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Preliminary Phytochemical Screening of Eight Selected Medicinal Herbs Used for the Treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya

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Abstract: Medicinal plants are an important source of phytochemicals that offer traditional medicinal treatment of various ailments. In Kisii region, southwest Kenya, amongst the indigenous herbs used as phytomedicines for the treatment of diabetes, malaria and pneumonia are *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis Peruvian, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica.* A study was carried out on these plants in the year 2011 to 2012. The objective was to test for the presence of phytochemical compounds in the eight selected traditional medicinal herbs. Leaf samples of the selected herbs growing in the ecological conditions of the Kisii region were collected, washed, air-dried and milled. The samples were extracted with one solvent namely water. The aqueous portion of the extract was used for phytochemical analysis to determine the presence of bioactive compounds. Results showed that saponins, tannins, steroids, terpenoids and flavonoids are present in all the eight herbs investigated. Herbs with cardiac glycoside in their leaves are *Carissa spinarum, Senna didymobotrya, Physalis peruviana, Bidens pilosa* and *Leonotis nepetifolia* while those without are *Urtica dioica, Warburgia ugandensis* and *Toddalia asiatica*. Plants with alkaloids are *Warburgia ugandensis* and *Physalis peruviana.* The anthraquinones are present in *Carissa spinarum and Bidens pilosa.* The presence of pytochemicals in the herbs confirms their potential as medicinal plants. The herbs vary in their phytochemical constituents hence potential as medicinal plants.

Key words: Medicinal herbs % Phytochemicals % Diabetes % Malaria % Pneumonia

INTRODUCTION

Medicinal herbs constitute effective sources of antimicrobial and antioxidant natural products [1]. Medicinal herbs are an important source for the therapeutic remedies of various ailments [2]. Since time immemorial, different parts of medicinal herbs have been used to cure specific ailments in Kenya [3]. In the Kisii region, the leave decoction of *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica,* are locally used for the treatment of diabetes, malaria and pneumonia [4]. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties [5]. The plants produce these chemicals to protect themselves but recent research demonstrates that they can protect humans and animals against diseases [6]. A number of phytochemical are known, some of which include: alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids and terpenoids. 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. Phytochemicals are responsible for medicinal activity of plants and they have protected human from various diseases [7]. Phytochemicals are basically divided into two groups that are primary and secondary metabolites based on the function in plant metabolism. The major constituents of phytochemical are consist of carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and among others [8]. The phytochemical

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constituents are playing a significant role in the identification of crude drugs. There is widespread interest in evaluating drugs derived from plant sources. This interest mainly arises from the belief that green medicine is safe and dependable, compared to costly synthetic drugs which are invariably associated with adverse effects [9]. Natural antimicrobials have been often derived from plants, animal tissues or microorganisms. The adverse effects of the drugs available today, necessitate the discovery of new harmless pharmacotherapeutic agents from medicinal plants [10, 11].

The objective of the present study is to test for the presence of various phytochemicals in eight selected traditional medicinal herbs from Kisii region, southwest Kenya, used in treatment of diabetes, malaria and pneumonia.

MATERIALS AND METHODS

Sample Collection and Preparation: In this study the leaves of the *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica* were collected from Kisii region, southwest Kenya. The verification of the herbal species was done by the Botanist; Egerton University. The leaves of the validated medicinal herbs were then collected from their site in Kisii region and air-dried for twelve weeks to obtain constant weight. The dried sample was cut into smaller pieces and then ground into fine particles with a grinder at the Department of Food Science and Technology, Faculty of Science, Jomo Kenyatta University of Agriculture and Technology. The powdered sample was bagged in black plastic bags and stored in an air-tight container for further work.

Phytochemical Screening of Crude Extracts Procedure: The leaves of the herbs were tested for bioactive compounds according to standard procedures.

Test for Cardiac Glycosides: 1.0 g of extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayered with 1 ml of concentrated sulphuric acid (H_2SO_4).

Test for Alkaloids: Two millitre of aqueous sample extract was measured using a measuring cylinder and equal volume of ethanol containing 3 % tartaric acid was added and shaken. Then few drops of marquin's reagent were added into the mixture. The formation of precipitate any form indicated the presence of alkaloids. The same procedure was repeated for ethanol extract.

Test for Saponins: In determining the presence or absence of saponins, 0.5 g of the dried extract was placed in a test tube and 3ml of distilled water added and boiled for fifteen minutes. The content was filtered and the filtrate shaken vigorously. The same procedure was repeated for the other dried extracts of the eight selected herbs.

Test for Tannins:

- Two millitres of sample aqueous extract was measured by measuring cylinder and equivalent volume of potassium hydroxide was added. The same procedure was repeated for the other extract.
- Two millitres of sample aqueous extract was measured by measuring cylinder and equivalent volume of 2ml of 10% sodium chloride was added to the extract. The mixture was filtered and divided into two different test tubes. Four drops of lead acetate solution was added to one of the test tubes and four drops of ferric chloride to the other. This was repeated for the aqueous extracts and ethanol of the other herbs.

Test for Steroids and Triterpenoids: 2.0 g of the dried aqueous extract was dissolved in 10 ml chloroform and filtered. 2 ml of filtrate was measured and placed in two different test tubes. Two drops of concentrated H_2SO_4 was added to one of the test tubes and five drops of acetic anhydride followed by five drops of conc. H_2SO_4 were added to the other test tube for confirmation. The same procedure was repeated for the other herbal extracts.

Test for Flavonoids: About 2 g of the powdered leaves were completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone in a water bath. The mixture was filtered while still hot. The filtrate was cooled and used.

Sodium Hydroxide Test: Five ml of 20% sodium hydroxide was added to equal volume of the detanned water extract. The same procedure was repeated for the dried extracts of other herbs.

Test for Anthraquinones: About 0.5 g of the extract was taken and placed into a dry test tube and then 5 ml of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate shaken with an equal volume of 100% ammonia solution.

Data Collected: The change of colour was observed when the test reagent was added to the prepared sample for the phytochemical test. The result was recorded as present (+) or absent (-) depending on the outcome of the test.

Data Analysis: The calculated t value was obtained by comparing the sum of positive (+) and negative (-) results. The critical t value is obtained from significant test table using number of samples. Differences between the critical t-value and calculated t-values of the bioactive compounds of the herbal extracts were computed. For all the eight herbal species investigated, the null hypothesis was retained because the calculated t-value was more than the critical t-value at p # 0.05.

RESULTS AND DISCUSSION

Phytochemical Screening: The preliminary phytochemical screening results of *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens Pilosa, Leonotis nepetifolia and Toddalia asiatica* leaves extract, showed the presence of various bioactive secondary metabolites constituents (Table 1). Phytochemical study of *Carissa spinarum* and *Bidens Pilosa* leaves extract showed that the leaves contained cardiac glycoside, saponins, tannins, steroids and terpenoids, flavonoids and anthraquinones while alkaloids were absent. The phytochemical test results obtained indicated that *Urtica dioica* and *Toddalia asiatica* leaves extract have saponins, tannins, steroids and terpenoids and flavonoids while alkaloids, cardiac glycoside and anthraquinones were absent. The results also showed *Senna didymobotrya* and *Leonotis nepetifolia* leaves extract have cardiac glycosides, saponins, tannins, steroids and terpenoids and flavonoids as alkaloids and anthraquinones were absent. The test results of *Warburgia ugandensis* leaves extract indicated that alkaloids, saponins, tannins, steroids and terpenoids and flavonoids were present while cardiac glycoside and anthraquinones were absent. However, results obtained show *Warburgia ugandensis* (bark) extracts had cardiac glycoside, saponins, tannins, steroids and terpenoids and flavonoids whereas alkaloids and anthraquinones were absent. The *Physalis peruviana* leaves extract results indicated that cardiac glycoside, alkaloid, saponins, tannins, steroids and terpenoids and flavonoids were present while anthraquinones were absent.

The observations and inferences made in the phytochemical tests are presented in the following subsections.

Cardiac Glycoside: In this study a brown ring was observed at the interface in the water extracts tested for the cardiac glycoside indicating the presence of a de-oxysugar characteristic of cardenolides in the extract of *Carissa spinarum, Senna didymobotrya, Physalis peruviana, Bidens Pilosa* and *Leonotis nepetifolia*. It was also observed that no brown ring formed at the interface in the extracts of *Urtica dioica* and *Warburgia ugandensis* indicating absence of cardiac glycoside in their extracts.

Alkaloids: In this study a yellow precipitate was observed in the water extracts of *Warburgia ugandensis* (leaves) and *Physalis peruviana* indicating the presence of alkaloids in the two herbs whereas a clear yellowish solutions (with no precipitates) was observed in the water extract of *Carissa spinarum, Urtica dioica, Warburgia ugandensis* (bark), *Senna didymobotrya, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica* indicating the absence of alkaloids in these herbs.

Table 1: Phytochemical screening of water extract from selected medicinal herbs

Pytochemical constituents \ Plant samples	cardiac glycoside	alkaloid	saponin	tannin	steroid	flavonoid	anthraquinone
Carissa spinarum	+	-	+	+	+	+	+
Urtica dioica	-	-	+	+	+	+	-
Warburgia ugandensis (leaves)	-	+	+	+	+	+	-
Warburgia ugandensis (bark)	+	-	+	+	+	+	-
Senna didymobotrya	+	-	+	+	+	+	-
Physalis peruviana	+	+	+	+	+	+	-
Bidens pilosa	+	-	+	+	+	+	+
Leonotis nepetifolia	+	-	+	+	+	+	-
Toddalia asiatica	-	-	+	+	+	+	-

Key: (+) = indicate present, (-) = indicate absent

Tannins: In this study a yellow precipitate was observed for the water extracts for *Carissa spinarum, Urtica dioica, Warburgia ugandensis* (leaves), *Warburgia ugandensis* (bark), *Senna didymobotrya, Physalis peruviana, Bidens Pilosa, Leonotis nepetifolia* and *Toddalia asiatica* indicating presence of tannins in all herbs analysed. Bluish-green colouration (with ferric chloride) was also observed in the water extracts of the same samples indicating presence of tannins.

Saponins: Results indicated that frothing which persisted on warming was observed in the water extracts of *Carissa spinarum, Urtica dioica, Warburgia ugandensis* (leaves), *Warburgia ugandensis* (bark), *Senna didymobotrya, Physalis peruviana Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica* indicating the presence of saponins in the herbs. The same extract with few drops of olive oil formed a soluble emulsion, confirming the presence of saponins in all the selected eight herbs.

Flavonoids: In this study a yellow colourations was observed with water extract of *Carissa spinarum, Urtica dioica, Warburgia ugandensis* (leaves), *Warburgia ugandensis* (bark), *Senna didymobotrya, Physalis Peruvians, Leonotis nepetifolia* and *Toddalia asiatica* indicating the presence of flavonoid.The yellow colourations was not observed with the extract of *Bidens pilosa* indicating flavonoids were absent.

Steroids and Terpenoids: The results indicated that a reddish brown ring at the interface was observed with the water extracts of *Carissa spinarum, Urtica dioica,* (*Warburgia ugandensis* (leaves), *Warburgia ugandensis* (bark), *Senna didymobotrya, Physalis peruviana, Bidens Pilosa, Leonotis nepetifolia* and *Toddalia asiatica*, indicating the presence of steroids and terpenoids.

Anthraquinones: The results showed a pink violet or red colouration was observed for water extracts in the ammoniacal layer (lower layer) indicated the presence of free anthraquinones in the extracts of *Carissa spinarum* and *Bidens Pilosa* while there was no pink violet or red colouration observed in *Urtica dioica, Warburgia ugandensis* (leaves), *Warburgia ugandensis* (bark), *Senna didymobotrya, Physalis peruviana, Leonotis nepetifolia* and *Toddalia asiatica* indicating that the anthraquinones were absent.

The medicinal value of the herbal secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body [12-15]. The most important of these secondary metabolites include alkaloids, saponins, tannins, glucosides, steroids and terpenes, flavonoids and anthraquinones [16-18]. Phytochemical screening of eight medicinal herbs studied showed that all the leaves have saponins, tannins, steroids and flavonoids. The maximum number of secondary metabolites was found in *Bidens Pilosa, Carissa spinarum* and *Physalis peruviana,* followed by *Warburgia ugandensis* (leaves), *Warburgia ugandensis* (bark), *Senna didymobotry* and *Leonotis nepetifolia*. The minimum number of secondary metabolites was recorded in *Urtica dioica* and *Toddalia asiatica.* The alkaloids have been investigated for many pharmacological properties including antiprotozoal, cytotoxic, antidiabetic [19] and anti-inflammatory [20] properties, but there are only few reports about their antimicrobial properties. The herbs with alkaloids in the present study are *Warburgia ugandensis* (leaves) and *Physalis peruviana* that are used to cure asthma. The saponins are glycosides occurring widely in plants. The saponin is used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hypercholesterolemia, hyperglycemia, antioxidant, anti-cancer, anti-inflammatory, central nervous system activities [20] and weight loss. It is also known to have antifungal properties [21].

The plants having saponins are *Carissa spinarum, Urtica dioica, Warburgia ugandensis* (leaves), *Warburgia ugandensis* (bark), *Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica*. Plant steroids are known to be important for their cardiotonic activities, possession of insecticidal, anti-inflammatory, analgesic properties, central nervous system activities and antimicrobial properties [21]. They are also used in nutrition, herbal medicine and cosmetics. In this study the steroids are present in *Carissa spinarum, Urtica dioica, Warburgia ugandensis* (leaves), *Warburgia ugandensis* (bark), *Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica.* Tannins exhibit antidiabetic, anti-inflammatory, antibacterial and antitumor activities [21]. It was reported that certain tannins were able to inhibit HIV replication selectively besides use as diuretics [21]. The herbal tannins have been recognized for their pharmacological properties. Glycosides were reported to exhibit anti-diabetic characteristics [22]. Cardiac glycosides are known to hamper the Na+ /K+ pump [22]. This results in an increase in the level of sodium ions in the myocytes which then

enhance the level of calcium ions. This consequently increases the amount of Ca^{2+} ions available for contraction of the heart muscle, which improves cardiac output and reduces distention of heart and thus is used in the treatment of congestive heart failure and cardiac arrhythmia.

CONCLUSIONS

All the selected eight herbs investigated have bioactive compounds namely saponins, tannins, steroids and flavonoids. The bioactive compound cardiac glycoside is present in six selected herbs but absent in *Urtica dioica*, *Warburgia ugandensis* (leaves) and *Toddalia asiatica*. The alkaloids are present in the herbs *Warburgia ugandensis* (leaves) and *Physalis peruviana* but absent in the other six selected herbs. Further studies are needed with these herbs to isolate, characterize and elucidate the structure of the bioactive compounds of the herbs which are responsible for the antimicrobial activity and other medicinal value.

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