

**UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
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**COMPARISON OF THE PHYTOCHEMICAL
COMPOSITION BETWEEN COOKED AND UNCOOKED
AMARANTH PLANT**

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**A Thesis Proposal submitted in partial fulfilment for the
award of the degree of Bachelor of Pharmacy, University of
Nairobi.**

MARCH, 2010

DECLARATION

I declare that this proposal is of my own origin and has not been used in any other Institution for the purpose of acquiring a degree.

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DEDICATION

To my family for their sacrifice, love, prayers, moral and financial support and to my friends James Kiyukia, Naji Said, Eric Osolo, Kenneth Irungu, Munga, Stephen and Sin Hassan for the constant support they have given me in the pursuit of this dream.
Thank you.

ACKNOWLEDGEMENT

First and foremost to God Almighty for the wisdom, strength and guidance He has given me all through.

Secondly, to my supervisor, Dr. Kefa Bosire, without whom this project would not be a success.

Great thanks to technical staff of the school of Pharmacy and Botany department, University of Nairobi who contributed to the success of this project. Special thanks to Mr Mwalikumbi, Mr Masengo, Mr Samoei and Mr Mugo for their end less effort to guide me through the laboratory work.

ABSTRACT

Phytochemical studies were carried out on fresh, dry and boiled *Amaranthus* powdered leaves by use of sequential extraction method. The chloroform and methanol sequential extracts of *Amaranthus* were then studied by use of thin layer chromatography and their percentage yield determined.

The results obtained showed that the dry and freshly powdered leaves contained alkaloids, saponins, tannins and phenols. Thin layer chromatography gave good separation with both chloroform 100% mobile phase and chloroform: methanol(95:5) mobile phase.

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CHAPTER ONE

LITERATURE REVIEW

1.1 Uses of *Amaranthus* plant

It is used as a green vegetable in Kenya and its grain is used in ground form as flour for use in bread, noodles, pancakes, cereals, granola, cookies and other flour based products.

The leaf juice has been used by mitishamba doctors as a blood cleanser and as an immunobeneficiary in AIDS patients.

1.2 Botanical description of *Amaranthus* plant

Kingdom: Plantae

2 Division: Magnoliophyta

3 Class: Magnoliopsida

4 Order: Caryophyllales

5 Family: Amaranthaceae

6 Genus: *Amaranthus*

7 Species: *Amaranthus muricatus* (African Amaranth)

Amaranth is also known as terere in Kikuyu language, chepkerta among the kalenjin and mchicha in Kiswahili.

1.3 Characteristics

Amaranth is a herbal plant that grows up to 1.7 metres and has a broad leaf with a flower head that has tiny seeds galore. The leaves may come in many colours including green, red, white, orange and pink. The leaves are oval 2-4 inches long starting out as green or dark red and changing to a bright yellow, orange or fluorescent pink at the top.

It is a glabrous plant.



Fig1.2Amaranth leaf

Fig 1.1Amaranth leaf



Fig 1.3Amaranth leaf and grain

1.3.1 Distribution in Kenya

It is cultivated in western Kenya region of Busia, in central Kenya Nyeri and in the whole of coast province.

1.3.2 Distribution in the rest of the world

Has its origin in the Americans.

Amaranthus species were grown as the principle grain crop by the Aztecs 5,000 to 7,000 years ago, prior to the disruption of the South American civilization by the Spanish Conquistadors. Synonyms such as "mystical grains of the Aztecs," "super grain of the Aztecs," and the "golden grain of the Gods" were used to describe the nutritious amaranth grain. The leaves and grains were noted to be nourishing to infants and to provide energy and strength to soldiers on extended trips. While the early civilizations were aware of these nutrient factors by experience, it would take six centuries for modern biochemistry to confirm these facts. While the amaranths were the principle species used on the South American continent, amaranth have been cultivated as a vegetable crop by early civilizations over 2,000 years ago, and continue to be used essentially world-wide even at the present day (NAC 1985). Vegetable *Amaranthus* spp. were and are presently utilized for food from such diverse geographic areas as southwestern United States, China, India, Africa, Nepal, South Pacific Islands, Caribbean, Greece, Italy, and Russia. While various species of grain and vegetable types can be distinguished, often both the grain and leaves are utilized from individual types for use as both human and animal food (Saunders and Becker 1984; Tucker 1986). Present American production is estimated to be between 2,000 to 3,000 ha with the largest production in the Great Plains area, particularly Nebraska, with numerous smaller production areas throughout the Midwest. The stimulus for the present American production and marketing was initiated by the Rodale Foundation and the Rodale Research Center in the mid-1970s. The interest stimulated by the Rodale Foundation led to the establishment of the American Amaranth Institute in Bricelyn, Minnesota, and numerous Amaranth marketing companies, several of which deal exclusively in the purchase, milling, and distribution of amaranth products. In approximately only 15 years, American amaranth has gone from an obscure plant to a recognized plant.

1.3.3 Botany

The genus *Amaranthus* consists of approximately 60 species, however, only a limited number are of the cultivated types, while most are considered weedy species. *Amaranthus* germplasm is available in 11 countries (Sauer 1967; Toll and von Sloten

1982). Several thousand germplasm accessions are available in the United States at either the Rodale Research Institute, or the USDA North Central Regional Plant Introduction Station at Iowa State Univ., Ames. A taxonomic key for the cultivated species of *Amaranthus* has been developed by Feine-Dudley (Grubben and von Sloten 1981).

The species grown as vegetables are represented primarily by *A. tricolor*, *A. dubius*, *A. lividus*, and *A. creuntus*. *Amaranthus palmeri* and *A. hybridus* were utilized by natives of early civilizations in the southwestern part of the United States. The weed amaranth, comes from *A. retroflexus* and is considered one of the worlds worst weeds (NAC 1984). The genetic and plant breeding characteristics among the cultivated *Amaranthus* spp. has been considered elsewhere (Kulakaw and Jain 1991; Weber and Kauffman 1990).

1.3.4 Research work done on *Amaranthus* plant

Several studies have shown that amaranth may be of benefit for those with hypertension and cardiovascular disease; regular consumption reduces blood pressure and cholesterol levels, while improving antioxidant status and some immune parameters due to vitamin C and A that are found at significant levels with trace quantities of vitamin B. Amaranth appears to lower cholesterol via its content of plant stanols and squalene that form water soluble cholesterol which is readily excreted from the body.

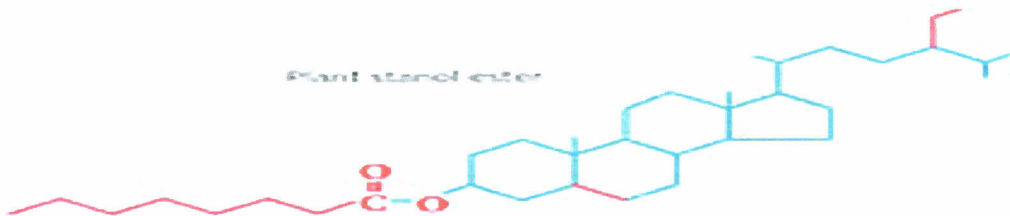


Fig 1.4

The photochemical components predicate that it may not only be useful due to its dietic value but also medicinally and pharmacologically. Saponin is used in the manufacturing of shampoos, insecticides and various drug preparations and synthesis of steroid hormones. Phenols and phenolic compounds within the leaves have been used as disinfectants and remain the standard to which other bactericides are

compared. Alkaloids on the other hand are known to play some metabolic role and control development in living systems and have a protective role in animals. Tannins have been shown to lower available protein by antagonistic competition and can therefore illicit protein deficiency syndrome, kwashiorkor.

1.4 OBJECTIVES

- a) To obtain amaranth plant from its natural habitat.
- b) To isolate plant leaves from whole plant.
- c) To carry out milling process of dry, fresh and boiled leaves separately.
- d) To carry out sequential extraction of the leaves using chloroform followed by methanol.
- e) To evaluate the phytochemical changes that occur after cooking of amaranth and compare with raw plant phytochemistry.
- f) To carry out thin layer chromatography on both chloroform and methanol extracts

1.5 Justification of the study

Amaranth plant has not been well studied to determine the phytochemical changes during cooking process or between the time period from which it had been harvested to when it is cooked.

The plant has been known to be used by patients who visit traditional doctors that give the plant as a remedy for a cleansing of blood without knowing its physiochemical properties and effects on body but believe it is better used when uncooked than cooked. Thus patients blend the leaves or crush the leaves in a mortar and pestle, add water and use it raw without any boiling or cooking.

It has been considered to be having immunobeneficiary properties and mitishamba doctors use it for AIDS patients to boost their immunity although don't have proof of this function and the compounds involved.

CHAPTER TWO
EXPERIMENTAL

2.1 Materials, reagents and equipment

2.1.1 Materials

These include;

- (i) Fresh, dry and boiled powdered leaves of *Amaranthus* plant
- (ii) Pestle and mortar
- (iii) Beakers
- (iv) Filter funnel and papers
- (v) Silica gel G 254
- (vi) Aluminium foil

2.1.2 Reagents

These were of analytical grade and included;

- (i) Chloroform
- (ii) Methanol
- (iii) Vanillin 1% in sulphuric acid
- (iv) Lead subacetate solution
- (v) Meyer's reagent
- (vi) Dragendorff's reagent
- (vii) Iodine

2.1.3 Equipment

- (i) Weighing balance
- (ii) Oven
- (iii) Rotary evaporator
- (iv) Iodine chamber
- (v) UV spectrophotometer
- (vi) Developmental tanks
- (vii) TLC plates

2.2 Experimental procedure

2.2.1 Plant collection

The plant was collected from a shamba in Kasarani and the samples confirmed by Mr. Mwalukumbi of Pharmacognosy department.

2.2.2 Drying and milling

The leaves were isolated from the plants and some were air dried for a period of two weeks to get dry leaves while others were stored carefully in a water surrounding to prevent drying. After the two week period, some of the fresh leaves were boiled for thirty minutes after which they were left to dry overnight in an oven. The fresh, dried and boiled leaves were then milled separately using a milling machine for the dry and boiled leaves while the fresh leaves were milled using a pestle and mortar.

2.2.3 Extraction

About 15 g of powdered dried leaf, 15 g of powdered fresh leaf and 15 g of powdered boiled leaf were put in separate beakers and into each beaker 250ml of chloroform added. They were then left for 48 hours to allow for extraction. The distillate was then filtered from crude extract using a filter paper and filter funnel into a separate beaker for each extract. To each of the beakers with crude extract 250ml of methanol was put and left to extract for another 48 hours. The distillate was then filtered into different beakers using a filter paper and filter funnel. The extracts were then reduced to quantity using a rotary evaporator and weight of each extract yield determined using a weighing balance.

2.2.4 Thin layer chromatography

Exactly 35 g of silica gel was weighed then suspended in 2:1 v/v 100ml chloroform to methanol which was then transferred into a staining chamber. Two microplates were then dipped to 3/4, removed, wiped on the sides and left to dry. On one microplate samples from the chloroform extract were spotted 4X and on the other microplate samples from the methanol extract were spotted and air dried.

In two separate staining chambers 100% chloroform and 95:5 chloroform to methanol respectively was set for solvent development. The microplate with

chloroform extract was dipped into the 100% chloroform chamber while the microplate with methanol extract was dipped into the 95:5 chloroform to methanol chamber.

2.2.4.1 Preparation on larger plates

20cm×20cm glass plates were used. The glass plates were cleaned with distilled water and dried. A smooth slurry was then prepared by mixing 35g of silica with 100 ml of distilled water. The slurry was poured in the desaga spreader and passed over the plates at uniform speed to receive a uniform coating of adsorbent layer. The plates were then allowed to air dry and activated by heating in the oven at 105°c for 1 hr.

The plates were then spotted with the samples from the plant extracts and developed in mobile phases of 100% chloroform and the other of chloroform and methanol in the ratio of 95:5 respectively. The developed plates were left standing to dry, visualised with naked eye, sprayed with 1% vanillin in sulphuric acid, visualised, put under UV spectrophotometer at 365nm, visualised, then placed inside an iodine box and visualised with all separations being circled. The plates were documented by tracing on a tracing paper and Rf values calculated.

2.2.5 Phytochemical tests

2.2.5.1 Test for tannins

About 2 g of the powdered dry, boiled and fresh leaf were boiled separately in 20 ml of distilled water in a boiling tube for 5 minutes. The contents were then filtered and a few drops of 0.1 % ferric chloride added and observations made for brownish green or blue black coloration.

2.2.5.2 Test for saponins

Foaming test

About 0.5 g of the powdered dry, boiled and fresh leaf were boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. This indicated the presence of saponins.

2.2.5.3 Test for alkaloids

The Dragendoff's reagent test

About 100 mg of dried, boiled and fresh powdered leaf was extracted by warming in a water bath with 2 ml of 1% sulfuric acid for two minutes. This was then filtered and the excess acid neutralized with dilute ammonia solution. 5 ml of chloroform was added into the filtrate then the chloroform layer was separated and washed with a little water. The chloroform was evaporated to dryness and a residue obtained. This residue was dissolved in 0.25 ml of 1% sulfuric acid and to this solution was added one drop of Dragendoff's reagent. Presence of alkaloids would be indicated by formation of a yellow precipitate.

2.2.5.4 Test for glycosides

About 1.0 g of the powdered dry, boiled and fresh leaf were extracted separately with 10 ml of 70% alcohol by heating on a water bath for 5 minutes. They were then cooled and filtered. To the filtrates, 10ml of water was added and 5 drops of strong solution of lead sub acetate added. The contents were filtered and 10% sulfuric acid added drop wise till no more precipitate was formed. The solutions were filtered and extracted with 2 successive 5ml portions of chloroform. The 2 chloroform extracts were combined, washed with 1ml of distilled water and then separated. chloroform The layer was filtered through a small plug of cotton wool. The chloroform extract

was divided into 2 equal parts for the test of unsaturated lactone ring of the aglycone (kedde test) and the test for 2-deoxy sugar (keller-killiani).

(i) Test for unsaturated lactone ring of the aglycone (kedde test)

The chloroform extract was evaporated to dryness and 1 drop of 90% alcohol and 2 drops of 2 % 3,5-dinitrobenzoic acid in 90% alcohol added. 20% NaOH solution made the extract alkaline. The development of a purple color showed the presence of a beta unsaturated lactone ring in the aglycone.

(ii) Test for 2-deoxy sugar (keller-killiani test)

The chloroform extract was evaporated to dryness and 0.4 ml glacial acetic acid containing a trace of ferric chloride added. The solution was then transferred to a test tube and 0.5 ml of concentrated sulfuric acid carefully added downside of the test tube. Presence of 2-deoxy sugar was shown by a green blue color in the upper acetic acid layer.

CHAPTER THREE
RESULTS AND DISCUSSION

3.1 RESULTS

Table 1: Weights of extracts and percentage yields using 15g samples

| Extract | Weight of empty flask + extract (g) | Weight of empty flask(g) | Weight of extract(g) | Calculation of % yeild | % yeild |
|----------------------------|-------------------------------------|--------------------------|----------------------|------------------------|---------|
| Chloroform (dry leaves) | 41.16 | 40.84 | 0.32 | $0.32/15 \times 100$ | 2.13 |
| Chloroform (fresh leaves) | 41.70 | 40.84 | 0.62 | $0.62/15 \times 100$ | 4.13 |
| Chloroform (boiled leaves) | 40.90 | 40.84 | 0.06 | $0.06/15 \times 100$ | 0.4 |
| Methanol (dry leaves) | 33.92 | 33.31 | 0.61 | $0.61/15 \times 100$ | 4.1 |
| Methanol (fresh leaves) | 34.21 | 33.31 | 0.9 | $0.9/15 \times 100$ | 6.0 |
| Methanol (boiled leaves) | 33.46 | 33.31 | 0.36 | $0.36/15 \times 100$ | 2.4 |

3.1.1 Phytochemical test results.

Table 2: Table of results for phytochemical tests

| Test | Observations | Inference |
|---------------------|---|-----------------------------|
| Test for tannins | A white precipitate was observed in both dry and fresh extract with no precipitate observed in boiled extract | Tannins were present |
| Tests for alkaloids | A yellow precipitate was observed in both fresh and dry extracts with no precipitate observed with boiled extract | Alkaloids were present |
| Tests glycosides | purple blue precipitate was not observed for all extracts | Glycosides were not present |
| Tests for saponins | The solution foamed on shaking for both fresh and dry extracts with no foam presence in the boiled extract | Saponins were present |

3.i.2 Thin layer chromatography

Comparative TLC of Amaraunthus leaf extracts using visual, UV, iodine, and 1% vanillin in H₂SO₄

Key:

a- dry extract

b- fresh extract

c- boiled extract



Fig 1.5 TLC of chloroform extracts using 100% chloroform as mobile phase



Fig 1.6 TLC chromatogram of methanol extracts using chloroform to methanol (95:5) mobile solvent

Table 3: Rf values of chromatogram

| | Rf values | |
|---|------------------|-------------------------------|
| | | |
| Chloroform Extract in 100% chloroform | dry | 0.76; 0.75; 0.59; 0.36 |
| | fresh | - |
| | boiled | 0.81; 0.8; 0.59; 0.16 |
| Methanol extract in 95:5 chloroform:methanol | dry | 0.92 |
| | fresh | 0.87; 0.47 |
| | boiled | 0.47; 0.45 ; 0.16 |

3.2 DISCUSSION

Phytochemical tests showed that the powdered leaves of both fresh and dried amaranthus contain alkaloids, tannins and saponins but no glycosides.

On yield comparison the results showed that on drying for a period of time there is phytochemical loss of about two percent thus nutritional value tends to reduce. After boiling of the leaves, results show that there is near complete photochemical loss as the components are extracted into the water and leaf left without most of the components. This indicated that fresh amaranth leaves are most nutritious with chloroform extract yielding 4.13% while the methanol extract yielding 6%. Boiled amaranth tends to have the least amount of phytochemicals which are lost in the water extract with the chloroform extract yielding 0.4% and methanol extract yielding 2.4%. Methanol proved to be the best extract of amaranth phytochemicals as there was a two percent increase in extraction compared to the chloroform extract.

Thin layer chromatography with chloroform to methanol (95:5) as mobile solvent yielded spots with each sample compared to 100% chloroform. This indicated the extracts had both polar and non polar solvents with the polar components achieving a better separation with chloroform to methanol (95:5).

3.3 CONCLUSION

The chromatograms obtained indicate that the most suitable mobile phase for separation for amaranth leaf extracts is a mixture of methanol and chloroform as the leaf is shown to contain polar compounds which are phenols.

3.4 RECOMMENDATIONS

Further work should be done on whole plant as it also contains grains which may contain nutritive value and health benefits

Extractions should be done using more polar mobile solvents as many of the plant components are actually polar.

CHAPTER FOUR

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