TITLE: Phytochemical Screening for Active Compounds in Aloe Vera, Fiscus Auriculata, Melaleuca Cajuputi, Adiantum Pedatum and Carica Papaya.

Key Words: Phytochemical Screening, Active Compounds

INTRODCUTION

Among the many challenges that have swept across the planet earth today is that of the discovery of drugs that will help to fight the many diseases that exist and others still to come. In this endeavor herbs are proving worth facing the challenge. This can be attributed to modern research that has found out that plants contain bioactive (biologically active) compounds that play various roles when introduced into the body system. These bioactive compounds are called phytochemicals. Phytochemicals are different from vitamins and minerals in that they have no known nutritional value.

By definition, phytochemicals are, "a large group of plant derived compounds hypothesized to be responsible for much of the disease protection" (Heneman, 2008). The word "Phytochemical" is a compound word made of two words, "Phyto" meaning "plant" in the Greek language and the word "chemical". Hence is a lay-man's definition phytochemicals can be defined as non-nutritive chemical compounds that can be found in plant foods. Plant sources of phytochemicals include; fruits, vegetables and grains. "They accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds" (Saxena et al, 2013). With this knowledge phytochemical screening therefore refers to a test for identifying the presence of active compounds in a particular plant sample and active compounds are those compounds that trigger a biological response when introduced in the body.

Phytochemicals otherwise called phytoconstituents are known to be responsible for the color, flavor, odor and general appearance of plants. Research has also shown that some phytochemicals act as defense mechanism compounds "responsible for protecting the plant against microbial infections or infestations by pests" (Abo et al., 1991) and other environmental hazards. An example of such is those of the phenolic group. Concerning the total number of phytochemicals, scientists have found out that there are over 4, 000 known phytochemicals in plants. In their diversity phytochemicals are "classified according to their chemical structures and functional properties" (Webb, 2003).

As earlier stated, tens of thousands of phytochemicals have been discovered by researchers. Some among these so many phytochemicals include; alkaloids, glycosides, terpenes, phenols, tannins, steroids, flavonoids, saponins, anthraquinoes, essential oils, stylbenes, triterpenoids, lignans, xanthophylls, phytosterols, etcetera.

While each class or group of phytochemicals has its own mechanism of functioning, general ways in which phytochemicals affect the body include; as antioxidants, hormonal actions, stimulation of enzymes, interference with DNA replication, as antimicrobial, physical actions, anti-carcinogens, anti-diabetic, anti-ulcers, anti-inflammatory, multifunctional targets and much more to be discovered. By having such actions phytochemicals achieve great results in the fight against many diseases such as cancer, diabetes, cardiovascular diseases, microbial diseases and infections, physical pain, coughs, stomatitis, scabies, rheumatisms, etcetera. However, the use and functioning of phytochemicals should not be limited to drugs only as they are also used in the manufacture of a great many chemicals needed for use by man such as soaps. Even animals benefit from the use of these phytochemical.

The future with phytochemicals is bright as increasing research continues to unearth the numberless benefits that can be got from phytochemicals. Bioengineering is also increasing the possibilities of having new kinds of plants with higher levels of phytochemicals. Among such research that is helping in the furthering of phytochemicals include practicals that are being carried out by undergraduate students in various Universities around the world. This report focuses on the results that were got during a practical carried out for the analysis of the presence of various phytochemicals in five different plants; Fiscus Auriculata (Elephant Ear Fig Tree or Roxburgh Fig), Aloe Vera, Adiantum Pedatum (Maindenhair Fern), Melaleuca Cajuputi (Cajuputi) and Carica Papaya (Paw-paw) . Phytochemicals that were screened for are; Flavonoids, Steroids and Saponins.

MATERIALS

1. Samples
    - Carica Papaya (fruit, dried and crushed)
    - Fiscus Auriculata (Fruit, dried and crushed)
    - Adiantum Pedatum (Leaves, dried and crushed)
    - Aloe Vera (Leaves, dried and crushed)
    - Melaleuca Cajuputi (Leaves, dried and crushed)

2. Apparatus Used
    - Test tubes
    - Beakers
    - Pipettes
    - Heating coil

- Droppers
- Spatulas
- Filter papers
- Petri Dishes

3. Reagents and Chemicals Used
    - Ethanol
    - Dimethyl ether
    - Acetic anhydrate
    - Concentrated Sulphuric acid
    - Magnesium coil
    - Concentrated hydrochloric acid
    - Distilled water

PROCEDURE

1. Sample Collection and Preparation

   The samples were prepared from five different plant sources namely; (1) Aloe Vera (Leaves), (2) Carica Papaya (Fruit), (3) Fiscus Auriculata (Fruit), (4) Adiantum Pedatum (Leaves) and (5) Melaleuca Cajuputi. The parts (that is the leaves and fruits) of these various plants were all collected from Bugema demonstration forest. After the collection from the forest, the samples were thoroughly cleaned with clean tap water to remove dust and foreign materials then sliced thinly into pieces in preparation for drying. Due to limited time the samples were oven dried at 40^0 C for seventy-two (72) hours. Each sample well labeled to avoid mixing that could give

inaccurate results. The natural method is by allowing the plants in shade till completely dry. This helps in the preservation of the phytochemicals.

After the drying, each sample was reduced to powder using a blender. This was in preparation for the actual experiment.

2. Test Procedure for each plant Sample

    a) Steroids

    For each plant sample the following procedure was followed in the test for steroids:

    - 3 grams of each plant sample (Fiscus Auriculata, Aloe Vera, Adiantum Pedatum, Melaleuca Cajuputi and Carica Papaya) was mixed with 30ml of ethanol and the mixture was then boiled.
    - The boiled mixture was then filtered into test-tubes and the residue for each plant sample was extracted from the filter paper by washing with dimethyl ether.
    - The extracted residue was later left to dry on petri dishes. Acetic anhydride was then added to the dried extract and stirred.
    - Lastly a few drops (3-5) of concentrated Sulphuric acid were dropped onto the mixture and observations were made.

    b) Flavonoids

    For each plant sample the following procedure was followed in test for the flavonoids;

- 10ml of the filtrate in the test for steroids for each plant sample (Fiscus Auriculata, Aloe Vera, Adiantum Pedatum, Melaleuca Cajuputi and Carica Papaya.) was put into test-tubes.
- To each test-tube for each plant sample about 1cm of Magnesium coil was added followed by three (3) drops of Concentrated Hydrochloric acid.
- The solution was then let to settle for about 10 minutes and observations were made.

c) Saponins

For each plant sample (Fiscus Auriculata, Aloe Vera, Adiantum Pedatum, Melaleuca Cajuputi and Carica Papaya) the following procedure was followed in the test for saponins;

- 10ml of the filtrate in the test for steroids for each plant sample (Fiscus Auriculata, Aloe Vera, Adiantum Pedatum, Melaleuca Cajuputi and Carica Papaya.) was put into test-tubes. Distilled water was added to each test-tube until approximately three-quarters (3/4) full.
- The solution was then shaken vigorously and let to stand for about 20 minutes.
- Observations were then made.

RESULTS: Observations and Interpretation of Observations.

1. FLAVONOIDS

SAMPLE	RESULTS/ OBSERVATIONS	INTERPRETATION OF OBSERVATIONS
1. Aloe Vera	➢ Green clear solution remained green but with precipitate and with slow effervescence.	➢ Flavonoids absent.
2. Carica Papaya	➢ Yellow solution remained yellow but gave off bubbles of a gas.	➢ Flavonoids present.
3. Fiscus Auriculata	➢ Yellow solution with precipitate suspended in the solution with slow effervescence.	➢ Flavonoids present.
4. Adiantum Pedatum	➢ Green clear solution remained green with precipitate. The precipitate disappeared on shaking. Fast effervescence was also observed.	➢ Flavonoids absent.
5. Melaleuca Cajuputi	➢ Green solution remained green with precipitate and fast effervescence was observed.	➢ Flavonoids absent.

2. STEROIDS

SAMPLE	OBSERVATIONS	INTERPRETATION OF OBSERVATIONS
1. Aloe Vera	➢ Green residue remained green.	➢ Steroids absent.
2. Carica Papaya	➢ Black residue remained black.	➢ Steroids absent.
3. Fiscus Auriculata	➢ Brown residue turned to dark brown	➢ Steroids present.
4. Adiantum Pedatum	➢ Residue turned black.	➢ Steroids absent.
5. Melaleuca Cajuputi	➢ Pale green residue turned to a dark brown solution.	➢ Steroids present.

3. SAPONINS

SAMPLE	OBSERVATIONS	INTERPRETATION OF OBSERVATIONS
1. Aloe Vera	➢ Green precipitate with many bubbles and a stable white foam	➢ Saponins present.
2. Carica Papaya	➢ Clear yellow solution remained yellow.	➢ Saponins absent.
3. Fiscus Auriculata	➢ Pale yellow precipitate	➢ Saponins

	with little foam and some bubbles given off	absent.
4. Adiantum Pedatum	➢ Clear green solution with a stable foam at the top.	➢ Saponins present.
5. Melaleuca Cajuputi	➢ Pale green solution with stable foam at the top.	➢ Saponins present.

SUMMARY OF RESULTS

SAMPLES	FLAVONOIDS	STEROIDS	SAPONINS
1. Aloe Vera	Absent	Absent	Present
2. Carica Papaya	Present	Absent	Absent
3. Fiscus Auriculata	Present	Present	Absent
4. Adiantum Pedatum	Absent	Absent	Present
5. Melaleuca Cajuputi	Absent	Present	Present

DISCUSSIONS

1. Flavonoids

   In the fives samples (Fiscus Auriculata, Aloe Vera, Adiantum Pedatum, Melaleuca Cajuputi and Carica Papaya) tested for flavonoids, two of these samples namely Carica Papaya and Fiscus Auriculata showed the presence of flavonoids These results coincide with the results from research work done by Hossain et' al (2013), Aiyelaagbe et' al (2009) and Rattana et' al (2010). The results of the mentioned researchers showed a yellow color for the presence of flavonoids. Other researchers consulted however showed a red-brown color, White-pitch, Green-dark brown and light brown-dark brown for the presence of flavonoids. The difference can be attributed to varying methods used in the identification of these phytochemicals. The methods used in phytochemical screening vary depending on the plant type being screened or the type of phytochemical being screened for. "Flavonoids are widely distributed in plants, fulfilling many functions. Flavonoids are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentations in petals designed to attract pollinator animals. In higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. They may act as chemical messengers, physiological regulators, and cell cycle inhibitors" (Wikipedia, 2016). Plant sources of flavonoids include; "Apples, citrus fruits,onions,soybeans and soy products (tofu,soy milk, edamame,etc.),coffee and tea" (Villanova University).

2. Steroids

   Among the five samples (Fiscus Auriculata, Aloe Vera, Adiantum Pedatum, Melaleuca Cajuputi and Carica Papaya) screened for the presence of steroids, only two of these samples namely; Aloe Vera and Melaleuca Cajuputi showed a red-brown

color for the presence of steroids. According to Hossain et' al (2013) research the upper layer in the test tube turns into red and Sulphuric acid layer showed yellow with green fluorescence. The strange color changes noticed in other samples during the experiment on the part of steroids can be linked to the experimental errors that were experienced during the preparation of the samples. "Steroids (anabolic steroids) have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness" (Maurya *et al.*, 2008; Madziga *et al.*, 2010).

3. Saponins

Saponins were found present in three plant samples namely; Aloe Vera, Adiantum Pedatum and Melaleuca Cajuputi. The presence of saponins in a particular sample is determined by an emulsion and formation of a stable form at the surface of the sample mixture. According the Journal of King Saud University (2015), formation of persistent froth at the surface of a sample mixture is evidence of the presence of saponins. The form during our experiment was not as much. This can be attributed to experimental errors that include; too much heat that could have led to over drying of samples hence disturbing the integrity of phytochemicals present in the particular plant sample. "Initially, saponins are known to be derived from plants, but recent studies show that they are also present in marine organisms. They are commonly found in the soapwort plant, whose roots were once used as soap. They can also be derived from soapberries, maples, horse chestnuts and ginseng. Saponins can be extracted from the bark, leaves, stems, bulbs, fruits and flowers of these plants. Saponins by nature serve as anti-feedants. They also act as a shield against the penetration of microbes and fungi into the plant which can cause the latter's sickness and death. Other saponins further nutrient absorption and improve digestion

especially to animals. They are however, bitter, and are hence not that appealing to the taste preferences of most warm-blooded animals (including humans)" (Hanz, 2016).

CONCLUSION

The results of the experiment showed presence of different phytochemicals in the five different plant samples screened. Flavonoids were present in Carica Papaya and Fiscus Auriculata, Steroids were present in Fiscus Auriculata and Melaleuca Cajuputi, and Saponins were present in Aloe Vera, Adiantum Pedatum and Cajuputi. Irrespective of the experimental errors, the practical was a success as the aim of identifying phytochemicals in the five plant extracts namely; Fiscus Auriculata, Aloe Vera, Adiantum Pedatum, Melaleuca Cajuputi and Carica Papaya, was achieved.

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