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PHYTOPHARMACOLOGICAL ANALYSIS OF METHANOLIC-AQUA EXTRACT (FRACTIONS) OF *SENNA DIDYMOBOTRYA* ROOTS

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Abstract: The study was carried to analyze the phytochemical constituents of the *Senna didymobotrya* roots. The plant samples were extracted using methanol and water in the ratio of 9:1. The crude extract was disintegrated using different solvents according to their polarity. The solvents used were chloroform, ethyl acetate, butanol and water respectively. All the extracts were subjected to phytochemical study using standard procedures. From the study the of crude extract of *Senna didymobotrya* was found to contain tannins, saponins, terpenoids, flavonoids, phenols, alkaloids, steroidal rings but steroids were found to be absent. The presence of these important phytochemicals in the plant roots is a scientific justification of the use of the plant in the traditional treatment against various diseases affecting humans and animals. However, more research needs to be done to identify the specific compounds, there structural formulas and their medicinal values.

Keywords: Phytochemicals, Roots, Herbs, Medicine, Senna.

INTRODUCTION

Research on medicinal plants is of great importance taking in to account the old and new problems emerging day by day. Medicinal plants are available in nature and the grand's have information about their medicinal value traditionally. The information about natural healing methods was passed from grand's to children and grand children from generation to generation. With growing knowledge on technology and civilization this information transfer is no longer taken seriously in the society hence endangering the knowledge on traditional methods of treatment with one of them being the use of medicinal plants. This calls for a great need to have the knowledge on medicinal plants reserved and kept for future reference [1].

With increase in diseases caused by the modes of leaving and emerging drug resistant microbes, back -to-nature is becoming a common acronym to many people in the world today. The use of plants in the past tells clearly the fascinating relationship between mankind and plants since ancient times. Due to lack of clear knowledge on the mode of treatment of certain plants, people in the past have attributed the healing of diseases using medicinal herbs to supernatural forces due to their indisputable healing capability [2].

After many years of using medicinal herbs by humankind the isolation of active compounds such as morphine, quinine and alkaloids 200 years ago ushered in the dawn of a new era in the use of medicinal plants and marked the beginning of modern research in the use of plants to cure diseases [3]. According to Ameyaw [3], many plants in Africa such as *Cassia siame* Lam, *Nauclea latifolia* Benth, *Cryptolepis sanguinolenta* (Lindl), *Azadiracha indica* and *Jatropha gossypifolia* have been tested for antimalarial properties. Many of the traditionally used plants in the African continent tested have shown great potential in biological activity [4].

Out of the 600 species of medicinal plants from 125 families tested against *P. allinaceum* in chicks, *P. cathemerium* and *P. lophurae* in duckling 33 species were found to possess high potential in the treatment against the microbes, with the highest been plants from amaryllidaceae and simaroubaceae family [5]. Medicinal plants have since ancient times been used to treat many illnesses which affect humankind even today. Many traditionalists have done this for quite some time and therefore prevented many deaths in the past few decades. However, this has been done with little scientific proof on the efficiency and the effects of the extracts on the affected individuals. Herbal medicine is still a matter of argument in the current world with many still doughting its efficiency. This has been due to greedy practitioners who want to become wealthy by pretending to know much about the diseases which their clients claim to have, hence leading to the application of wrong treatment and administration of wrong drugs which do not cure the patient and therefore leading to worsening of the situation or even death of the victim [6]. Much scientific data needs to be provided in order to create the needed confidence in the use of medicinal plants.

Due to their composition plants have been known to possess multiple medicinal properties hence enabling them to have several uses in the pharmaceutical industry. Studies on several plants have been done all

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over the world and plants have shown great potential in the treatment of diseases affecting both humans and animals. Study reports on potentilla fulgens have shown the plant to have anti-hyperglycemic, hypoglycemic, anti-hyperlipidemic, antitumor, antioxidant, anti-inflammatory and anti-ulcerogenic properties [7]. The use of medicinal plants is as old as man [8]. In the past few decades medicinal plants have been tested extensively and found to have several pharmacological uses such as, antibacterial activity, antifungal activity, anti-diabetic activity, anticancer activity, antioxidant activity, hepatoprotective activity, haemolytic activity, anti-inflammatory activity, larvicidal activity, anthelmintic activity, central nervous system activity and pain relief activity [9 & 10]. Many side effects associated with allopathic medicines and dependencies are common reasons why many people are hospitalized today. In order to counteract the effects, many people are now turning to nature in pure form to prevent and cure diseases using natural medicinal herbs or natural health alternatives [11].

The herb *S. didymobotrya* locally nicknamed as mursik plant is a species of flowering plants in the legume family. The plant grows to a maximum height of 30-90 cm, tolerates light frost and is hardly attacked by disease or pests [12]. According to Tabuti [13], the charcoal of the stem is used to preserve milk. Root decoction of the plant was used for the treatment of malaria, other fevers, jaundice and intestinal worms. The root or leaf mixed with water is used to treat skeletal muscle and venereal diseases. The stem, leaves and roots of the plant are also used to treat fungal, bacterial, parasitic infections, hypertension, hemorrhoids, sickle cell anemia, and inflammation of fallopian tubes, fibroids and backache. In women they are used to stimulate lactation and to induce uterine contraction and abortion [14].

The methanol extract of *S. didymobotrya* stem *showed* bacterial growth inhibition with *E. coli* being mostly inhibited [12]. According to the study carried out on the stem by Nyaberi *et al.,* [12], the presence of either tannins or alkaloids could be attributed to the antibacterial activity of *S. didymobotrya* stem.

Senna didymobotrya leaves have being found to have 100% mortality on immature mosquitoes at 250mg/l concentration of leaves extracts [14]. This evidence confirms the pharmaceutical significance of Senna leaves against malaria larvae and therefore making the plant a potential malaria fighting species. This study was conducted to analyze the chemical composition of the roots of *Senna didymobotrya* roots.

MATERIALS AND METHODS

Sample Collection and Preparation:

The herb was randomly collected in the natural forest around University of Eastern Africa, Baraton. The plant 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 500 grams of the powdered roots of the *Senna didymobotrya* was placed in 2000 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 solvent was evaporated using rotary vacuum evaporator (R-11) with a water bath 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. The crude extract was then tested for the presence of phytochemicals. It was then disintegrated using chloroform, ethylacetate, butanol and water respectively. The obtained samples were concentrated using the rotavapor and subjected to the same study using standard procedures.

Qualitative phytoconstituents analysis:

The extracts phytoconstituents analysis for identification of bioactive chemical constituents was done using standard procedures [15, 16 & 17].

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 1 % of $FeCl_3$ was added to the filtrate and observations made. Brownish green coloration indicated the presence of tannins.

2. Saponins:

The crude extract was mixed with 5 ml of water and vigorously shaken. The formation of stable form indicated the presence of saponins.

3. Flavonoids:

A portion of the aqueous extract was added in a test tube. To this, 5 ml of dilute ammonia and 2 ml of concentrated sulphuric acid were added. The appearance of a yellow color indicated the presence of flavonoids.

4. Terpenoids:

The 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 indicated the presence of terpenoids.

5. Glycosides

Salkowsks' test: The 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 colour indicated the presence of steroidal ring (glycone portion of 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 to the test tube. A resulting precipitate confirmed the presence of alkaloids.

7. Steroids:

Liebermann Burchard reaction: About 2 g of the extract was put in a test tube and 10 ml of chloroform added and filtered, 2 ml of the filtrate was mixed with 2 ml of a mixture of acetic acid and concentrated sulphuric acid was added along the side of the test tube. Blue green ring indicated the presence of steroids.

8. Phenols:

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

RESULTS AND DISCUSSION

Table.1: Methanol-water crude extract

Phytochemical	Observation	Inferences
Tannins	Blue-black color	Present
Saponins	Stable foam	Present
Terpenoids	Gray color	Present
Flavonoids	Yellow color	Present
Phenols	Black color	Present
Alkaloids	Precipitate	Present
Steroids	No blue green ring	Absent
Steroidal rings	Red-brown	Present

Table.2: Chloroform extract

Phytochemical	Observation	Inferences
Tannins	No color change	Absent
Saponins	Stable foam	Present
Terpenoids	Grey coloration	Present
Flavonoids	No yellow color	Absent
Phenols	No color change	Absent
Alkaloids	No precipitate	Absent
Steroids	No blue green ring	Absent
Steroidal rings	No blue green ring	Absent

Table.3: Ethyl acetate extract

Phytochemical	Observation	Inferences
Tannins	Brown green	Present
Saponins	Stable foam	Present
Terpenoids	Grayish color	Present
Flavonoids	Yellow color	Present
Phenols	No color change	Absent
Alkaloids	No precipitate	Absent
Steroids	no blue green ring	Absent
Steroidal rings	Red-brown color	Present

Table.4: Aqueous extract

Phytochemical	Observation	Inferences
Tannins	Blue-black	Present
Saponins	Stable foam	Present
Terpenoids	Grey color	Present
Flavonoids	Yellow color	Present
Phenols	No color change	Absent
Alkaloids	Precipitate	Present
Steroids	No blue-green ring	Absent
Steroidal rings	Red-brow	Present

Table.5: Butanol extract

Phytochemical	Observation	Inferences
Tannins	Brown green color	Present
Saponins	Stable foam	Present
Terpenoids	Grayish color	Present
Flavonoids	Yellow color	Present
Phenols	No color change	Absent
Alkaloids	No precipitate	Absent
Steroids	No blue green ring	Absent
Steroidal rings	Red-brown color	Present

The crude extract of Senna didymobotrya was found to contain tannins, saponins, terpenoids, flavonoids, phenols, alkaloids, steroidal rings but steroids were found to be absent (Table 1).

The chloroform extract was found to contain only saponins and terpenoids but tannins, flavonoids, phenols, alkaloids, steroids and steroidal rings were not detected (Table 2). The extract of ethyl acetate was found to contain tannins, saponins, terpenoids, flavonoids, and steroidal rings but phenols, alkaloids and steroids were found to be absent (Table 3). The aqueous extract was found to contain the highest percentage of the phytochemicals in which tannins, saponins terpenoids, flavonoids alkaloids and steroidal rings were found to be present however phenols and steroids were found to be absent (Table 4). The butanol extract Phytochemical analysis indicated the presence of tannins, saponins, terpenoids, flavonoids, steroidal rings but phenols, alkaloids and steroids were found to be absent (Table 5).

Alkaloids which are secondary metabolites, they can be defined as a cyclic compound which have nitrogen in a negative oxidation state. They affect the chemical transmitters' action of the nervous system. They also have other pharmacological activities such as analgesic,

antispasmodic, antihypertensive effects and anti-arrhymic effects and antibacterial. Cryptolepine a major alkaloid in *S. acuta* was found to be an antimalarial agent [18]. Cryptolepine has also being used clinically to treat malaria, colic and stomach ulcers [19], and also used in anticancer drugs. According to Karou [20], much study has being done on pharmacological properties of alkaloids and proved to have antiprotozoal, cytotoxic and anti-inflammatory properties.

The presence of alkaloids in the plant justifies its' medicinal value. Alkaloids have been isolated from different plants and their medicinal values tested. The most important use of alkaloids already known with its originality from plants is the use of alkaloids compounds in the treatment of malaria. According to Ameyawn and Duker-Eshon [3], many of the antimalarial drugs used today are quinoline derivatives manipulated from cinchona species bark [21]. Alkaloids have being identified for their functions which include analgesic, antiplasmodic and antibacterial activity [22]. According to Ayitey [23], bitter leaves containing alkaloids are capable of reducing headache associated with hypertension.

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 [14]. They have been found to treat hypercholesterolemia, hyperglycemia, antioxidant, anti-inflammatory, central nervous system activities, anticancer and weight loss [14]. They are used to stop bleeding, treating wounds and ulcers as it helps red blood cells to precipitate and coagulate [24]. 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 [24].

Saponins are used by the folkloric remedies of Kashmir (India) in treating wounds [25], this is because of their ability to cause red blood cells coagulation and therefore help in blood clotting, treating wounds and enteric ulcers problems [26]. Saponins have also been used to prevent hypercholesterolemia and antibiotic activity, the anti-inflammatory and anti-diabetic.

Flavonoids are used as antioxidants because of their ability to scavage free radicals such as peroxide and hydroperoxide of lipid hydroxyl hence inhibiting oxidation that lead to degenerative diseases [27]. They can be used as anti-diabetic. According to Namki [28], flavonoids can be used to prevent synthesis of off flavours that are caused by fat oxidation. Flavonoids have been found to have antibacterial activity due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [29]. Flavonoids are produced by plant in response to microbial infection and studies have shown that they have antibacterial activity against a wide range of micro-organisms [30]

Flavonoids are secondary metabolites with polyphenolic structure and synthesized in plants, through polypropanoid pathway [31]. 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 [32]. 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, rotin, catechin and its derivatives and the oligomeric proathocyanidins (OPCS) have shown in vitro studies to inhibit the oxidation of low-density lipoproteins (LDL).

Tannins are also secondary metabolites in plants. They are glycosides of gallic or protocatechvic acids. There astringent property makes them useful in preventing diarrhea and controlling hemorrhage due to their ability to precipitate proteins, mucus and constrict blood vessels [6]. 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 [33]. This shows how traditional medicinal plants rich in tannins can be used to control this dangerous disease. Tannins have also shown antiparasitic effects [34]. According to Bajal [35], tannins can also be used to protect the kidney since when taken the poliovirus, herpes complex virus and various enteric viruses are inactivated. Foods rich in tannins can be used to treat hereditary hemochromatosis which is a hereditary disease

characterized by excessive absorption of dietary iron. According to Chung [36], many tannin molecules have been shown to reduce the mutagenic activity of a number of mutagens. 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 growths of many fungi, yeast, bacteria and viruses have been 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 immune responses. The dosage and kind of tannins are critical to these effects [36].

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 Na^+/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 [37]. The unexpected results relating cardiac glycosides with anticancer properties have created a great interest in this secondary metabolite. This has lead to clinical trial of cardiac glycosides based drugs in clinics [38].

Terpenoids have medicinal value such as ant-carcinogenic, antimalarial, antimicrobial and diuretics activity [39 & 40]. Evaluation of the ant-inflammatory activity of three different Capaiba oleoresins showed that the crude extract of the plant and its' fractions of hexane, dichloromethane and methanolic extracts of *C. cearensis, C. reticulata and C. multijuga* have anti-inflammatory potential [41]. Terpenoids have also shown a great potential in treatment against disease causing microorganisms. Terpenoids have exhibited antibacterial activity against *E.coli, Staphylococcus, Pseudomonas aeruginosa* [42], *Proteus mirabilis* [42], *Klebsiella pneumoniae* [42 & 43], methillin-resistant S.aureus, *Staphytococcus epidermidis* [43], *Listeria monocytogenes* [44], Enterobacter cloacae, yeast *Candida albicans* and fungi, *Aspergillus flavus* [45]. Terpenoids have also been found to reduce the growth of melanoma cells on mice after oral administration [46]. Terpenoids have been proved scientifically to kill mosquito larvae. Terpenoids extracted from *Camellia reticulata* species have shown potential in killing *Aedes aegypti* [47].

CONCLUSION

The presence of the important phytochemicals in the plant roots is a scientific justification of the use of the plant in the traditional treatment against various diseases affecting humans and animals. The study is in conformity with earlier results in which the plant leaves were found to contain the same important phytochemicals however, in addition to the compounds found in the leaves, the roots were also found to contain terpenoids which were found to be absent in the leaves. This difference shows that the roots may have more medicinal potential than the leaves [48]. More research needs to be done to justify this and also find the specific phytochemicals which gives the plant its medicinal value.

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