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PHYTOCHEMICAL AND BIOACTIVITY EVALUATION OF *SENNA DIDYMOBOTRYA* FRESEN IRWIN USED BY THE NANDI COMMUNITY IN KENYA

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Abstract: Medicinal plants play a vital role in the traditional treatment of various diseases in Africa and the world at large. In Nandi county rift valley province in Kenya *Senna didymobotyya* is one of the most valued plants traditionally, this is mainly attributed to the famous mursik milk in which the plant is used as a preservative. A study was carried out to investigate the bioactivity of the plant leaves. Organism tested were Escherichia coli, Salmonella typhi, *Enterobacter aerogenes*, Klebsiella sp., *Streptococcus pyogenes* isolated from clinical specimens, and *Proteus vulgaris* and *Bacillus cereus*, obtained as standard isolates from commercial source. From the study *Senna didymobotrya* was found to contain secondary metabolites, saponins, flavonoids, tannins, phenols, steroids, steroidal ring (glycone portions of the glycoside), steroidal nucleus (glycone portion of the glycoside) and cardiac glycosides. The plant leaves were found not to contain alkaloids, terpenoids and anthroquinones. The bioactivity of the plant extract observed in this study can be attributed to the presence of these phytochemicals. Results from the bioassay Senna extract showed the highest zone of inhibition was obtained against Bacillus cereus at 500 mg/ml and its minimum inhibitory concentration was 31.25 mg/ml. The extract was also active against all the organisms at 500 mg/ml. Its minimum inhibitory concentration was 250 mg/ml for *Streptococcus pyogenes*, *Escherichia coli* and *Proteus vulgaris*.

Keywords: *Senna didymobotyya*, Phytochemical, Antibacterial, Medicinal herbs

INTRODUCTION

The use of plants to treat diseases has been practiced all over the world since ancient times. Investigation of plants contents has lead to discovery of artificial medicines used today. Over 80% of the people in developing countries are using medicinal plants to treat the illnesses which affect them in daily basis (Ganga, 2012).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 this country 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 population have one time in their life used medicinal plants (Adongo *et al.*,2012). According to Kokwaro (2009), 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. This has lead 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 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 (Nyaberi, *et al.*,2013). According to Tabuti (2007), 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 (Maobe, 2013).

The methanol extract of *S. didymobotrya* stem *showed* an inhibition with *E. coli* being mostly inhibited (Nyaberi, 2013). According to the study carried out on the stem by Nyaberi *et al.*, the presence of either tannins or are alkaloids could be attributed to the antibacterial activity of the *S. didymobotrya* stem.

Senna didymobotrya leaves have being found to have 100% mortality on immature mosquitoes at 250 mg/l concentration of leaves extracts (Maobe, 2013).

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This evidence confirms the pharmaceutical significance of Senna leaves against malaria larvae and therefore making the plant a potential malaria fighting species. According to Mir (2013), medicinal value of plants lies in some chemical substances that produce a definite physiological action in the human body. The discovery of chemical constituents has led to the discovery of many drugs used today. The use of medicinal plants is becoming widely used due to their inability to cause side effects and combat antibiotic resistant microorganisms (Sukirtha *et al.,* 2012). Some plants however have being found to contain some components which are poisonous to human beings and therefore plant materials have to be investigated and proven efficacious and safe (Mir, 2013). According to Smirth (2009), the extracts of plant materials can contain some toxicants and therefore they should be processed properly before using them for medicinal purposes, research done by Smirth shows *Senna-siamea* which belongs to the same genus with *Senna didymobotrya* contains oxalate and phytate toxins therefore proper preparation of the plant before using and using the correct amounts are essential factors for using any plant extract. According to Kokwaro (2009) some components such as certain types of resins are poisonous and therefore are used locally for poisoning purposes. The death of Socrates, one of the greatly known philosophers in the planet in 399 BC, is believed to have being due to the consumption of *Conium maculatum* which contains the alkaloid coniine. Consumption of these plants can be fatal and therefore regardless of the percentage of such components in plants much care needs to be taken.

Much research has being done to investigate the chemical composition of medicinal plants this has led to the discovery of many organic compounds which can be synthesized in the laboratory and even commercialized. According to Ganga (2012), about 25% of the modern medicines are derivatives of plants. The non-nutritive plant components are referred to as phytochemicals, which can be divided in two major categories primary and secondary. Primary constituting of carbohydrates, proteins and chlorophyll and the secondary consisting of tannins, alkaloids, saponins, steroids, flavonoids, terpenoids and anthroquinonines. (Maobe, *et al.,* 2013). 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 (Kokwaro, 2009). Physiological activities of phytochemicals have being found to include cancer prevention, antibacterial, antifungal, anti-oxidative, hormone action and enzyme stimulation.

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 (Kokwaro, 2009). According to Mir (2013) the use of alkaloids, saponins and tannins as antibiotics has been scientifically justified.

Majority of the pharmacologically active chemical compounds where found mainly in ethanol extracts which is contrary to previous researches which had affirmed the traditional way of extracting these compounds using water (Iqbal, 2012).

MATERIALS AND METHODS

Sample collection and preparation

The herb *Senna didymobotrya* was randomly collected in the natural forest around Baraton University in Nandi County. The samples were collected and identified by a taxonomist in the Biology Department, Baraton University. The leaves 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 *Senna didymobotrya* was place 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 24h 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.

Qualitative phytochemical analysis

The extracts phytochemical analysis for identification of bioactive chemical constituents was done using standard procedures by Trease (1989) and Evans (1989), Harborne (1973) and Sofowara (1983).

1. Tannins

A sample of 0.5 g 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 indicated the presence of tannins.

2. Saponins

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

3. Flavonoids

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

4. Terpenoids

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

1. Glycosides.

a. Salkowsks's 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 colour indicated 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 2 ml of acetic acid. The mixture was cooled in ice and observations made. A colour change from violet to blue to green indicated 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 indicated the presence of cardiac glycoside.

2. Alkaloids

The crude extract was mixed with 1% of HCl in a test tube. The test tube was then heated gently a few drops of Mayers and Wagners reagents were added by the side of the test tube. A resulting precipitate confirmed the presence of alkaloids.

3. Steroids

Liebermann's Burchard reaction: About 2g of the solvent 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. Blue green ring indicated the presence of steroids.

4. **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 indicated the presence of phenols.

5. Anthroquinones

Borntreger's test: A sample of 5 gm of the extract was put in a test tube and 10 ml of benzene added. The mixture was shaken and filtered. 5 ml of ammonia solution was added to the filtrate and the mixture shaken. Presence of violet color in the ammoniacal phase (lower phase) indicated the presence of anthroquinones.

Bioassay Study:

Method and Procedure: The procedure and methods used for the preparation of the bacterial suspensions in this study was adopted from that used by Andrew, (2004).

Preparation of the Bacterial Suspension: The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard. The McFarland standard was prepared by dissolving 0.05 g of $BaCl_2$ in 50 ml of water to obtain a 1% solution of Barium chloride (w/v). This was mixed with 99.5 ml of 1% sulfuric acid solution. 3-5 identical colonies of each bacterium was taken from a blood agar plate (Himedia) culture and dropped in Mueller Hinton broth (Himedia). The broth culture was incubated at 37°C for 2-6 hours until it achieved turbidity similar to the 0.5 McFarland standards. The culture that exceeded the 0.5 McFarland standard were each adjusted with the aid of a UV spectrophotometer to 0.132A° at a wavelength of 600 nm in order to obtain an approximate cell density of $1x10^8$ CFU/ml.

Preparation of the Extract Concentrations and Antibiotic: Stock solutions for the extracts were prepared by dissolving 500 mg in 1 ml of dimethylsulfoxide (DMSO). A serial double dilution was prepared for each extract to obtain 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.63 mg/ml, 7.81 mg/ml, 3.91 mg/ml and 1.95 mg/ml respectively. An antibiotic control was made by dissolving 1µg of Augmentin in 1 ml of sterile distilled water. DMSO served as a negative control.

Determination of bioactivity of the Extract: Brain heart infusion agar plates were prepared by the manufacturer's instruction. 0.1 ml of each of the prepared bacterial suspension for the test was transferred to 2 plates for each organism to give a duplicate for each concentration and organism. Five wells were drilled in each agar plate. Three of the wells were filled with the extract dilution and the other wells were filled with Augmentin and DMSO control respectively. The wells were labeled on the underside

of the plate. The plates were incubated at 37°C for between 24 to 48 hours and the zones of inhibition were measured in millimeters with the aid of a ruler.

RESULTS AND DISCUSSION

Table.1: Phytochemical results

Phytochemical	Observation	Inferences
Tannins	Blue –black color observed	Presence of tannins
Saponins	Stable foam	Presence of saponins
Flavonoids	Magenta – red color observed	Presence of flavonoids
Terpenoid	No gray color observed	Terpenoids absent
Glycosides: (a)Liebermann's test	(a)Blue to green color observed	(a) Presence of steroidal nucleus
(b) Salkowsk's test	(b)Red – brown color observed	(b)Presence of steroidal ring
(c) Keller –Kilani test	(c)Brown ring at the inter phase	(c)Presence of cardiac glycoside
Alkaloids	No precipitate observed	Absence of alkaloids
Steroids	Blue – green ring was observed	Presence of steroids
Phenols	Blue- green color observed	Presence of phenols
Anthroquinones	No violet color at lower phase	Absence of anthroquinones

The phytochemicals found in this plant can be attributed to its medicinal value. Saponins can be used as mild detergents and intracellular histochemistry staining to allow antibody access to intercellular proteins (Maobe, 2013). They have being found to treat hypercholesterolemia, hyperglycemia, antioxidant, anti-inflammatory, central nervous system activities, anticancer and weight loss (Maobe, 2013). They are used to stop bleeding, treating wounds and ulcers as it helps red blood cells to precipitate and coagulate (Okwu, et. al, 2006). They can reduce the uptake of some nutrients such as glucose and cholesterol at the gut causing hypocholesterolemic effect and thereby relieve the liver metabolic burden (Smirth, 2009). 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 (Just *et al.*,1998).

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 (Kokwaro, 2009). 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 (Argal, 2006). This shows how traditional medicinal plants rich in tannins can be used to control this dangerous disease. Tannins have also shown antiparasitic effects. According to Bajal et al (1988), tannins can also be used to protect the kidney by inactivating herpes complex virus and various enteric viruses. It has also being discovered that tannins have anti-inflammatory and antiulcer activity in rodents, a clear indication that they can be used treat against inflammation. 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 (1998) 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 anti-oxidative 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, produce liver necrosis and module immune responses. The dosage and kind of tannins are critical to these effects (Chung, 1998).

Flavonoids are secondary metabolites with polyphenolic structure and synthesized in plants, through polypropanoid pathway (Ali, 2011). 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 (Sharma, 2006). 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).

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. (Schatzmann, 1965).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 (Newmann, 2008).

Table.2: Antimicrobial Activity of the Methanol Extract of *S. didymobotrya* Extract (500 mg/ml) Against Selected Microorganisms. Zones of Inhibition (mm).

Organisms	Senna Extract	Augmentin	DMSO Control
Salmonella typhi	12.50 ± 0.563	31.67 ± 0.667	0.00 ± 0.00
Klebsiella sp.	14.33 ± 0.211	29.67 ± 0.558	0.00 ± 0.00
Bacillus cereus	19.00 ± 0.258	29.00 ± 0.516	0.00 ± 0.00
Streptococcus pyogenes	11.67 ± 0.494	31.00 ± 0.577	0.00 ± 0.00
Escherichia coli	12.17 ± 0.477	39.17 ± 1.17	0.00 ± 0.00
Proteus vulgaris	10.83 ± 0.477	30.83 ± 0.477	0.00 ± 0.00
Enterobacter aerogenes	10.33 ± 0.615	32.00 ± 0.683	0.00 ± 0.00

The *S. didymobothrya* leaf extract showed the highest average zone of inhibition against Bacillus cereus (19.0 mm). This was followed by Klebsiella sp (14.33 mm), Salmonella typhi (12.50 mm), Escherichia coli (12.17 mm), Streptococcus pyogenes (11.67 mm), Proteus vulgaris (10.83 mm) and *Enterobacter aerogenes* (10.33 mm). A zone of inhibition greater than 8 mm was considered as active. A one-way analysis of variance (ANOVA) showed that there was a significant difference in the zones of inhibition among the extracts and augmentin for all the microorganisms ($P<0.001$). On further comparison with the Tukey's pair wise comparison test, it was shown that the zones of inhibition for augmentin antibiotic was significantly higher than all the extract concentrations ($P<0.001$). It also showed that the zone of inhibition for Bacillus cereus was significantly higher than all the other bacterial organisms ($P<0.001$). Klebsiella sp. showed significantly higher zones of inhibition compared to Proteus vulgaris ($p<0.01$) and *Enterobacter aerogenes* ($p<0.05$) but was not significantly different from all the other organisms ($p>0.05$).

Table.3: Tukey's Multiple Comparison of the Zones of Inhibition among the Bacterial organisms treated with S. didymobotrya and the Antibiotic Control

COMPARISON	P-value	Significance
Salmonella typhi vs Klebsiella sp	0.639	NS
Salmonella typhi vs. Bacillus cereus	0.000	S
Salmonella typhi vs Streptococcus pyogenes	0.999	NS
Salmonella typhi vs Escherichia coli	1.000	NS
Salmonella typhi vs. Proteus vulgaris	0.769	NS
Salmonella typhi vs *Enterobacter aerogenes*	0.368	NS
Klebsiella sp. vs Bacillus cereus	0.000	S
Klebsiella sp vs Streptococcus pyogenes	0.107	NS
Klebsiella sp. vs Escherichia coli	0.308	NS
Klebsiella sp. vs Proteus vulgaris	0.006	S
Klebsiella sp. vs *Enterobacter aerogenes*	0.001	S
Bacillus cereus vs Streptococcus pyogenes	0.000	S
Bacillus cereus vs Escherichia coli	0.000	S
Bacillus sp. vs Proteus vulgaris	0.000	S
Bacillus sp. vs *Enterobacter aerogenes*	0.000	S
Streptococcus pyogenes vs Escherichia coli	1.000	NS
Streptococcus pyogenes vs Proteus vulgaris	0.999	NS
Streptococcus pyogenes vs E. aerogenes	0.943	NS
Escherichia coli vs Proteus vulgaris	0.999	NS
Escherichia coli vs *Enterobacter aerogenes*	0.639	NS
Proteus vulgaris vs *Enterobacter aerogenes*	1.000	NS
Salmonella vs Augmentin	0.000	S
Klebsiella sp vs Augmentin	0.000	S
Bacillus cereus vs Augmentin	0.000	S
Escherichia coli vs Augmentin	0.000	S
Streptococcus sp and Augmentin	0.000	S
Proteus vulgaris vs Augmentin	0.000	S
Enterobacter aerogenes vs Augmentin	0.000	S

Key: S = Significant; NS = Not Significant;

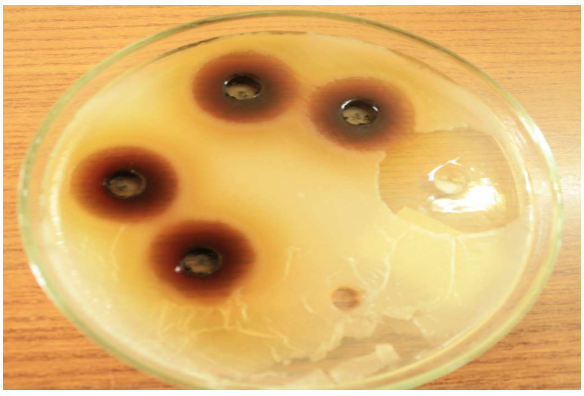

Plate.1: Zone of Inhibition of *S. didymobothrya* against Klebsiella sp. with a positive antibiotic control and DMSO control.

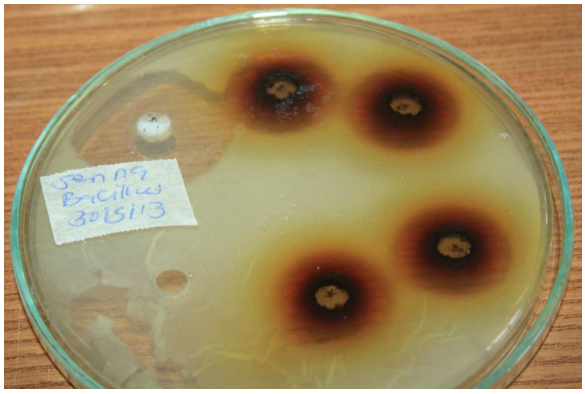

Plate.2: Zone of inhibition of *S. didymobothrya* Extract against *Bacillus cereus*

The agar well diffusion method was used to determine the minimum inhibitory concentrations for the extracts. The minimum inhibitory concentrations for *Bacillus cereus* was 31.25mg/ml, the best and lowest concentration to destroy the organism. The other organisms that were able to show MIC values were Streptococcus pyogenes and Proteus vulgaris both at 250mg/ml. All other organisms showed zones of inhibition only at 500 mg/ml. These results have shown that it is very possible to limit the spread the Bacillus and Streptococcus infection using the concentrations of the crude extracts indicated.

CONCLUSION

From this study it is clear that *Senna didymobotrya* leaves has a great potential in limiting the spread of Bacillus and Streptococcus and to some extent all the other microorganisms in which the plant was tested against. The medicinal value of the leaves can be greatly associated to the phytochemicals which were found in the plant. From the study saponins, flavonoids, tannins, phenols, steroids, steroidal ring (glycone portion of the glycoside), steroidal nucleus (glycone portion of the glycoside) and cardiac glycosides were found present in the plant leaves but alkaloids, terpenoids and anthroquinones were found to be absent in the plant leaves. Further study needs to be done to come up with the specific compounds in the plant which are active against the microbes.

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