Phytochemical Constituents of Kalunay Leaves and Roots (*Amaranthus spinosus* L.)

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Abstract

Investigation of the phytochemical constituents of both the leaves and roots of the Kalunay (Amaranthus spinosus L.) was carried out using various solvents of ethyl acetate, ethanol, water and reagents. The qualitative tests of both the leaves and roots manifested the presence of alkaloids, and saponins. Alkaloids indicated yellowish precipitates while saponins showed a formation of more than 1.2 cm of "honeycomb froth". It was noted, however, that only fresh concentrated root extract manifested the presence of glycosides. All the other tests on fats and oils, flavanoids, triterpenes, sterols, leucoanthocyanins as well as resins showed negative results. In the concentrated root extract of Kalunay, a formation of brick-red precipitate in the hydrolyzed sample was noted; thus, it showed the presence of glycosides.

THe plant materials (leaves and roots) were collected in the wasteland of Don Alejandro Quirolgico (Lusong West), Caoayan, Ilocos Sur.

Different qualitative test namely filter paper test (for fats and oils), Wagner's Test (alkaloids), Fehling's Test (glycosides), Bate-Smith & Metcalf Test (flavanoids and leucoanthocyanins), Froth Test (saponins), Liebermann-Burchard Test (sterols and triterpenes) and Phloroglucinol Test (resins) were used.

There was an interesting result of the presence of glycosides in the root extract of the kalunay plant while it was negative in the leaf extract.

It is therefore, recommended that other substances in both the leaves and roots of the plant be analyzed. The sensitivity of bacterial and fungal strains to the plant materials be tested; and the pharmacological assay of the plant should be done.

Introduction

Background of the Study

The Philippines is well known for its rich natural resources and a wide variety of plants grow abundantly. Kalunay *(Amaranthus spinosus* L.) is one of the many varieties that grow and taken for granted.

The plant is described as stout, erect, smooth, branched herb, 0.4 to 1 m high. Stems with slender axillary spines. The presence of spines differentiates it from kolitis *(Amaranthus viridis)*. Its leaves are glabrous, long-petioled, oblong to oblong ovate, or elliptic-lanceolate, 4 to 10 cm long, obtuse and alternate. Its flowers are very numerous, stalkless, green or greening-white, about I mm long and born in dense, axillary clusters and in elongated terminal axillary spikes. Sepals, 5 or 1-3, ovate to linear, often aristate. Its petals, scarious, bracts, linear, bristle-pointed and as long as the sepals or longer. Its fruits are utricles, wrinkled, nearly as long as the sepals. Its seeds, minute, black and shining. (Tucker, 1986).

Kalunay *(Amaranthus spinosus L.)* is generally considered a pest plant of the farmers. Aside from being a wee, is also a host to may pest insects. During the planting season, they are pulled out and thrown away. However, in Asia and some parts in America, several specdies are being raised. In Guaemala, Peru, India and Nepal, they are being cultivated. One of the important reputed findings of some of the amaranth species is the protein content ot its grain which is 30% higher than the seeds such as wheat, rye and oats. It is found throughout the Philippines at lowlands and low altitudes. A common weed on roadsides and wastelands. (http://plants.usda.gov/java/profile)

Several species are raised for amaranth grain in Asia and the Americas. Ancient amaranth grains stilt used to this day include the three species, Amaranthus caudatus, Amaranthus cruentus, and Amaranthus hypochondriacus. Although amaranth was (and still is) cultivated on a small scale in *parts* of Mexico, Guatemala, Peru, India, and Nepal, there is potential for further cultivation in the U.S and tropical countries and it is often referred to as "the crop of the future. It has been proposed as an inexpensive native crop that could be cultivated by indigenous people in rural areas for several reasons: l) easily harvested, 2) produces a lot of fruits (and thus seeds) which are used as grain, 3) highly tolerant of arid environments which are typical of most subtropical and some tropical regions, and 4) large amounts of protein and essential amino acids, such as lysine. Due to its weedy life history, amaranth grains grow very rapidly and their large seedheads can weigh up to I kilogram and contain a half-million seeds. Amaranthus species are reported to have a 30% higher protein value than other cereals, such as rice, wheat flour, oats, and rye. (Tucker, 1986)

Since very little or no value is given to the lowly kalunay *(Amaranthus spinosus L)* and considered just a weed, this study had been conceptualized to identify the phytochemical constituents of the plant which eventually could be used both in the field of nutrition as well as medicine.

Objective of the Study

This study was conducted to determine the phytochemical constituents of kalunay leaves and roots extract. The extracts were tested with different qualitative tests namely filter paper test (for fats and oils), Wagner's Test (alkaloids), Fehling's Test (glycosides), Bate-Smith and Metcalf Test (flavanoids and leucoanthocyanins), Froth Test (saponins), Liebermann-Burchard Test (sterols and triterpenes) and Phloroglucinol Test (resins).

Scope and Delimitation

This study was delimited to the determination of the phytocbemical constituents of both the leaves and roots of the kalunay *(Amaranthus spinosus* L.). This study was conducted at the Biotech Laboratory, University of Northern Philippines on September, 2009. The phytochemical analysis was delimited to the qualitative rather than quantitative screenings.

Review of Related Literature

A species of the amaranth has been used as traditional food plant in Africa, this vegetable has potential to improve nutrition, boost food security, and foster rural development and support sustainable landcare. Amaranth grain is a crop of moderate importance in the Himalaya. It was one of the staple foodstuffs of the Incas. and it is known as kiwicha in the Andes today. It was also used by the ancient Aztecs. who called it huautli, and other Native America peoples in Mexico to prepare ritual drinks and foods. To this day, amaranth grains are toasted much like popcorn and mixed with honey. molasses or chocolate to make a treat called alegria (literally, "joy" in Spanish). Amaranth was used in several Aztec ceremonies, where images of their gods (notably Huitzilopochtli) were made with amaranth mixed with honey. The images were cut to be eaten by the people. This looked like the Christian communion to the Roman Catholic priests. so the cultivation of the grain was forbidden for centuries. Because of its importance as a symbol of indigenous culture, and because it is very palatable. easy to cook. and its protein particularly well suited to human nutritional needs, interest in grain amaranth (especially A. cruentus and A.

hypochondriacus) was revived in the 1970s. It was recovered in Mexico from wild varieties and is now commercially cultivated. It is a popular snack sold in Mexico City and other parts of Mexico, sometimes mixed with chocolate or puffed rice, and its use has spread to Europe and other parts of North America. Amaranth and guinoa are called pseudograins because of their flavor and cooking similarities to grains. These are dicot plant seeds, and both contain exceptionally complete protein for plant sources. Besides protein, amaranth grain provides a good source of dietary fiber and dietary minerals such as iron, magnesium, phosphorus, copper, and especially manganese. (http://plants.usda.gov/java/profile)

In Indonesia and Malaysia. leaf amaranth is called bayam, while the Tagalogs in the Philippines call the plant kulitis. 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 and variations on this transliteration in various dialects). In Vietnam, it is called rau den and is used to make soup. There are two species popular as edible vegetable in Vietnam: den do- amaranthus tricolor and den com or den trang- amaranthus viridis. In East Africa, Amaranth leaf is known in Swahili as mchicha. It is sometimes recommended by some doctors for people having low red blood cell count. Also known among the Kalenjin as a drought crop (chepkerta). 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[clarification needed] 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. In Greece, Green Amaranth (Arnaranthus viridis) is a popular dish and is called vleeta. It's boiled, then served with olive oil and lemon like a salad, usually alongside fried fish. Greeks stop harvesting the (usually wild-grown) plant when it starts to bloom at the end of August The flowers of the 'Hopi Red Dye' amaranth were used by the Hopi Amerindians as the source of a deep red dye. There is also a synthetic dye that has been named "amaranth" for its similarity in color to the natural amaranth pigments known as betalains. This synthetic dye is also known as Red No. 2 in North America and E123 in the European Union. In some parts of America the genus also contains several well-known ornamental plants, such as A. caudatus (love-lies-bleeding), a native of India and a vigorous, hardy annual with dark purplish flowers crowded in handsome drooping spikes. Another Indian annual, A. hypochondriacus (prince's feather), has deeply-veined lance-shaped leaves, purple on the under face, and deep crimson flowers densely packed on erect spike. Amaranths are recorded as food plants for some Lepidoptera (butterfly and moth) species including the Nutmeg and various case-bearers of the genus Coleophora: C. amaranthella, C. enchorda (feeds exclusively on Amaranthus), C. immortalis (feeds exclusively on Amaranthus), C. lineapulvella and C. versurella (recorded on A. spinosus). (Tucker, 1986)

A study of the betacyanins and phenolic compounds from kalunay *(Amaranthus spinosus L)* had been conducted by Sttintzing, et al., 2005. Stem bark extracts of Boerhavia erecta L. (erect spiderling) and Amaranthus spinosus L (spiny amaranth), two

wild growing weed plants used in traditional African medicine, werecharacterized with respect to their phenolic profile including the betalains. While the mainbetalains in A. spinosus were identified as amaranthine and isoamaranthine, the major betacyaninsin B. erecta were betanin, isobetanin together with neobetanin. The latter showedhigher betalain concentrations amounting to 186 mg/100 g, while the former contained 24 mgbetacyanins in 100 g of the ground plant material. Extracts of A. spinosus were found tocontain bydroxycinnamates, quercetin and kaempferol glycosides, whereas catechins, procyanidinsand quercetin, kaempferol and isorhamnetin glycosides were detected in B. erecta. Theamounts of these compounds ranged from 305 mg/100 g for A. spinosus to 329 mg/100 g forB. erecta. (Stintzing, et. al., 2005)

"Kalunay plants are a very good sourcesof yitamins including vitamin A, vitamin K, vitamin B6, vitamin C, riboflavin, and folate, and dietary minerals including calcium, iron, magnesium, phosphorus, potassium, zinc, copper, and manganese. Because of its valuable nutrition, some farmers grow amaranth today. 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 amaranth greens is often discouraged, particularly for consumption by small children, as the nitrates in the leaves can be converted to nitrites, similarly to spinach. (http://plants.usda.gov/java/profile).

As a weed

Not all amaranth plants are cultivated. Most of the species from Amaranthus are summer annual weeds and are commonly referred to as pigweeds. These species have an extended period of germination, rapid growth, and high rates of seed production and have been causing problems for farmers since the mid-1990s. This is partially due to the reduction in tillage, reduction in herbicidal use and the evolution of herbicidal resistance in several species where herbicides have been applied more often. The following 9 species of Amaranthus are considered invasive and noxious weeds in the U.S and Canada: A. albus, A. blitoides, A. hybridus, A. palmeri, A. powellii, A. retroflexus, A. spinosus, **A.** tuberculatus, and A. viridis.

hi tropical Africa and elsewhere Amaranthus spinosus leaves and young plants are collected for home consumption as **a** cooked, steamed or fried vegetable, especially during periods of drought. Leaves are occasionally found for sale on markets. In Uganda and Kenya it commands a lower price than, for example, Arnaranthus dubius Mart ex Thell. because of its spines and because it is not much liked. Its use is declining, and it is acquiring the status of a famine food. It has a bitter taste and is usually eaten in small quantities as a substitute when no other vegetables are available. Amaranthus spinosus is also used as forage and said to increase the yield of milk in cattle. However, the spines can cause injury to the mouths of grazing animals and cases of poisoning in cattle have also

been reported. In Uganda the ash of burnt Amaranthus spinosus plants is used as a tenderizer in cooking tough vegetables such as cowpea leaves and pigeon peas. The ash is also used as a vegetable salt and in southern Africa it is used as a snuff, alone or with tobacco. Amaranthus spinosus has numerous medicinal uses. The root is known as an effective diuretic. In South-East Asia a decoction of the root is used to treat gonorrhoea and is also applied as an emmenagogue and antipyretic. In many countries, including those in Africa, the bruised leaves are considered a good emollient and applied externally in cases of eczema, bums, wounds, boils, earache and haemorroids. The plant ash in solution is used to wash sores. Plant sap is used as an eye wash to treat ophthalmia and convulsions in children. In Malaysia Amaranthus spinosus is used as an expectorant and to relieve breathing in acute bronchitis. In mainland South-East Asia, it is also used as a sudorific, febrifuge, antidote,to snake poison, galactagogue, and to treat menorrhagia. Some tribes in India apply Amaranthus spinosus to induce abortion. (http: / / bicn.com / wei / resources / nerp / wrs / ch3.htm)

The nutritional value of Amaranthus spinosus is comparable to that of other vegetable amaranths. Amaranthus leaves contain per 100 g edible portion: water 84.0 g, energy 176 kJ (42 kcal), protein 4.6 g, fat 0.2 g, carbohydrate 8.3 g, fibre 1.8 g, Ca 410 mg, P 103 mg and Fe 8.9 mg (Leung, W.-T.W., Busson, F. & Jardin, C., 1968). Cases of spontaneous poisoning in cattle by Amaranthus spinosus have been reported, particularly after severe droughts when few other forages were available. It was suggested that Amaranthus spinosus caused renal failure. The roots contain a.-spinasterol and some saponins. Sterols, n-alkanes, fatty acids and free alcohols have been found in petroleum-ether extracts of the herb. The flavonoid rutin has been found in the aboveground parts in a concentration of up to 1.9%, and traces of hydrocyanic acid in the leaves. The considerable amount of potassium in the leaves might explain the diuretic properties. A lectin has been isolated from the seeds. Its reaction was non-specific in general: it reacted with human and various animal erythrocytes. Its unique carbohydrate specificity will prove useful in biochemistry.

Methodology

Fresh plant materials were cut into smaller pieces and extracted with ethanol, then soaked for 48 hours. It was extracted exhaustively with the same solvent. The combined filtrates were concentrated in water bath at 50° C to obtain semi-solid extract.

Test for Fats and Oils

A piece of strip of filter paper was moistened with the extract and dried. A greasy appearance after drying indicates the presence of fats and oils.

Test for Alkaloids (Wagner's Test)

Ten (10) ml of alcoholic extract was evaporated to syrupy and the residue was heated on boiling water with 5 ml of 2 N HCL. After cooling, NaCl was added to prevent false positive result. Add enough 2 N HCI to wash, the mixture was filtered. Two (2) ml of the filtrate, 2-3 drops of Wagner's reagent were added. Formation of a brick red precipitate indicates the presence of alkaloids. Another 2 ml of the filtrate, 2-3 drops of Dragendorffs reagent were added. Fonnation of orange precipitate indicates the presence of alkaloids

Test for Glycosides (Fehling's Test)

Ten (10) ml of alcoholic extract was dissolved in I0 ml distilled water and filtered. Two ml of the filtrate was placed in test tube as sample I, 1 ml diluted HCl was added. Another 2 ml of the filtrate waw placed in test tube as sample 2, nothing was added. Then the two test tubes were placed in boiling water bath for 5 minutes. Then the 2 test tubes were coiled. Both were neutralized with anhydrous sodium carbonate until no more effervescence was produced. Then Fehling's A solution was added to the two test tubes and was heated over a water bath for 2 minutes. An increase in the amount of brick red precipitate in hydrolyzed sample was indicative of the presence of glycosides.

Test For Flavanoids/Leucoanthocyanins (Bate-Smith and Metcalf Test

Ten (I 0) ml of the plant extract was evaporated to incipient dryness over a water bath cooled to room temperature. The residue was defatted with 9 ml of hexane and water (2: I). The defatted extract was diluted with I0 ml of 80% ethyl. The mixture was filtered and the filtrate was divided into 2 test tubes: test tube I, 0.5 ml of con. HCL was added

then wanned into water bath for 15 mins. The gradual development of a strong red or violet color is indicative of the presence of leucoanthocyanins.

Test tube 2 was treated with a few drops of concentrated HCL and magnesium turnings (0.5g). The presence of flavanoids was indicated if pink or magentared color developed within 3 min.

Test For Saponins (Froth Test)

The alcoholic extract was dissolved with 10 ml of distilled water, a stopper was placed and shaken. vigorously for 30 seconds. This was allowed to stand for ten minutes and classified for siphoning content as follows: no froth = negative; froth less than 1cm = **weakly** positive; froth 1.2cm high = positive; and froth greater than 2cm = strongly positive.

Test For Triterpenes and Sterols (Liebermann-Burchard Test)

Two (2) ml of fruit extract was dissolved in acetic anhydride. The soluble portion was decanted and to this, 1-2 drops of concentrated sulfuric acid was added. A pink to red color **was an** indication of triterpenes and blue color if Sterols is present.

Test For Resins (Phloroglucinol Test)

Ten (IO) ml of the alcoholic extract was added with 2 drops of alcoholic phloroglucinol test solution and concentrated H_2SO_4. Formation of red color indicates the presence of resins.

Results and Discussion

200 g fresh Kalunay (leaf) extracted with 600 ml of 95% Ethanol produce 50 ml concentrated extract.

Qualitative Test	Results	Bi0active Substances
Filter Paper Test	No greasy appearance	Absence of fats and oils
Wagner's Test	**Formation of brick red precipitate**	**Alkaloids**
Fehling"s Test	No brick red precipitate in the hydrolyzed sample	Absence of Glycosides
Bate-Smith & Metcalf Test	No formation of pink or magenta-red color	Absence of Flavanoids
Froth Test	**Formation of more than 1.2cm of "honeycomb froth"**	**Saponins**
Liebermann-Buchard Test	No pink to red color was fonned	Absence of Triterpenes
Liebermann-Buchard Test	No formation of blue color	Absence of Sterols
Bate-Smith & Metcalf Test	No formation of a strong red or violet color	Absence of Leucoanthocyanins
Phloroglucinol Test	No formation of red color	Absence of Resins

The table shows that only saponins and alkaloids were present in the leaf extract and all the other tests showed negative results.

200 g fresh Kalunay (roots) extracted with 600 ml of 95% Ethanol produce 50 ml concentrated extract.

Qualitative Test	Results	Bioactive Substances
Filter Paper Test	No greasy appearance	Absence of fats and oils
Wagner's Test	Formation of a brick red precipitate	Alkaloids
Fehlings Test	Formation of a brick red precipitate in the hydrolyzed sample	Glycosides
Bate-Smith & Metcalf Test	No formation of pink or magenta-red color	Absence of Flavanoids
Froth Test	Formation of more than 1.2cm of "honeycomb froth"	Saponins
Liebermann-Burchard Test	No pink to red was formed	Absence of Triterpenes
Liebermann-Burchard Test	No formation of blue color	Absence of Sterols
Bate-Smith & Metcalf Test	No formation of strong red or violet color	Absence of Leucoanthocyanins
Phloroglucinol	No formation of red color	Absence of Resins

The table shows that only saponins and alkaloids were present in the leaf extract and all the other tests showed negative results. Only in the root extract wherein the presence of glycosides is noted.

Conclusion

1. Kalunay leaves extract contain a trace of alkaloids and saponins.
2. Kalunay root extract contain a trace of alkaloids, saponins and glycosides.

Recommendation

1. Other substances be analyzed in both the leaf and roots of the plant and sensitivity of bacterial and fungal strains to the plant materials be tested and phannacological assay on the plant should be done.

2. The sensitivity of bacterial strains to Kalunay extract of both leaves and roots must be tested.

3. Other phannacological effects such as anti-inflammatory and diuretic effects of the leaves and root extract must be tested.

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