EXTRACTIVE VALUE, ASH VALUE AND MOISTURE CONTENT OF *AMARANTHUS DUBIUS* LEAVES ("DODO") IN BUSHENYI _ ISHAKA TOWN COUNCIL, SOUTH WESTERN UGANDA

RESEARCH SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF A BACHELOR OF PHARMACY

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DECLARATION

I, **JECINTA NJUGUNA** hereby declare that this is my original work and findings and that it has never been submitted to any university or any higher institution of learning for the same award or otherwise by any other person.

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DEDICATION

This report is dedicated to my family especially my beloved parents with whose support I accomplished this study, also to my almighty God for his guidance, support and blessings throughout my studies.

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I wish to acknowledge all those people who helped me in one way or another towards completion of these report

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ABSTRACT

Amaranthus dubius is a plant that grows in most tropical parts of the world and usually found in most sub-humid parts of Uganda. Extractive values, ash values and moisture content are methods employed to authenticate herbal preparations which cannot be assayed by any other means. The values obtained can help one know whether the preparation was adulterated or not. The study involved determining the extractive, ash values and moisture content of *Amaranthus dubius* leaves. The Proximate analysis showed the percentage yield of extractive value, total ash, water-soluble and water insoluble as 5%, 27.5%, 17.5% and 10% respectively. Percentage moisture content was highest being 15.8% and lowest 10%. Temperature of the room should be considered and be maintained to the required temperature. Results indicated that A. *dubius* leaves were contaminated with foreign matter which could be soil, excreta or sand. Plants that are grown on mud soil are likely to be contaminated with soil than those grown on loamy soil.

There is need to determine level of adulteration if any. Plant materials should be entirely free from visible signs of contamination by moulds or insects, and other animal contamination, including animal excreta. Results indicated that A. *dubius* contain moisture which could lead to deterioration, proliferation of organisms thereby rendering the plant unfit for human consumption.

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CHAPTER ONE

1.1 INTRODUCTION

The extractive values are useful in evaluating the amount of active constituents extracted with solvents from a given amount of plant material and thus help in identification of the plant material. Extraction is removal of soluble material from an insoluble residue either liquid or solid, by treatment with a liquid solvent, the solvent used is in a position to dissolve appreciable quantities of substances desired. The controlling factor in the rate of extraction is normally the rate of the diffusion of the solute through the liquid boundary layer or layers at the interface, (Trease and Evans Pharmacognosy,1998).It is employed for materials for which as yet no suitable chemical or biological assay exists, (WHO, 1998).

Ash content of the drug is the residue remaining after incineration as a form of adulteration of which many times the plant is mixed with various substances like sand, soil, chalk powder or other different inorganic contents. Incineration is done to burn out all organic matter and ash value is a criterion to judge the identity, quality or purity of crude drugs/plant material especially in the powdered form, (Trease and Evans Pharmacognosy 1998).

Moisture content is achieved by heating the powder in an oven to a constant weight at 105°C and moisture content should be minimized in order to prevent decomposition of crude drug either due to chemical change or microbial contamination. Presences of water or volatile materials do contribute to weight loss and materials containing little volatile materials, direct drying to constant weight is used. Also, presence of moisture will lead to the activation of enzymes and given

favourable conditions, to the proliferation of living organisms, (Trease & Evans Pharmacognosy, 1998).

Pharmacognostic studies are required to validate the usage of leaves of *Amaranthus dubius* as food supplement. These studies help in identification and authentication of the plant material. This information could be helpful in the identification and preparation of a monograph on the plant. Hopefully, the determination of these characteristics will aid future investigators and researchers in their pharmacological analysis of this species. The physico-chemical standards, such as ash values, extractive values, will be useful to identify the authenticity of the species even from the crushed or powdered plant materials. The information obtained from the ash values and extractive values are useful during the time of collection and also during extraction process. One of the locally available, under-exploited but potentially high vitamins food sources in Uganda is amaranthus species. It has been reported that vegetables (amaranthus) supplies part of the protein, vitamins and minerals needed in diet as well as roughage which promote digestion and prevent constipation. In East Africa, Amaranthus leaf is known in Chewa as ' *Bonongwe'*, and in Swahili as '*mchicha'*. Also known among the Kalenjin as a drought crop ('*chepkerta'*).

1.1.2 BACKGROUND

Amaranthus dubius is a branched herb up to 1metre or more, resembling the spiny amaranth, *A. spinosus,* but without spines. Leaves are simple, long petiolate alternate, usually with an ovate lamina to 8cm long, veins conspicuous underneath. Flowers are borne in clusters in the avils and in terminal branched heads or spikes, fruits are covered by bracts and bracteoles which are the more visible structures of the flowering part, seeds are black and shiny. It's a common herb in most towns, rural and commonly found on cultivated land ,roadsides and flood plains, leaves and tender

shoots are used as a vegetable sometimes cooked with more bitter vegetables and is a popular choice for improving the taste of many traditional leafy vegetables.

Amaranthus is especially rich in lysine, an essential amino acid that is lacking in diets based on cereals and tubers. They are a very good source of vitamins including vitamin A, vitamin K, vitamin B6, vitamin C, riboflavin, and folate, and dietary minerals including calcium, ions, magnesium, phosphorus, potassium, zinc, copper, and manganese. However their moderately high content of oxalic acid inhibits the absorption of calcium and zinc, and also means that they should be avoided or eaten in moderation by people with kidney disorders, gout, or rheumatoid arthritis. Reheating cooked amaranthus greens are often discouraged, particularly for consumption by small children, as the nitrates, in the leaves can be converted to nitrites, similarly to spinach. Amaranthus seeds, like buckwheat and quinoa, contain protein that is unusually complete for plant sources. Most fruits and vegetables do not contain a complete set of amino acids, and thus different sources of protein must be used. Its seeds have protein content greater than that of wheat.

1.1.3 PROBLEM STATEMENT

Quality control is essential for all products consumed by man including plants. Many plants have elaborate methods for assay of active constituent but not all. For those plants which cannot be assayed; other methods such as extractive values can be used to determine their quantity. Also all plants are at risk of decaying if the moisture content exceeds 15%. Ash values help in determining the amount of adulteration present in a plant.

1.1.4 GENERAL OBJECTIVE

To establish the pharmacognostical value/properties of *Amaranthus dubius* in South Western Uganda using quantitative pharmacognostic methods.

1.1.5 SPECIFIC OBJECTIVE

- ✓ To determine the percentage cold extractive value
- ✓ To determine the ash values, and
- ✓ To determine the moisture content of Amaranthus *dubius leaves*.

1.1.6 JUSTIFICATION

Pharmacognostical value is essential in determining quality and purity of plant products. From results obtained, information can be disseminated on issues resulting to proper cultivation, time of harvesting, preparation and storage. The major aspect also relates to the preparation methods. It was prudent enough to understand the nutrient contents and suggest the proper Amaranthus species preparation literally cooking procedure.

1.2 ACRONYMS AND ABBREVIATIONS

REG NO – Registration number

WHO – World Health Organization

C – Celsius

C – Crotalaria

ADF – acid detergent fibre

UIP – undegraded intake protein

M – mass

A - Amaranthus

CHAPTER TWO

2.1 LITERATURE REVIEW

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. Analytical techniques for providing information about the total mineral content are based on the fact that the minerals (the "analyte") can be distinguished from all the other components (the "matrix") within a food in some measurable way. The most widely used methods are based on the fact that minerals are not destroyed by heating, and that they have a low volatility compared to other food components. *Amaranthus dubius* are among the most important leafy vegetables in the continent, a fact attributed to their hardiness and, hence, wide occurrence, low susceptibility to disease, low pest incidence, low labor input (making them easy to cultivate), ease in cooking and high nutritional value. *Amaranthus dubius* grow well on most soils rich in organic matter and require moist soils, warm and sunny conditions. Land should be prepared to a fine tilth due to the small seed size which creates the friable texture needed by fragile roots of the young plants. *Amaranthus dubius* are fast growing and known to suppress nematodes and are therefore suitable for intercropping with vegetables that are susceptible to nematodes such as tomatoes and potatoes but also Jew's mallow and African nightshades. They can be planted together with spider plant, nightshades or Ethiopian kale (Maundu, 1999). Locally, the vegetables are mostly found in rural areas where people have no access to fast foods but they just consume them not knowing of their importance in their bodies. In Indonesia and Malaysia, leaf amaranthus is called '*bayam*', while the Tagalogs in the Philippines call the plant '*kulitis*'. In Karnataka

state in India it is used to prepare Hulli. Palya, Maggigayhulli and so on. In Tamilnadu State, it is regularly consumed as a favorite dish, where the greens are steamed, and mashed, with light seasoning of salt, red chillis and cumin. It is called '*keerai masial*'. In Andhra Pradesh, India, this leaf is added in preparation of a popular dal called '*thotakura pappu*'. In China, the leaves and stems are used as a stir-fry vegetable and called '*yin choi*'. In Vietnam, it is called '*rau dền*' and is used to make soup (Willis, 1984).

In West Africa such as in Nigeria, it is a common vegetable, and goes with all Nigerian carbohydrate dishes. It is known in Yoruba as '*efo tete* or *arowo jeja*' ("we have money left over for fish"). In Congo it is known as '*lenga lenga* or *biteku teku*'. In the Caribbean, the leaves are called '*callaloo*' and are sometimes used in a soup called pepperpot soup. The more important species include *A. cruentus* (synonym *A. hybridus*), which is widely distributed and used throughout the continent; cultivated widely in humid West Africa, mostly in urban and peri-urban areas, *A. dubius* is important in East and Southern Africa, occasionally with large leaf forms; *A. lividus (A.blitum),* commonly used from northern Tanzania, western Kenya and Uganda to Cameroon; and *A. hypochondriacus*, which together with *A. cruentus*, constitute most of the local seed amaranths. The vegetable is commonly grown in East Africa especially in Uganda. Several studies have shown that like oats, amaranthus seeds or oil 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 is a new crop with ancient history. Members of *Amaranthus* spp. have been grown for centuries for vegetable and grain in different parts of the world. Amaranthus is consumed as vegetable in Africa, Caribbean, China, Greece, India, Italy, Nepal, and South Pacific Islands (Stallknecht

& Schulz-Schaeffer, 1993). *Amaranthus* species most commonly utilized as vegetable have short plants with wide leaves and small inflorescence (Huang 1980). However, it is common to find same plant type used both for leaf and grain (Saunders & Becker, 1984; Tucker, 1986).

In New Zealand, dried seeds of myristica fragrans deprived from its seed coat and with or without a thin coating of lime were used for making beer. Nutmeg is the dried kernel of the seed of myristica Fragrans. Pharmacognostical and preliminary phytochemical studies of leaves of Tridax procumbens were done whereby the plant was collected; its parts, colour, size, shape, odour, taste appearance margin, apex, petiole, texture and fracture were all identified. Later, ash and extractive value were done with various reagents and examples of solvents used in extractive process are haxen, alcohol and water, (Teutonico & Knorr, 1985).

In Indian department of pharmacognosy, determination of extractive value of plant *Argyreia nervosa* was performed and analysis of powdered vegetable drugs were also carried out and used the plant for prevention of diseases because the plant is highly nutritious. In India, they studied a plant Euphorbia hirtal, by determination of physicochemical parameters using foreign organic matter, total ash value, acid insoluble upto quantitative estimation of active constitute. They later concluded that its leaves were used to induce vomiting. (Singh & Whitehead, 1993).

Similarly, in the United State of America they did evaluation of the fruit methanolic extractive, research on human food and their nutritive value which was found to be used in prevention of diseases once they consume it continuously. The importance attached to vegetable consumption depends on the community in question.

The awareness in the United States of the potentiality of amaranthus, mainly as a grain and to a lesser extent as a vegetable crop, was generated by the Rodale Research Center, Kutztown, Pennsylvania in the mid-1970s. Immigrants from the countries where vegetable amaranthus is consumed widely, however, were the real impetus behind creating a demand for this vegetable in the United States. Since then, it has been realized that amaranthus can also fill a void for fresh leafy vegetables during the summer months (Makus & Davis 1984). Amaranthus leaves are comparable to spinach (*Spinacia oleracea* L.) in taste (Abbott & Campbell 1982). They are also a good source of dietary fiber and contain high amounts of protein, vitamins, and minerals (Makus & Davis, 1984). However, unlike spinach, an ideal season for producing amaranthus in the temperate climate is during the hot months of the summer season. In addition, research has shown that green yields from amaranthus produced at different locations in the United States are high enough to make commercial exploitation feasible (Berberich, 1980; Campbell & Abbott 1982). Eating of unrefined grains, vegetables, fruits and legumes were found to aid in clearing the gallbladder and hasten the gall stones. Seed proteins of Crotalaria species like *C. juncea* yielded amino acids, ash, starch, sugar, fibre, phosphorous, ether extractive and calories. They were found to have nutritional quality in enrichment of ions in the body. Several studies (Stordahl, 1999; Lehmann, 1990; Pond & Lehmann, 1989; Senft, 1979; Cheeke & Bronson, 1979; Odwongo & Mugerwa, 1980; Yue, 1987) have shown that amaranthus qualities are superior to those of the common cereals and forage crops. Arguably, the most important nutritional quality of a grain is its protein content and quality. Amaranthus protein levels range from 13 to 19% in the grain (Lehmann, 1990; Pedersen, 1987) and from 12 to 27% for the whole plant (Stordahl, 1999; Mugerwa & Bwabye, 1974; Marten & Andersen, 1975). The protein quality of

amaranthus grain combined with its productivity (Stordahl, 1999; Campbell & Abbott, 1982; Clark & St. Jean, 1984) compares favorably with more commonly used grains. The proteins of wheat, corn, and rice are deficient in the essential amino acid lysine and the sulfur-containing amino acids methionine and cysteine.Amaranthus, however, is rich in both lysine (Bressani, 1987) and sulfur-containing amino acids (Senft, 1979).

Cheeke & Bronson (1979) found that amaranthus leaves and stems were higher in hemicellulose and ash and lower in acid detergent fiber (ADF) than alfalfa (*Medicago sativa* L.). They also found a greater amount of protein bound to the cell wall constituents in amaranthus than in alfalfa and comfrey (*Symphytum officinale* L.). This suggests that amaranthus may have a higher bypass protein value. Bypass protein or rumen undegraded intake protein (UIP), if available in the lower gut, can be of great value in livestock production because rumen microbes may degrade high-quality protein and escape protein is more efficiently used in postruminal digestion as long as it contains essential amino acids (Van Soest, 1994). Increasing the UIP percentage in the diets of growing heifers improves feed efficiency and increases body weight gain (Tomlinson et al., 1997) and milk yield (Vagnoni & Broderick, 1997).

Pond & Lehmann (1989) cited *Amaranthus cruentus* (Zimbabwe cultivar PI 482049) as a potential feed resource for ruminants based on its high protein content, low cellulose, and the absence of toxic substances in the vegetative fractions of the plant. However, some amaranthus can have toxic levels of nitrates and oxalates (Cheeke & Bronson, 1979). Toxicity can result from nitrates in forages. Poisoning may result from accumulation of nitrates and/or oxalates in plants growing under stress, especially if drought conditions occur during a period of heavy nitrate uptake by the plant. Dietary nitrate is converted to nitrite, then ammonia by rumen bacteria.

Toxicity occurs when the rate of conversion of nitrate to nitrite is higher than the conversion of nitrite to ammonia. Once absorbed into the blood, nitrite will bind to hemoglobin, forming methemoglobin. Since methemoglobin is less efficient in oxygen transport, animals will literally suffocate (Vough, 1991). Adams, 1992 reported that nitrate content above 1 to 3% on a dry matter basis can cause acute toxicity in animals. However, (Vough et al. 1991) reported that toxicity usually occurs when cattle consume large amounts of forage containing 1.76% or more nitrate ion on a dry matter basis.

It is a herb with a branched, multiple upright stem and ovate or rhomboid-ovate, dark-green leaves 5-6cm long belonging to family Amaranthaceae, subfamily Amaranthoideae, genus Amaranthus and subgenus Amaranthus whose Nomen number is 101534. It is hardy and frost tender. It is in leaf from April to October, in flower from July to September, and the seeds ripen from August to October. The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by Wind. The plant is self-fertile. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well-drained soil, prefers acid, neutral and basic (alkaline) soils. It cannot grow in the shade and requires moist soil. Leaves - cooked used as a potherb, they are considered to be very palatable. Seed – cooked rather small, but very nutritious, it can be ground and used as a powder. The seed can be cooked whole, and becomes very gelatinous like this, but it is rather difficult to crush all of the small seeds in the mouth and thus some of the seed will pass right through the digestive system without being assimilated.

If the plant is cut back and not uprooted, it may continue to grow for 120-300 days and provide successive harvests.

Leaves and tender stems are boiled in water and prepared much like spinach, said to be excellent treatment for anaemia, tiredness, constipation and poor nutrition. . *Amaranthus dubius* can be employed to assist with healing of rashes of the skin and boils, migraine headaches, diarrhoea, leucorrhoea, soothing the stomach, as a diuretic, for bladder distress, excessive menstruation, haemorrhoids and dysentery.

A.dubius is widely distributed in Central Southern America, northern America, Caribbean, northern South America, Brazil and west south America, (Adams, C.1972).

CHAPTER THREE

METHODOLOGY

3.1 PLANT COLLECTION, IDENTIFICATION AND PREPARATION

Amaranthus dubius leaves were collected in Ishaka, Bushenyi district, South Western Uganda, in the morning from the garden assisted by garden owner nearby Kampala International University-Western campus. Collection was made in the month of September 2009. Fresh leaves of the plant were dried by placing them under a shade and then reduced to fine powder by grinding using a metallic motor and pestle in Kampala International University pharmacy laboratory.

3.2 METHODS AND PROCEDURES

Water- soluble ash, water-soluble extractive and loss on drying methods will be used to determine ash value, extractive value and moisture content respectively.

3.3 DETERMINATION OF COLD EXTRACTIVE VALUES

4grams of *Amaranthus dubius* powder were weighed in weighing papers using a weighing balance and transferred to a three dried 250ml conical flasks, three 100ml graduated flask were filled with chloroform water. The weighing papers were washed out and the washing poured together with the remainder of the solvent into the conical flask. The samples were prepared in triplicate. The conical flasks together with the samples were weighed to obtain the total weight. Flask was corked and shaken frequently for 6 hours, then allowed to stand for 18 hours. The samples were filtered into 50ml cylinders. When sufficient filtrate was collected, 25mls of the filtrate was transferred to a pre-weighed 250ml beaker. It was allowed to evaporate to dryness on a water-bath and complete drying in an oven at 100degrees for 6 hours, allowed to cool in a dessicator and weighed.

The percentage yield was calculated as follows;

25 ml of chloroform- water extract = x g of residue

100 ml of chloroform-water extract = 4 xg of residue

Where x is weight of beaker + residue – weight of empty beaker.

3.4 DETERMINATION OF ASH VALUES

4 grams in triplicate of the powdered samples were ignited to ash, cooled in a dessicator for up to 24 hours and weight of total ash was taken. They were then dissolved in 200mls of distilled water and stirred. The mixture was filtered using a known weight of filter paper and the residue obtained was dried together with the filter paper and the weight of the insoluble residue taken. The results were subtracted from the total ash to obtain the amount of water soluble ash in the drug.

3.5 MOISTURE CONTENT

4grams in triplicate of the powdered sample were weighed into weighed crucibles, and then dried in the oven at 100degrees until constant weight. Then allowed to cool in a dessicator and watch. Loss in weight was recorded as moisture content.

3.6 DATA ANALYSIS

Results were expressed as mean ± standard deviation and bar graphs.

CHAPTER FOUR

RESULTS

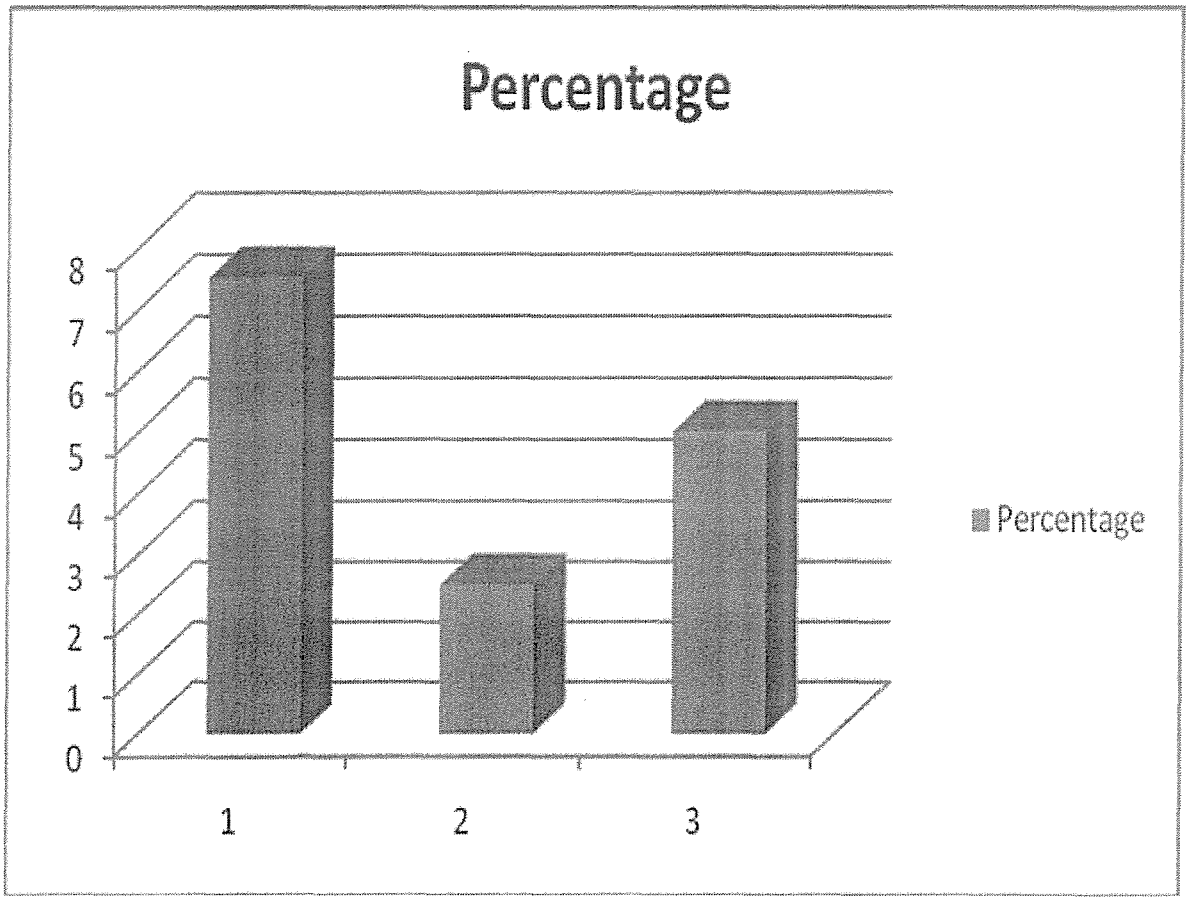
EXTRACTIVE VALUES

4.1 COLD EXTRACTIVE VALUES

The result of the cold extractive value as determined is presented in bar graph 1. Values are in triplicate.

Mean weight of residue is 0.2 ± 0.1
Mean Percentage yield (%) is 5%.

Bar graph 1:

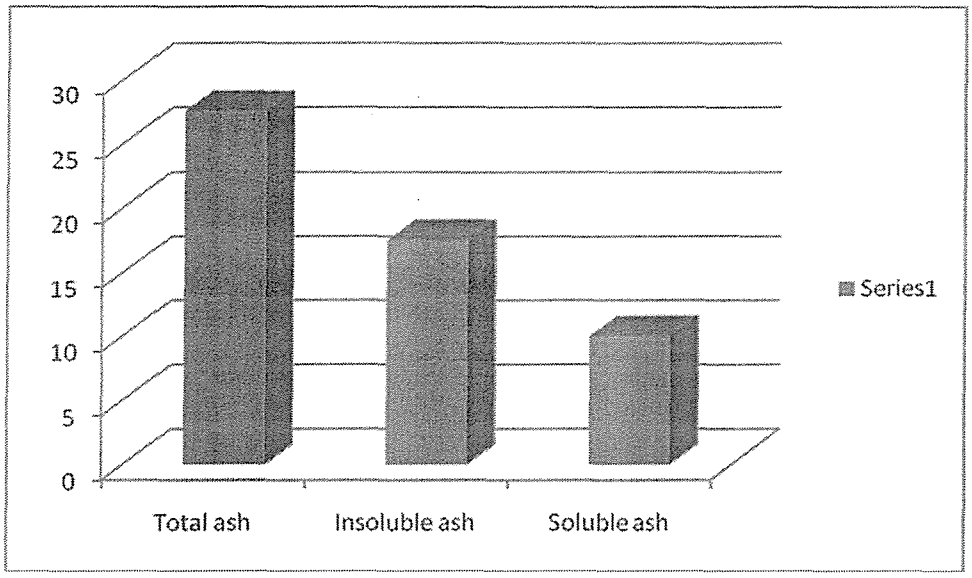


Graph showing percentage yield of extractive values of three samples, 1, 2, 3 indicating 7.5%, 2.5% and 5% respectively.

4.2 ASH VALUES

Results of the ash value are presented in Table 1 in the appendices.

Bar graph 2:



Graph showing percentage total ash, insoluble ash, soluble ash of three samples total ash being high with 27.5% and soluble ash of 10%.

4.3 MOISTURE CONTENT

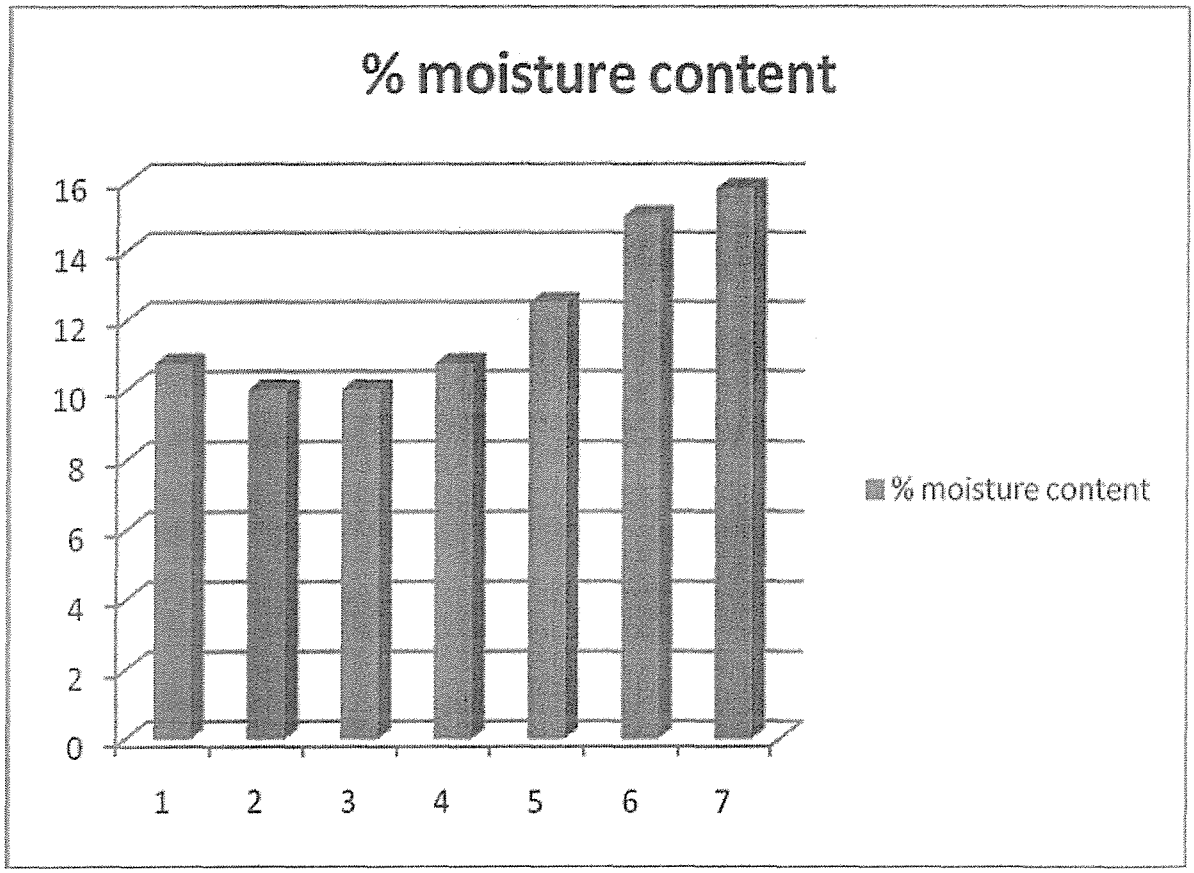
The result of the moisture content is presented in Table 2 in the appendices.

The moisture content in Amaranthus dubius is calculated as follows;

% Moisture = $M_{initial} - M_{dried} / M_{initial} * 100$

Where M is the Mass.

Bar graph 3: Shows percentage moisture content



Highest percentage moisture content is 15.8% while lowest is 10%

CHAPTER FIVE

5.1 DISCUSSION

In the present investigation values of solvent extractive indicated variability among the three samples used using the same method of extraction. Although the variation is minimal it could have been as a result of temperature which actually affects solubility therefore with cold maceration, variability can occur depending with the place the maceration was done and the temperature of that particular place. Successive extractive values are highest as seen with aqueous extract indicating the possibility of considerable content of polar compounds in the leaves. Duration of maceration can bring about variability in results as well as method of extraction used. Bar graph 1 revealed extractive potency of the leaves in water giving yield of 5% which was not close to the recommended value of between 20% and 30%, (Abushwireb et al., 2007).

Percentage total ash, insoluble ash and soluble ash obtained were 27.5%, 17.5% and 10% respectively, these parameters detect amount of organic and inorganic material in the plant sample. Recommended level should not exceed 12% (Seader et al., 1998). The ash values are high indicating presence of foreign matter in the plant material which could be contaminated with soil or sand. The significance of ashing the drug is to remove all traces of organic matter that may otherwise interfere with the analytical determination and the ash obtained normally consists of carbonate, phosphates and silicates of sodium, potassium, calcium and magnesium.

The recommended moisture content level of plant material should not exceed 15% and if it exceeds, the material is predisposed to decaying, degradation or decomposition, (Trease and Evans Pharmacognosy, 1998). Percentage moisture content for *Amaranthus dubius* were found to high or exceeded by 0.8%.Lowest

percentage were 10 while high were 15.8%.This is an indication that the plant contains moisture.

5.2 CONCLUSION

During extraction, room temperature should be considered and be maintained to the required temperature. Data show that the leaves of A. *dubius* were contaminated with foreign matter which could be soil or sand. Plants that are grown on mud soil are likely to be contaminated with soil than those grown on loam soil.

Contamination of plant raw materials can occur and so there is need to determine level of adulteration if any. Plant materials should be entirely free from visible signs of contamination by moulds or insects, and other animal contamination, including animal excreta. Results indicated that A. *dubius* contain moisture which could lead to deterioration, proliferation of organisms thereby rendering the plant unfit for human consumption.

5.3 RECOMMENDATION

From the results obtained, A. *dubius* is an important vegetable rich in amino acids (lysine), good source of vitamin A, K, Riboflavin, dietary minerals, (Bressani, 1987).

Also from the results obtained found that the Amaranthus species being consumed in Western Uganda, has a higher level of adulterant, therefore there is need for educating the public on proper cultivation, collection and preparation of the plant in order to maximize its potency.

They should be consumed fresh or immediately without storing them so as to avoid contamination. More work should be done on phytochemical screening so as to find other uses of the plant that has not been documented.

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APPENDICES

AMARANTHUS DUBIUS

Table 1: Percentage total ash, % insoluble ash.

Total ash	Mean weight of crucible + ash	Mean weight of crucible alone	Weight of ash	% Total ash
Sample A,B,C	24.5± 1.01	23.4±1.01	1.1	27.5
Insoluble ash	Mean weight of filter paper + insoluble ash	Mean weight of filter paper alone	Weight of ash	% insoluble ash
Sample A,B,C	1.67±0.12	0.97±0.21	0.70	17.5

Table 2: showing moisture loss and percentage moisture content

Sample	**Moisture loss at the following time**						
	2 hr	**4 hrs**	**6 hrs**	**8 hrs**	**10 hrs**	**12 hrs**	
Weight difference	0.43	0.4	0.4	0.43	0.5	0.6	0.63
% Moisture content	10.75	10	10	10.75	12.5	15	15.8