

PHYTOCHEMICAL CONSTITUENTS OF *SENNA DIDYMOBOTRYA* FRESEN IRWIN ROOTS USED AS A TRADITIONAL MEDICINAL PLANT IN KENYA

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ABSTRACT

Medicinal plants play an important role in the treatment of various illnesses in Kenya and the whole world. *Senna didymobotrya* is one of such plants used traditionally in Kenya to treat illnesses such as diarrhea, malaria, ringworm, jaundice and intestinal worm. The main aim of this study was to analyze the phytochemical composition of the plant roots. Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plant was carried out in crude root extracts using standard procedures. From the analysis, it was demonstrated that steroids, terpenoids, anthraquinones, tannins, saponins, glycosides, flavonoids, alkaloids and phenols were found in the roots of the plant.

Key Words: *Senna didymobotrya*, Phytochemical, Roots

1.0 INTRODUCTION

People all over the world have used plants as medicines from time immemorial (Korir *et al.*, 2012). It is estimated by WHO that 90% of the population, majority of this in developing countries, still rely on plant-based medicine for primary health care (Evans, 1997; WHO, 2002). Herbal drugs are prepared from various parts of the plants such as leaves, stem, roots, seeds, tubers or exudates (Mukherjee, 2002; Kokwaro, 2009). Due to their composition, plants have been known to possess multiple medicinal properties hence enabling them to have several uses in the pharmaceutical industry (Athoney *et al.*, 2014a). Studies on several plants have been done all over the world and plants have shown great potential in the treatment of diseases affecting both humans and animals (Silva and Fernandes, 2010; Anthoney *et al.*, 2014b).

The use of medicinal plants is as old as man (Anthoney *et al.*, 2013). 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 (Sukirtha *et al.*, 2012; Mir *et al.*, 2013). 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 (Deshpande, 2010). Many species of the plants belonging to the genus *Cassia* possess potential larvicidal, ovicidal, repellent activities against wide species of immature and adult vector mosquitoes (Govindarajan *et al.*, 2011b).

Senna didymobotrya is a potential medicinal plant and the medicinal values are explored well in many parts of the world by traditional practitioners (Nagappan, 2012). In Kenya, traditionally the Kipsigis community has been using these plants to control malaria as well as diarrhea (Korir *et al.*, 2012). The pastoralists of West Pokot peel the bark, dry the stem and burn it into charcoal that they use to preserve milk (Tabuti, 2007). In addition, it has been used to treat skin conditions of humans and livestock infections as well (Njoroge and Bussmann, 2007). It is also used in the treatment of animal diseases such as removal of ticks (Njoroge and Bussmann, 2006). In Congo, Rwanda, Burundi, Kenya, Uganda, Tanzania, root decoction of this plant has been used for the treatment of malaria, other fevers, ringworm, jaundice and intestinal worm (Nagappan, 2012). The root or leaf mixed with water or decoction of fresh parts has been used to treat abscess of the skeletal muscle and venereal diseases (Kamatenesi-Mugisha, 2004). The plant is also useful for the treatment of fungal, bacterial infections, hypertension, haemorrhoids, sickle cell anemia, a range of women's diseases such as inflammation of fallopian tubes, fibroids and backache, to stimulate lactation and to induce uterine contraction and abortion (Tabuti, 2007). The antibacterial activities of hexane extract against *Microsporium gypsum*, has been reported (Korir *et al.*, 2012). According to Reddy *et al.*, (2010), presence of phenolic compounds, flavonoids and carotenoids in the ethyl acetate extract of leaves are responsible for pronounced antibacterial activities.

A decoction or infusion from the leaves, stems and roots of *S. didymobotrya* is drunk as a laxative and purgative for the treatment of abdominal pains, while in large quantities it is taken as an emetic (Singh *et al.*, 2003). The leaf sap in water is given as a drink to treat diarrhoea, dysentery, and taken as a diuretic and emetic (Sunarno, 1997). A decoction made from the roots is used as an antidote for poisoning, to expel a retained placenta, and to treat East Coast fever and blackleg (Njoroge and Bussmann, 2007). Much research has not been done to test the phytochemical analysis of this plant. This study was carried out to investigate the presence of phytochemicals in the plant.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Doss and Anand, 2012). The plants produce these chemicals to protect themselves but recent research demonstrates that they can protect humans and animals against diseases (Doss and Anand, 2012). A number of phytochemicals are known, some of which include: alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids and terpenoids (Venkatesh *et al.* 2011; Doss and Anand, 2012). They do not only protect the plants but have enormous physiological activities in humans and animals. These include cancer prevention, antibacterial, antifungal, antioxidative, hormonal action, enzyme stimulation and many more (Venkataswamy *et al.*, 2010; Venkatesh *et al.*, 2011; Doss and Anand, 2012).

2.0 MATERIALS AND METHODS

2.1 Collection of *Senna didymobotrya* roots

The roots of *S. didymobotrya* were collected randomly from Bomet County during the month of October-November, 2012 and were authenticated. The plant materials were taxonomically identified by a taxonomist and the voucher specimens were preserved at the Centre for Biotechnology Research and Development of the Kenya Medical Research Institute (KEMRI), Nairobi for future reference.

2.2 Preparation of extracts

The sample preparation and extraction procedures were carried out as described by Harbone, (1994). The roots were washed, cut into small pieces and air-dried for three weeks under a shed. The dried specimens were shredded using an electrical mill in readiness for solvent extractions. Sequential extraction was carried out on the plant material with distilled water, dichloromethane, ethyl acetate, hexane and methanol as the solvent systems.

2.3 Solvent Extraction

300g of dried powder were added to 600ml of hexane in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220rpm for 24hrs. After 24hrs the supernatant was collected and the solvent evaporated. The residue obtained was collected and stored at 4°C in airtight bottles. The process was repeated sequentially for ethyl acetate, dichloromethane and methanol.

2.4 Aqueous Extraction

300g of dried powder were added to 600ml of distilled water in a conical flask and boiled on slow heat for 2hrs. It was then filtered using No. 1 Whitman filter paper and centrifuged at 5000 rounds per minute (rpm) for 10min. After 6hrs, the supernatant was collected at an interval of every 2 hrs pooled together and concentrated using a rotary evaporator. The residue obtained was collected and stored at 4°C in airtight bottles.

2.5 Phytochemical screening

Phytochemical screening was done on the crude root extracts to identify bioactive chemical constituents. Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plant was carried out in the crude root extracts using standard procedures as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973) as follows:

2.5.1 Steroids

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

2.5.2 Terpenoids:

5ml of aqueous extract of each plant sample is mixed with 2ml of chloroform (CHCl_3) in a test tube. 3ml of concentrated sulphuric acid (H_2SO_4) is carefully added to the mixture to form a layer. An interface with a reddish brown colouration is formed if terpenoids constituent is present.

2.5.3 Anthraquinones

About 5gm sample of the extract was put in a test tube and 10ml of benzene added. The mixture was shaken and filtered. 5ml of ammonia solution was added to the filtrate and the mixture shaken. Presence of violet color in the ammonical phase (lower phase) indicated the presence of anthraquinones.

2.5.4. Tannins

0.5g of powdered sample of each plant is boiled in 20ml of distilled water in a test tube and filtered. 0.1% ferric chloride (FeCl_3) is added to the filtered samples and observed for brownish green or a blue black colouration which shows the presence of tannins.

2.5.5. Saponins

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

2.5.6 Flavonoids

A few drops of 1% ammonia (NH_3) solution were added to the aqueous extract of plant sample in a test tube. A yellow coloration was observed if flavonoid compounds are present.

2.5.7 Glycosides

Salkowsks' test was used to investigate the presence of glycosides in the root extracts: The extract of the plant material was mixed with 2ml of chloroform and 2ml of concentrated sulphuric acid which were carefully added and shaken gently, then the observations were made. A red brown colour indicated the presence of steroidal ring (glycone portion of glycoside).

2.5.8 Alkaloids

100mg of powdered sample was dissolved in 5 ml of methanol and then filtered. Then 2ml of filtrate was mixed with 5ml of 1% aqueous HCl. One milliliter of mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent were added in one tube and occurrence of orange-red precipitate was taken as positive. To the second tube Mayer's reagent was added and appearance of buff-colored precipitate was taken as positive test for the presence of alkaloids.

2.5.9 Phenolic compounds

The extract (500mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

3.0 RESULTS

The phytochemical characteristics of *S. didymobotrya* crude root extracts using different solvents are summarized in the table 1. The results revealed the presence of medically active compounds in the plant roots. From the table, it can be seen that, tannins were present in all the *S. didymobotrya* crude root extracts though in higher amounts in the methanolic total crude root extract. Steroids were present in dichloromethane, methanol total and hexane crude root extracts. However, they were extracted in higher amounts by dichloromethane and low amounts by hexane crude root extracts. Terpenoids were extracted by all the tested solvents except methanol total.

Anthraquinones were moderately extracted by ethyl acetate and hexane crude root extracts of *S. didymobotrya* while in low amounts by dichloromethane crude root extract. On the contrary, they

were absent in methanol total, methanol successive and water crude root extracts. Saponins were highly extracted by methanol total and moderately by water crude root extracts and in low amounts by ethyl acetate extract while they were absent in dichloromethane, methanol successive and hexane crude root extracts. Flavonoids were extracted by all the crude root extracts except methanol successive crude root extract. However they were highly present in water crude root extract. Glycosides were shown to be present in all the crude root extracts except dichloromethane crude root extract. However they were highly extracted by methanol total crude root extract. Alkaloids were extracted by dichloromethane, methanol total and water crude root extracts while Phenols were extracted by all the *S. didymobotrya* crude root extracts except by ethyl acetate and water crude root extracts.

DISCUSSION

From the study results the roots of *S. didymobotrya* were found to contain steroids, terpenoids, anthraquinones, tannins, saponins, glycosides, flavonoids, alkaloids and phenols. This is in agreement with the study carried out by Kitonde *et al.*, (2014) on the roots that demonstrated the presence of Saponins, Terpenoids, Quinones and Flavonoids. This is also in conformity with a study carried out on the bark of *S. didymobotrya* by Korir *et al.*, (2012), which was found to contain terpenoids, anthraquinones, flavonoids, alkaloids and phenols.

Plant steroids are known to be important for their cardiotoxic activities, possess insecticidal and antimicrobial properties (Ngbede *et al.*, 2008; Anpin Raja *et al.*, 2011). They are also used in nutrition, herbal medicine and cosmetics (Anpin Raja *et al.*, 2011).

The terpenoids group show significant pharmacological activities, such as anti-viral, anti-bacterial, anti-malarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-cancer activities (Mahato and Sen, 1997)

Anthraquinones are the main active constituents in herbs often used to relieve constipation. They have an irritant or stimulating laxative effect on the large intestine (Menges *et al.*, 2001). Hence, a decoction or infusion from the leaves, stems and roots of *S. didymobotrya* is drunk as a laxative and purgative for the treatment of abdominal pains, while in large quantities it is taken as an emetic (Singh *et al.*, 2003).

Tannins are secondary metabolites in plants. They are glycosides of gallic or protocatechic 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 (Kokwaro, 2009). This is the reason why traditional healers use *S. didymobotrya* root extracts to rich in tannins to treat diarrhea. In addition tannins have also shown antiparasitic effects (Bajal, 1988).

The presence of Saponins shows the potential of *S. didymobotrya* root extracts to be used to produce mild detergents and intracellular histochemistry staining to allow antibody access to intercellular proteins (Trease and Evans, 1989). They have been found to treat hypercholesterolemia, hyperglycemia, antioxidant, anti-inflammatory, central nervous system activities, anticancer and weight loss (Trease and Evans, 1989). They are used to stop bleeding, treating wounds and ulcers as it helps red blood cells to precipitate and coagulate (Maobe *et al.*, 2013). This can be attributed to ability of saponins to bind with glucose and cholesterol molecules. Saponins have also been associated with inhibitory effect on inflammation (Maobe *et al.*, 2013). Saponins are used by the

folkloric remedies of Kashmir (India) in treating wounds (Foster and Duke, 1990), this is because of their ability to cause red blood cells coagulation and therefore help in blood clotting, treating wounds and enteric ulcers problems (Chiej, 1984). Saponins have also been used to prevent hypercholesterolemia and antibiotic activity, anti-inflammatory and anti-diabetic (Just *et al.*, 1998).

Flavonoids are used as antioxidants because of their ability to scavenge free radicals such as peroxide and hydroperoxide of lipid hydroxyl hence inhibiting oxidation that lead to degenerative diseases (Samatha *et al.*, 2012). They can be used as anti-diabetic. According to Marjorie, (1999), 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 (Yadav and Agarwala, 2011). 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 (Ghasemzadeh and Ghasemzadeh, 2011).

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 (Anthony *et al.*, 2014b) 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 been 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 (Newman *et al.*, 2008). 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 (Ngule *et al.*, 2013).

Alkaloids which are secondary metabolites, can be defined as cyclic compounds which have nitrogen in a negative oxidation state. They affect the chemical transmitters' action of the nervous system. According to Karou (2006), much study has been 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, (2009), many of the antimalarial drugs used today are quinoline derivatives manipulated from cinchona species bark (Okwu and Josiah, 2006). Alkaloids have been identified for their functions which include analgesic, antiplasmodic and antibacterial activity (Karou, 2006). According to Ayitey, (1977), bitter leaves containing alkaloids are capable of reducing headache associated with hypertension.

Phenols are associated with diverse functions, including nutrient uptake, protein synthesis, enzyme activity, photosynthesis; structural components and allelopathy (Wu *et al.*, 2000). Phenolics show an array of health promoting benefits in human health. The phenolic compounds, have biological and pharmacological properties especially their antimicrobial activity (Anpin Raja *et al.*, 2011), antiviral, anti-inflammatory and cytotoxic activity, the antimutagenic and anticarcinogenic activities (Mungole *et al.*, 2010). The medicinal herb is enriched with phenolic compounds that have

excellent antioxidant properties (Narayana *et al.*, 2001). Phenolics are active in curing kidney and stomach problems as well as helpful as anti-inflammatory in action (Shirwaikar *et al.*, 2003).

CONCLUSION

The presence of important pharmacological phytochemicals in the plant roots is an indication of the diverse medicinal importance of *S. didymobotrya*. Phenols, steroids, saponins, flavonoids, glycosides, tannins, anthraquinones, alkaloids and terpenoids were found in the roots of the plant. More research needs to be done to identify the specific bioactive compounds and to elucidate their chemical structures.

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Table 1. Phytochemical analysis of *Senna didymobotrya* crude root extracts

Phytochemicals	Dichloro-methane	Ethyl acetate	Methanol total	Methanol successive	Hexane	Water
Steroids	+++	-	++	-	+	-
Terpenoids	++	+	-	++	+++	+++
Anthraquinones	+	++	-	-	++	-
Tannins	++	+	+++	+	+++	+
Saponins	-	+	+++	-	-	++
Flavonoids	+	+	++	-	+	+++
Glycosides	-	+	+++	+	++	++
Alkaloids	+	-	++	-	-	++
Phenols	++	-	+++	+	+++	-

Key: +++= highly or greatly present; ++ = moderately or fairly present; + = less present (trace amounts); - = Not present

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