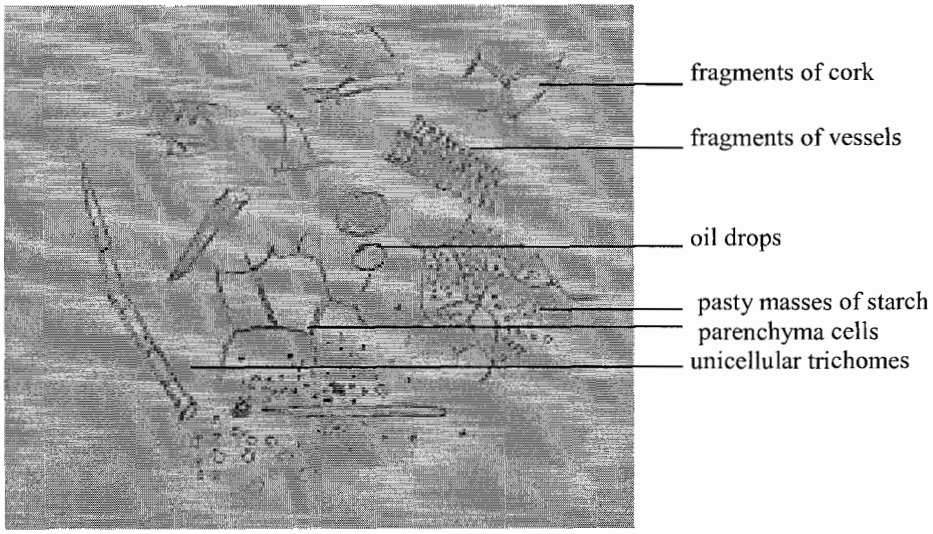


Plate 3. Microscopic characters of powdered plant material

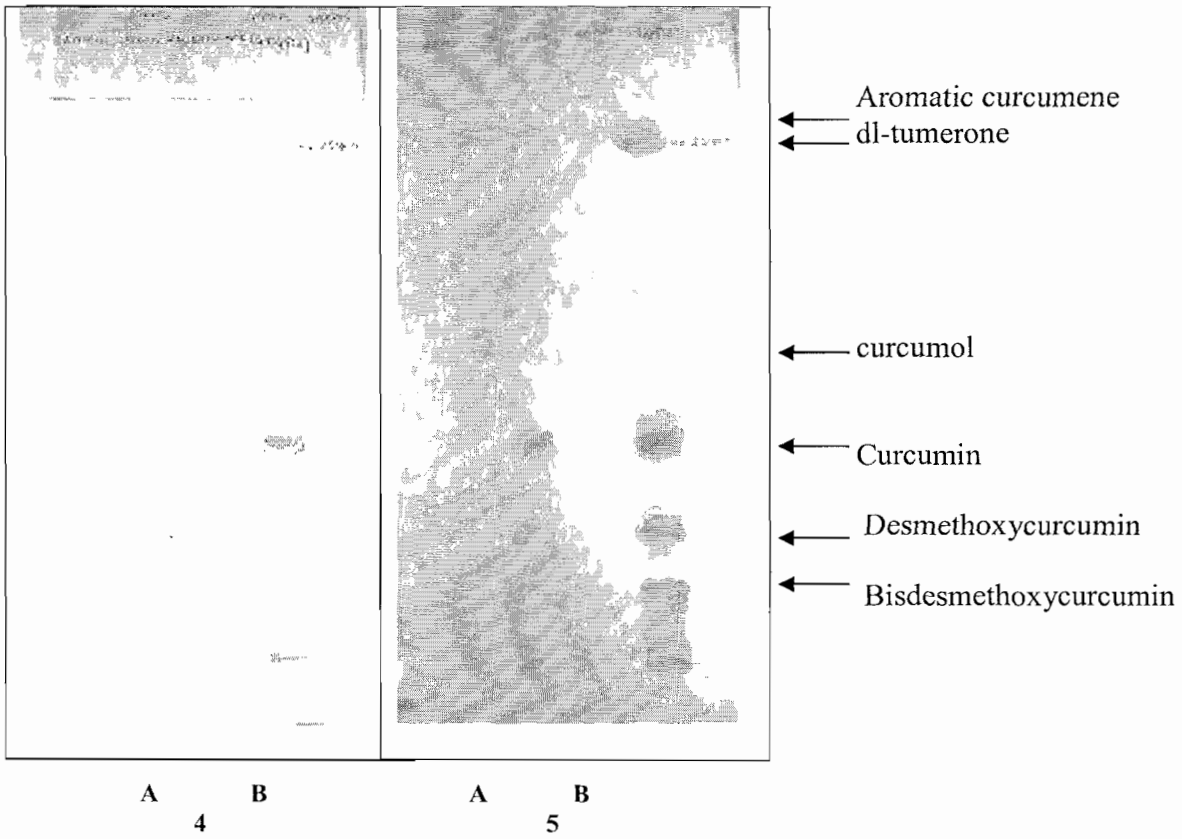


Fig. 1: TLC profiles for plate 4 and 5. *Solvent system:* Benzene:Chloroform:Ethanol (49:49:2), ***Visulization:*** **4,** UV; **5,** 10% w/v phosphomolydic acid in ethanol and heat to 105^{0}C 5min.
- **A** = Reference sample of curcumin,
- **B** = *C. longa* rhizome extract

Table 1: hR_f values of constituents in the methanol extract after detection with UV (Plate 4) and Phosphomolybdic acid (Plate 5).

Spot	hR_f Value	Detection with UV366 (Color)	Phosphomolybdic acid (Color)
1	5-8	Light brown	Brown
2	11-15	Yellow-brown	Orange
3	17-20	Yellow-brown	Orange-brown
4	21-24	Blue-green	Blue
5	28-34	Yellow-brown	Orange-brown
6	35-38	Blue-green	Blue
7	39-42	Yellow	Light yellow
8	44-46	-	Blue
9	48-51	-	Blue
10	52-53	-	Blue
11	57-60	-	Blue
12	62-66	-	Blue
13	71-74	-	Blue
14	80-85	-	Blue
15	87-90	-	Blue

Key: Spot 1, 4, 6-9, 11-13 = unknown ; 2 = bisdesmethoxycurcumin; 3 = desmethoxycurcumin 5 = *curcumin* ; 10 = curcumol; 14 = *dl*-turmerone; 15 = ar-curcumene (ar = aromatic).

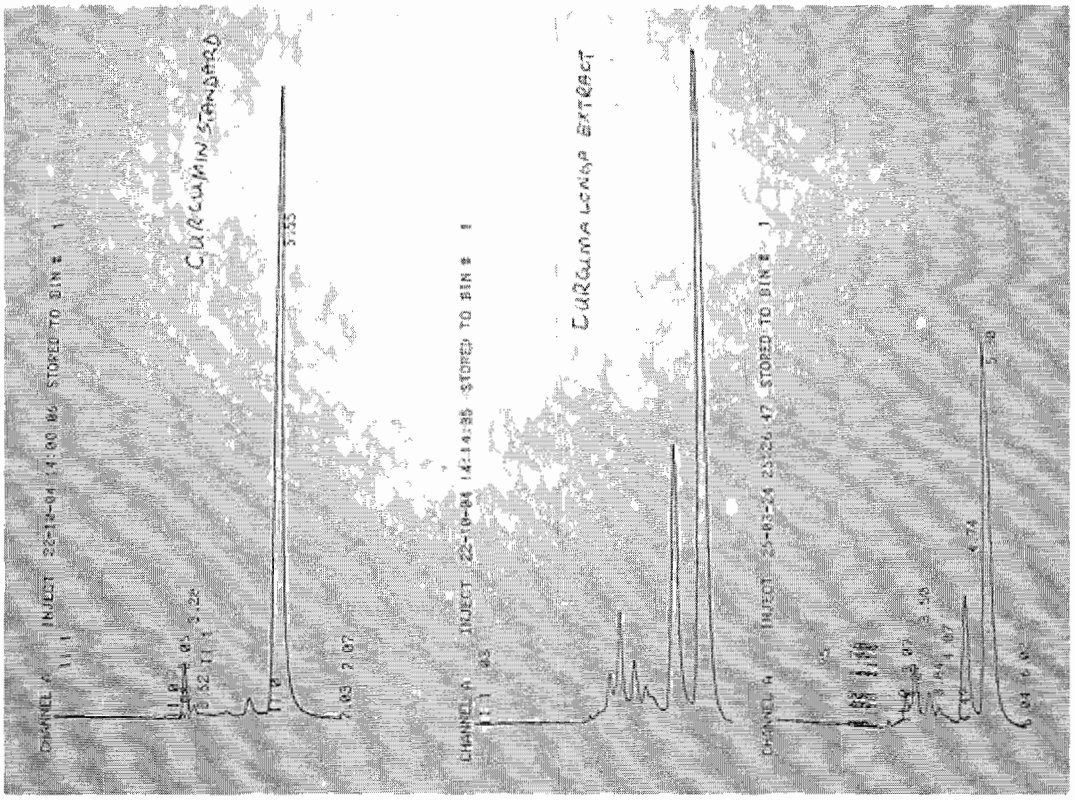


Fig. 2: HPLC of curcumin standard and *C. longa* extract.

Determination of total ash

An amount of 2.2613 g of powdered rhizome was placed in a crucible previously ignited to constant weight, spread in an even layer and ignited by gradually increasing the heat to 500 – 600C until the ash was white. It was then cooled in a desicator and weighed. The percentage of total ash was calculated per g of air-dried material as follows: Weight of ash. 0.1547g, % ash = 0.1547 x 100/2.2613= 6.84%.

Determination of acid insoluble ash

To the above crucible containing the total ash, 25 ml of hydrochloric acid (70 g/l) TS, was added and covered with a watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5ml of hot water and the liquid added to the crucible. The insoluble matter was collected on an ash less filter paper and washed with hot water until the filtrate is neutral. The filter paper containing the insoluble matter was then transferred to the original crucible dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a desicator for 30 minutes then weighed immediately. The content of acid-insoluble ash was calculated in mg per g of air-dried material. Weight of acid insoluble ash 0.031g Percentage of acid insoluble ash Percentage of acid insoluble ash is 0.031/2.2613 = 0.977%.

Water-soluble extractive

An amount of 3.6413 g of coarsely powdered plant material was placed in a glass-stoppered conical flask. It was then macerated with 100 ml of water for 6 hours shaking frequently and allowed to stand for 18 hours. The extract was then filtered immediately before an amount of 25 ml was measured and transferred to a flat-bottomed dish and evaporated to dryness on a water bath. It was then dried at 105 ^{0} C for 6 hours cooled in a desicator for 30 minutes and weighed immediately.

The extractible matter in mg per g of air-dried material was calculated as follows:

- Weight of powder taken 3.6413g
- Weight of water-soluble extractive 0.1202g
- 25 ml of extract is equivalent to 3.6413 x 25/100 = 0.910325g of powder.
- % water-soluble extractive is 0.1202 x 100/0.910325 =13.2040754 % = 13.2%

Alcohol soluble extractive

An amount of 3.6413 g of coarsely powdered plant material was placed in a glass-stoppered conical flask. It was then macerated with 100 ml of alcohol (90% alcohol BP, 1993) for 6 hours shaking frequently. It was allowed to stand for 18 hours then filtered before 25 ml was transferred to a flat-bottomed dish and evaporated to dryness on a water

bath. The residue was dried at 105 ^{0} C for 6 hours, cooled in a desiccator for 30 minutes and weighed immediately.
The percentage of alcohol soluble extractive in mg per g of air-dried material was then calculated as follows:

- Weight of powder taken 4.0511g
- Weight of water-soluble extractive 0.1729g
- 25 ml of extract is equivalent to 4.0511 x 25/100 = 1.012775g of powder, % alcohol-soluble extractive is 0.1729 x 100/1.012775 = 17.0719% = 17%

Chemical assays

Determination of volatile oil content

An amount of 50g of coarsely powdered dry rhizome was mixed with 500ml of distilled water in a 1L flask. The flask was then fixed with a volatile oil determination apparatus and heated on a hot plate for four hours then left to cool for 12 hours before the volume of distilled oil was measured. The volume of oil was recorded and drained. The material was redistilled for another six hours and again left to cool for 12 hours. The resulting volume of oil was recorded. The amount of oil content is determined by volume of oil per 100 g of plant material. Total amount of oil produced 2.7 ml. Volume of oil per 100 g of plant material is 2.7% of volume of oil per dried plant material is 5.4%.

Curcumin content

Preparation of standard solution: An amount of 2mg of curcumin was accurately weighed, placed in a volumetric flask with methanol to produce 5.0 ml. *Standard curcumin curve:* Five 10-ml volumetric flasks were added with 20, 40, 50, 60 and 80 µl, respectively, of standard curcumin solution and diluted to volume with methanol. Absorbance of the resulting standard solutions was measured relative to the blank at 420nm. The readings (table 2) were then plotted and a curve drawn (figure 4.)

Preparation of sample: About 300 mg of powdered Tumeric, accurately weighed, was transferred into a 10ml volumetric flask, added with tetrahydrofuran to volume and left at room temperature for 24 hours with frequent shaking. One ml of the clear supernatant liquid was diluted with methanol to produce 25ml. From this solution, 1ml was transferred into a 50ml volumetric flask, diluted to volume with methanol. The absorbance of the sample solution measured and by reference to the standard curcumin curve, the content of curcuminoids as curcumin was calculated in the sample. Amount of curcumin taken = 2.6 mg added with methanol to 5 ml. Amount of tumeric taken = 301.4 mg added with 10 ml tetrahydrofuran

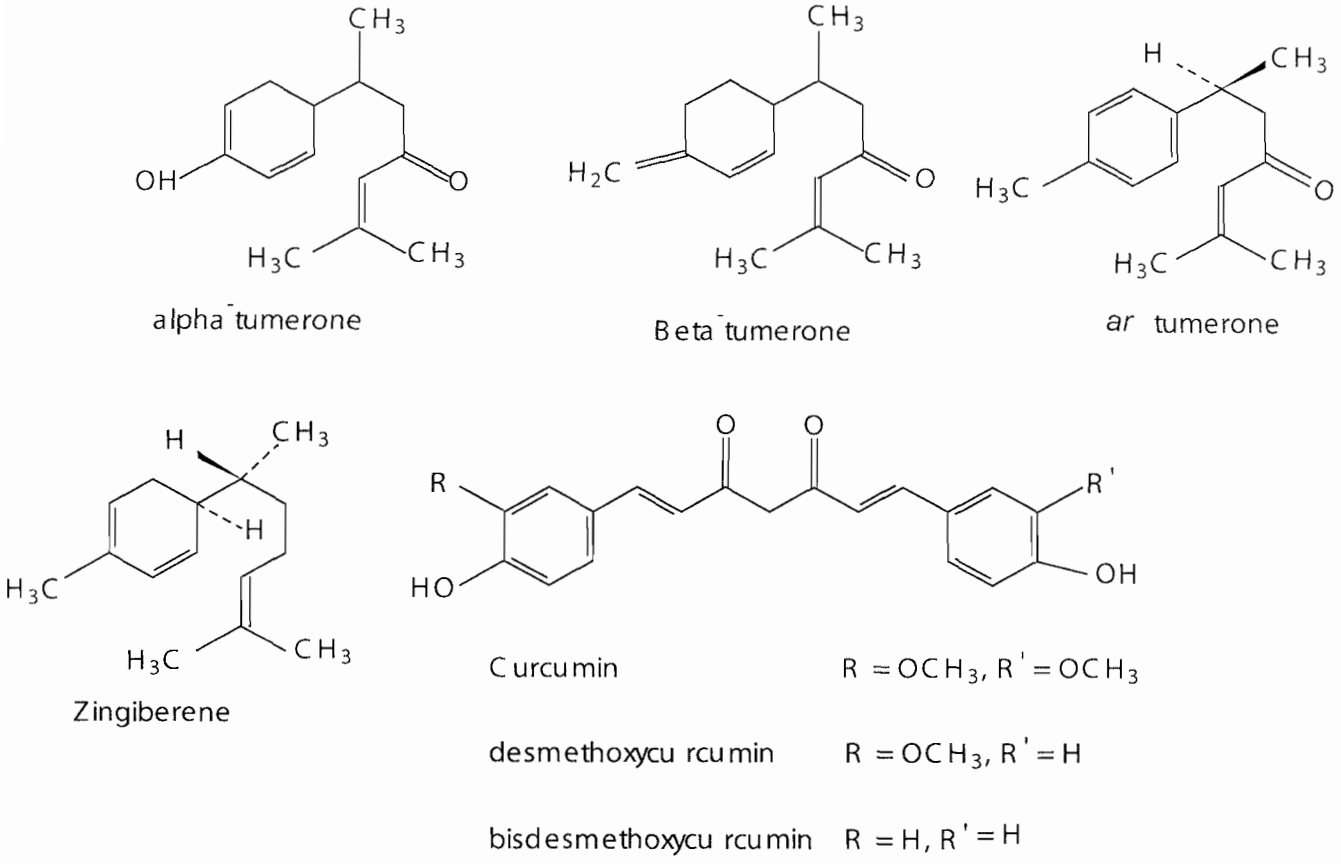


Figure 3. Major constituents of Curcuma longa rhoizome

Table 2. Absorbance readings for the standard

SN	Conc. (µl)	Absorbance for three readings	Average absorbance (nm)
1	Blank	-0.035, -0.019, -0.011, -0.009	-0.0185
2	20	0.055; 0.052	0.0535 + 0.0185 = 0.0720
3	40	0.183; 0.171	0.1770 + 0.0185 = 0.1955
4	50	0.229; 0.228	0.2285 + 0.0185 = 0.2470
5	60	0.333; 0.332	0.3325 + 0.0185 = 0.3510
6	80	0.403; 0.408	0.4055 + 0.0185 = 0.4240
7	Sample	0.139; 0.133, 0.133; 0.140	0.13625 + 0.0185 = 0.15475

The absorbance was adjusted by subtracting the blank (-0.0185).
The concentration of curcumin per ml in the experiment is 2.6/5 = 0.52 mg/ml.
The amount of curcumin in 20µl is 0.52mg/ml x 20/1000 = 0.00104mg/ml
The amount of curcumin in 40µl is 0.52mg/ml x 40/1000 = 0.00208mg/ml
The amount of curcumin in 50µl is 0.52mg/ml x 50/1000 = 0.00260mg/ml
The amount of curcumin in 60µl is 0.52mg/ml x 60/1000 = 0.00312mg/ml
The amount of curcumin in 80µl is 0.52mg/ml x 80/1000 = 0.00416mg/ml

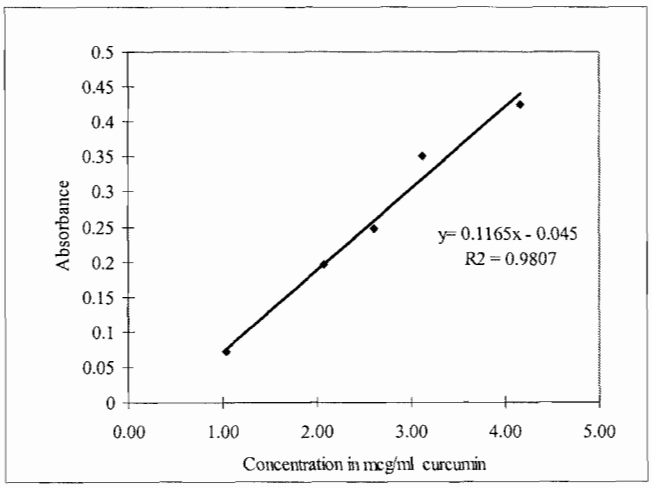


Figure. 4: Concentration/Absorbance curve for curcumin (µg/ml)

- *Calculations:* From the equation above, Y = 0.1165X – 0.045,
- Where Y = absorbance and X = concentration in µg/ml
- The absorbance for the sample, Y = 0.15475nm
- Therefore concentration of the sample (X) is 0.15475 = 0.1165X – 0.045
- X = (0.15475 + 0.045)/0.1165 = 1.711 µg/ml.

The amount of curcuminoids calculated as curcumin w/w:

i. 301.4 mg of powder was added with 10 ml of tetrahydrofuran to make 30.14mg/ml
ii. 30.14mg /ml was taken and completed to 25ml with methanol to make 1.2056 mg /ml
iii. 1 ml of this was diluted to 50ml resulting into 0.024112mg/ml = 24.11µg/ml of sample solution.

The percentage concentration of curcuminoids in terms of curcumin is therefore 1.711/24.112 x 100 = 7.1 %

Results and Discussion:

The importance of Tanzanian *Curcuma longa* as a raw material in the manufacture of drugs can not be questioned. The above quality control experiments were carried out using WHO monographs. Table 3 shows a comparison between the experimental results and WHO specifications. It can be seen that the rhizome meets the criteria.

Table 3: Comparison between experimental results and WHO specifications for *C. longa* rhizome

	Specification	Experimental	WHO requirement
1	Total ash	6.8%	Not more than 8%
2	Acid insoluble ash	0.98%	Not more than 1%
3	Water soluble extractive	13.2%	Not less than 9%
4	Alcohol-soluble extractive	17%	Not less than 10%
5	Percentage volatile oil	5.4%	Not less than 4%
6	Curcuminoids	7.1%	Not less than 5%

The specifications indicated in the WHO monograph are those necessary for efficacy and safety of the pharmaceutical formulations made from the plants.

The amount of extractable matter is important because it determines the amount of active constituents extracted with solvents from a given amount of plant material. The total ash following an ignition of medicinal plant material is designed to measure the total amount of material remaining which can be physiological ash derived from the plant tissue or non-physiological ash which is residue of the extraneous matter e.g. sand and soil which are contaminants. Acid insoluble ash measures the amount of silica present especially sand and siliceous earth. Excess water content in medicinal plants will encourage growth of microorganisms, fungi, insects and hydrolysis of active ingredients.

Cultivation of medicinal plants needs the use of pesticides like aldrin and dieldrin to protect the plant from insect attack. WHO gives a maximum residue limit of not more than 0.05mg/kg. Volatile oils are considered to be biologically active it is therefore important to determine the contnt in a given medicinal plant. Thin-layer chromatography (tlc) is an important method for determination of active ingredients present in a plant drug but particularly for qualitative determination of small amounts of impurities. Other parameters can differ according to specifications set by individual regulatory authority of eac h country. The anti-inflammatory activity of the rhizome is due to curcumin therefore its amount should be specified. In this experiment, the Tanzanian rhizome contains 7.1% total curcuminoids which is comparable with WHO specification of not less than 5%.

Conclusion and recommendation

It can be seen from the above study that the rhizome of *C.longa* growing in Tanzania meets the WHO specifications. The immediate study should be focused on the formulation

of the plant material for use by patients. This can be done directly on powdered rhizome, extract or volatile oil from the rhizome provided that at the end, the formulation remains effective and safe as a drug.

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