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QUALITATIVE ANALYSIS OF PHYTOCONSTITUENTS IN *TRAGIA BREVIPES* PLANT

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ABSTRACT

The study was conducted to analyze the phytoconstituents of *Tragia brevipes* plant leaves. The plant samples were extracted using methanol and water in the ratio 9:1. The phytoconstituents study was done using standard procedures [18, 19 and 20]. From the study *Tragia brevipes* was found to contain tannins, saponins, terpenoids, flavonoids, cardiac glycoside, phenols, steroidal nucleus and steroidal ring, but alkaloids and steroids were found to be absent in the plant extract.

Keywords: *Tragia brevipes*, Phytoconstituents, Medicinal herbs.

INTRODUCTION

Study on medicinal plants is becoming an important area of study. Micro-organisms are becoming resistant to drugs used to kill them, hence the need for alternative drugs to treat them. Many scientists have turned to plants to obtain these compounds. The use of medicinal plants to treat various types of diseases was very important in ancient days since there were no commercial medicines by then. The introduction of industrially produced drugs has however led many people to turn from plants to use these synthesized products. However the trend is changing with many people turning to plants for treatment.

Pharmacological studies report on endangered species *pontella fulgens* have indicated that the plant can be used as an antitumor, Anti-inflammatory, anti-hyperlipidemia, anti-hyperglycemic and hypoglycemic [1]. The antibacterial activities of plants such as senna didymobotrya plant have been associated with the presence of certain phytochemicals such as tannins and alkaloids [2, 3]. The plant showed clear zones of inhibition against *B.subtillis*, *E.coli*, *P.aeruginosa* and *C.albicans*. The plant also showed a great potential in the treatment against animal wounds [4]. All these activities against microbes have been closely attributed to the presence of certain bioactive compounds.

All chemicals found in plants are potential drugs, for example certain tree barks produce a chemical that discourage caterpillars from feeding on it, a good example being the Indian neem tree which keeps off desert locusts. The twigs are chewed by people in Serengeti national park in East Africa to prevent tooth decay. Plants produce more than 10,000 different compounds to prevent themselves against animals who feed on them. Almost half of all prescribed drugs contain chemicals produced by plants, fungi and bacteria or contain synthesized compounds in the laboratory that have being modeled after plants originating compounds [5].

The use of medicinal plants to treat diseases is as old as man. Medicinal plants have been used since ancient times to treat many illnesses. Research done shows that the concentration of these compounds in plants is directly related to their capability to treat certain illness [6]. Many of these non-nutritive secondary metabolites are found in plants which are even used for food. Over 80% of the plants in Nigeria used for treatment of malaria and other sicknesses are also used as food [7], there seem to be not much distinction between medicinal benefits of plants and their nutritive value.

The published WHO traditional strategy addressed

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the issues and provided a framework for countries to develop policies to govern medicinal plants use. The strategy put forward by WHO advocates the formulation of a policy by states as the first component of developing traditional medicine. India is one of the few countries which have started to develop such policies [8]. Over the past few years much research has being done and is still going on to prove scientifically the plants nutritional value and medicinal value. A good number of chemical compounds have being discovered from plants and found to have pharmacological value; this has lead to the development of over 25% of all the artificial medicines used today. Many of the traditional medicinal plants species used all over the world have being found to have great pharmacological value. Studies carried out throughout Africa confirm that indigenous plants are the main constituents of traditional African medicines.

Over 80% of the people in developing countries use medicinal plants to treat the illnesses which affect them from day to day [9].This can be attributed to poverty in these countries which has lead to inefficient health care system in hospitals and inadequate resources to access these facilities. People in these countries look for cheap and available medicines which are known traditionally to cure the illnesses. The use of herbal medicines in the western world is steadily growing with 40% of the population using plants to treat illnesses; while in Kenya 90% of the populations have one time in their life used medicinal plants [10]. The use of these plants in treatment of ailments is mainly based on the type of flora in that region.

Our environment is very rich with a great range of medicinal plant and this mainly explains the reason why our grand's lived for quite some time. They could stay in the bush during war for some time and even could use plants to treat ailments and wounds affecting soldiers in the battle ground. People all over the world should look around them especially in Africa were this information has not completely being replaced by industrial medicines, lest we forget this important aspect of treatment. In many communities in Africa they still consider the use of medicinal plants as an important part of their culture, just to mention, the Maasai community in Kenya still value their culture very much, the Kalenjin community and their medicinal fermented milk which is prepared mainly from medicinal plants such as *Senna didymobotrya stem* which previous studies have shown this plant to have a great potential in treatment of diseases such typhoid, diarrhea and food poisoning caused by *Salmonella typhi, E .coli* and *Bacillus cereus* respectively [2]. The reason why herbal medicine still remains a matter of argument is because of some greedy practitioners who want to become wealthy by pretending to know much about the treatment of every disease that their clients complain about [11]. This has led to administration of wrong drugs which do not cure a patient leading to death of the individual. Proper scientific evidence needs to be provided in order to create confidence in medicinal herbs. The increase of multi-resistant strains of bacteria calls for new discoveries of antibacterial classes and chemical compounds that can clearly inhibit these resistant strains, this is the reason why much research should be turned to plants which have been used since ancient times to treat many diseases [7]. The non-nutritive plant components are referred to as phytochemicals, which can be divided in two major categories primary and secondary, with the primary constituting of carbohydrates, proteins and chlorophyll and the secondary consisting of tannins, alkaloids, saponins, steroids, flavonoids, terpenoids and anthroquinones [12]. The secondary metabolites help the plant survive in the environment by protecting them against predators but research has shown that these metabolites can be used to treat diseases in both animals and humans [11]. The antibacterial activity of plants has been closely associated with the presence of these important compounds in the plant. The antibacterial activity of *Vernonia adoensis* leaves against *B.cereus, Klebsiella sp., Streptococcus pyogenes* and *Proteus vulgaris* was closely associated to the presence of phytochemicals in the plant leaves extract [13]. Physiological activities of phytochemicals have also been found to include cancer prevention, antifungal, antioxidative, hormone action and enzyme stimulation.

Natural bioactive compounds have been investigated in plants and their pharmacological effects analyzed. Secondary metabolites functions on growth, photosynthesis and other important plant activities have not been discovered but their medicinal values have been identified in most of them [14]. Phytochemicals have been used to a greater extend in Asia for various purposes such as treatment of diseases [15].

The lack of scientific knowledge on the phytochemical constituents, antibacterial, antioxidants and toxicological properties limits the use of traditional herbal medicine [3]. Phytochemicals can really improve the activity of the currently used drugs by acting as efflux of existing pump inhibitors. Many drug resistant microbes are emerging from time to time and causing the need to such for new antibiotics to kill and inhibit their growth. Phytochemicals have been associated with reduction of drug resistant forms of bacteria [16].

A big percentage of plants in the savanna and semi-arid areas of east Africa where Kenya is located contains alkaloids which have been associated with increase in renal secretion when ingested hence used as a diuretics and in the treatment of dropsy [11]. The use of alkaloids, saponins and tannins as antibiotics has been scientifically justified [6].

Majority of the pharmacologically active chemical compounds were found mainly in ethanol extracts which is contrary to previous researches which had affirmed the traditional way of extracting these compounds using water [17].

The roots of *Tragia brevipes* are used as purgative, given to women during labor period to increase contraction of the uterus. The leaves of the plant are rubbed on the knees and other joints to treat against rheumatism [11]. This study was carried out to investigate the presence of bioactive compounds in the plant.

MATERIALS AND METHODS

Sample Collection and Preparation

The herb was randomly collected in the natural forest around University of Eastern Africa, Baraton. The samples were collected and identified by a taxonomist in the Biology Department, Baraton University. The samples were thoroughly mixed and spread to dry at room temperature in the chemistry laboratory for about three weeks. They were then ground into fine powder and put in transparent polythene bags.

Extraction procedure

Using electric analytical beam balance fifty grams of the powdered leaves of the plant were placed in 1000 ml conical flask, methanol and water were then added in the ratio of 9:1 respectively until the leaves were completely submerged in the solvent. The mixture was then agitated for thorough mixing. The mixture was kept for 24 hours on a shaker for effective extraction of the plant components. The extract was filtered using Butchner funnel; Whatman no.1 filter paper and a vacuum and pressure pump. The filtrate was re-filtered again using the same apparatus. The solvents were evaporated using rotary vacuum evaporator (R-11) with a water birth at 40^{o}C. The extract was brought to dryness using vacuum and pressure pump at room temperature. The residue was then obtained and used for the experiment.

Qualitative phytochemical analysis

The phytoconstituents analysis of extracts for identification of bioactive chemicals was done using standard procedures [18, 19, and 20]

1. Tannins

About 0.5 g of the sample was put in a test tube and 20 ml of distilled water was added and heated to boiling. The mixture was then filtered and 0.1 % of $FeCl_3$ was added to the filtrate and observations made. A brownish green color or a blue black coloration indicates the presence of tannins.

2. Saponins

The crude solvent extract was mixed with 5 ml of water and vigorously shaken. The formation of stable foam indicates the presence of saponins.

3. Flavonoids

About 1 g of the plant extract was mixed with a few fragments of magnesium ribbon (0.5 g) and few drops of concentrated hydrochloric acid were added. A pink or magenta red color development after 3 minutes indicate presence of flavonoids.

4. Tarpenoids

The solvent extracts of the plant material was taken in a clean test tube 2 ml of chloroform was added and vigorously shaken, then evaporated to dryness. To this, 2 ml of concentrated sulphuric acid was added and heated for about 2 minutes. A greyish color indicates the presence of tarpenoids.

5. Glycosides

a. **Salkowsks' test:** The solvent extract of the plant material was mixed with 2 ml of chloroform and 2 ml of concentrated sulphuric acid was carefully added and shaken gently, then the observations were made. A red brown color indicate the presence of steroidal ring (glycone portion of glycoside)

b. **Liebermann's test:** The solvent extract of the plant material was mixed with 2 ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice and observations made. A color change from violent to blue to green, indicate the presence steroidal nucleus (glycone portion of the glycosides)

c. **Keller-Kilani test:** The solvent plant material extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$, the mixture was then poured into a test tube containing 2 ml of concentrated sulphuric acid. A brown ring at the interface of the two solutions indicates the presence of cardiac glycoside.

6. Alkaloids

The crude extract was mixed with 1% of HCl in a test tube. The test tube was then heated gently and filtered .To the filtrate a few drops of Mayer's and Wagner's reagents were added by the side of the test tube. A resulting precipitate indicates the presence of alkaloids.

7. Steroids

Libermann Burchard reaction: About 2 g of the solvent extract was put in a test tube and 10 ml of chloroform added and filtered. Then 2 ml of the filtrate was mixed with 2 ml of a mixture of acetic acid and concentrated sulphuric acid. Bluish green ring indicate the presence of steroids.

8. Phenols

The plants solvent extract was put in a test tube and treated with a few drops of 2% of $FeCl_3$ blue green or black coloration indicate the presence of phenols.

RESULTS AND DISCUSSION

Table 1. *Tragia brevipes* Results

Phytochemical	Observation	Inferences
Tannins	Blue black color	Present
Saponins	Formation of stable foam	Present
Terpenoids	Grey color	Present
Flavonoids	Magenta-red color	Present
Phenols	Black coloration	Present
Alkaloids	No precipitate	Absent
Steroids	No bluish green ring	Absent
Steroidal nucleus	Violet –blue –green color	Present
Cardiac glycosides	Formation of brown ring	Present
Steroidal ring	Red-brown color	Present

The presence of Saponins shows the potential of the plants to be used to produce mild detergents and in intracellular histochemistry staining to allow antibody access to intercellular proteins [12]. They have been found to treat hypercholesterolemia, hyperglycemia, antioxidant, anti-inflammatory, central nervous system activities, anticancer and weight loss [12]. They are used to stop bleeding, treating wounds and ulcers as it helps red blood cells to precipitate and coagulate [21]. This can be attributed to ability of saponins to bind with glucose and cholesterol molecules. Saponins have also being associated with inhibitory effect on inflammatory [22].

Tannins are also secondary metabolites in plants. They are glycosides of gallic or protocatechuic acids. Their astringent property makes them useful in preventing diarrhea and controlling hemorrhage due to their ability to precipitate proteins, mucus and constrict blood vessels [11]. This is the reason why traditional healers use plants reach in tannins to treat wounds and burns since they are able to cause blood clotting. Some tannins have being reported to inhibit HIV replication selectively besides the use of diuretics [23]. This shows how traditional medicinal plants rich in tannins can be used to control this dangerous disease. Tannins have also shown antiparasitic effects [24].Tannins can also be used to protect the kidney since when taken the poliovirus, herpes complex virus and various enteric viruses are inactivated [25]. Foods rich in tannins can be used to treat hereditary hemochromatosis which is a hereditary disease characterized by excessive absorption of dietary iron. Tannin molecules have been shown to reduce the mutagenic activity of a number of mutagens [26]. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property which is important in protecting cellular oxidative damage including lipid peroxidation. The growth of many fungi, yeast, bacteria and viruses has being proven to be inhibited by tannins. Tannins have also been reported to exert physiological effects, such as to accelerate blood pressure, decrease the serum lipid level, and produce liver necrosis and module immunoresponses. The dosage and kind of tannins are critical to these effects [26].

***Tragia brevipes* plant**

Flavonoids are secondary metabolites with polyphenolic structure and synthesized in plants, through polypropanoid pathway [14]. Flavonoids have being classified in to six sub-groups which include flavones, flavanol, flavanone, flava-3-ols, isoflavone and anthocynidin. Flavonoids are known to contain specific compounds called antioxidants which protect human, animal and plant cells against the damaging effects of free radicals. Imbalance between free radicals and antioxidants leads to oxidative stress which has being associated with inflammation, autoimmune diseases, cataract, cancer, Parkinson's disease, aging and arteriosclerosis. It also plays a role in heart diseases and neurodegenerative diseases. Flavonoids have also vasodilator activity a property which is useful in improving blood circulation in brain and in Alzheimer disease [27]. Leaf extract of *Ginkgo biloba* which contains flavonoids was used for improving blood circulation in brain varix. Several isoflavone can be used to improve blood circulation. Furanocoumarins can alter hexobarbital induced sleeping time and showed cytotoxic action and hence inhibited growth of tumor in mice. Free radicals including the hydroxyl, hydrogen peroxide, superoxide and lipid peroxide have being associated with a number of diseases such as cardiovascular disease, cataracts, diabetes, gastrointestinal inflammatory diseases, cancer, asthma, liver disease, macular degeneration, periodontal disease and other inflammatory processes. These oxidants are produced during normal body chemical processes. They can be damaged through free-radical damage. Flavonoids such as quercetin, catechin and its derivatives and the oligomeric proanthocyanidins (OPCs) have shown in vitro studies to inhibit the oxidation of low-density lipoproteins (LDL).

Glycosides another type of secondary metabolites are organic compounds from plants or animal sources in which a sugar is bound to a non-carbohydrate moiety. The

term Glycoside is a collective term used for compounds formed with a glycosidic bonding between a sugar and another compound other than sugar. Cardiac glycosides have being used traditionally as arrow poisons or as heart drugs. They are used to strengthen the heart and make it function properly under controlled therapeutic dose. Cardiac glycosides bind to and inhibit Na^+/K^+-ATPase, inhibition of N+/K+-ATPase raises the level of sodium ions in cardiac myocytes, which leads to an increase in the level of calcium ions and an increase in cardiac contraction force [28]. The unexpected results relating cardiac glycosides with anticancer properties has created a great interest in this secondary metabolite .This has lead to clinical trial of cardiac glycosides based drugs in clinics [29].

CONCLUSION

The presence important pharmacological phytochemicals in the plant leaves is an indication of the diverse medicinal importance of *Tragia brevipes*. From the study *Tragia brevipes* was found to contain tannins, saponins, terpenoids, flavonoids, cardiac glycoside, phenols, steroidal nucleus and steroidal ring, but alkaloids and steroids were found to be absent in the plant extract. More research needs to be done to identify the exact structures of the bioactive compounds and there effects in vivo. The use of the plant to treat various diseases also needs to be scientifically justified and the specific compounds isolated.

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