



Original Research Article

PRELIMINARY PHYTOCHEMICAL AND ANTIBACTERIAL SCREENING OF FRESH TETRADENIA RIPARIA LEAVES WATER EXTRACT AGAINST SELECTED PATHOGENIC MICROORGANISMSMutuku Chrispus Ngule^{1*}, Mueni Hellen Ndiku², F Ramesh³ and Makau Elijah Ngule⁴¹Department of Chemistry, University of Eastern Africa, Baraton, P.O. Box 2500, Eldoret -30100, Kenya.²Department of Family and Consumer Sciences, University of Eastern Africa, Baraton, Eldoret -30100, Kenya.³Department of Biological Sciences, University of Eastern Africa, Baraton, P.O. Box 2500, Eldoret -30100, Kenya.⁴Department of Biomedical Science and Technology, Maseno University, Maseno - 40105, Kenya.

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Abstract: The use of plants as a source of medicine is as old as man himself. Plants have been used since time immemorial to treat against various diseases affecting human beings all over the world. Before the invention of synthetic drugs, traditional medicine dominated the world. The study was conducted to analyze the antibacterial activity phytoconstituents of *Tetradenia riparia* plant. The samples were extracted using water solvent. The phytoconstituents study was done using standard procedures. The bioassay was done using well diffusion method. From the study, *Tetradenia riparia* was found to contain tannins, saponins, flavonoids, cardiac glycosides, phenols, alkaloids and steroidal rings, but terpenoids, steroids and steroidal nucleus were found to be absent in the plant extract. The fresh leaves water extract of the plant *Tetradenia riparia* inhibited the growth of all the microorganisms used. The zones of inhibition were high in *Salmonella typhi* (21.00±0.000), followed by *Staphylococcus epidermidis*, *Proteus vulgaris* and *Bacillus cereus* with inhibition zones of 18.67±0.577, 18.00±0.333 and 18.00±0.577 respectively. *Serratia marcescens* and *Serratia liquefaciens* had inhibition zones of 13.00±0.333 and 13.33±0.577 respectively. *Escherichia coli* had the least inhibition zone of 9.67±0.577. Penicillin which was used as the positive control inhibited the growth of all the microorganisms tested while DMSO (negative control) did not show any inhibition zones against any of the microorganisms tested. The current study may be a partial scientific justification of the plants use in the treatment against the infections caused by all the tested microorganisms.

Key Words: *Tetradenia riparia*, Phytochemicals, Medicinal herbs, antibacterial, leaves

INTRODUCTION

Pharmacological studies on plants have shown them to have antibacterial activity, antifungal activity, antitumor activity, anti-inflammatory activity, anti-hyperlipidemic, anti-hyperglycemic and hypoglycemic activity [1]. The antibacterial activity of plants has been associated with the presence of certain phytochemicals such as tannins and alkaloids [2-4].

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. Plants produce more than 10,000 different compounds to prevent themselves against pathogens. 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 [6].

Over 80% of the plants in Nigeria are used for treatment of malaria and other sicknesses. They are also used as food [7]. There seems to be no much distinction between medicinal benefits of plants and their nutritive value. The published WHO traditional

strategy addressed 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 been done and is still going on to prove scientifically the plants' nutritional and medicinal values. A good number of chemical compounds have been 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 ethnobotanical plants species used all over the world have been found to have great pharmacological value.

Over 80% of the people in developing countries use medicinal plants to treat the illnesses which affect them from time to time [9]. 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 also steadily growing with 40% of the population using plants as a source of medicine [10]. The use of these plants in treatment of ailments is mainly based on the type of flora in that region.

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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 [7 & 12].

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 search for new antibiotics to kill and inhibit their growth. Phytochemicals have been associated with reduction of drug resistant forms of bacteria [13-16].

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 diuretics and in the treatment of dropsy [11]. The use of alkaloids, saponins and tannins as antibiotics has been scientifically justified [6]. The plant *Tetradenia riparia* is a highly branched soft dioecious shrub which grows to a height of 1-3M. The stems of the plant are brittle and semi-succulent. It has sticky- aromatic foliage. The plant is mainly found in the wooden hillsides and stream banks of the coastal regions of Northern province of South Africa, Namibia, Angola, Botswana and East tropical Africa. Ethnobotanically the plant leaves are used by the Kisii community in the treatment against stomach problems and inflammation. The decoction of the plant leaves is also used to treat wounds and wound infections [17]. The current study was done to investigate the antibacterial

potency and the presence of important phytoconstituents in the plant.

MATERIALS AND METHODS

Sample Collection and Preparation

The leaves of the plant were randomly harvested in the month of July and August from the natural forest around University of Eastern Africa, Baraton. The samples were identified by a taxonomist in the Department of Biology, University of Eastern Africa, Baraton. A voucher specimen was prepared and stored in the department of biological sciences herbarium. The samples were thoroughly mixed and spread to dry at room temperature in the chemistry laboratory for about three weeks and then ground into fine powder. The powdered samples were stored in transparent polythene bags.

Extraction procedure

Using electric analytical beam balance fifty grams of the leaves sample was put in a conical flask and heated to boiling for 20 minutes. The extract was filtered using Butchner funnel; Whatman no.1 filter paper, 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 50°C. The extract was dried using vacuum and pressure pump at room temperature. The residue was obtained and used for the experiment [2].

Qualitative phytoconstituents analysis

The presence of phytochemicals (tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids, steroids and phenols) analysis was done using standard procedures [18, 19 and 20].

Bioassay Study

Bacteria source and media preparation: The bacteria used in the study were commercial pure cultures from Carolina biological supply company (USA). The colonies for use in the study were obtained from the pure cultures and then transferred into blood agar plates. The plates were then incubated at 37°C for 24 hours. The blood agar media was prepared according to the manufacturer's (Himedia, India) instructions. The plates were sterilized by the use of an autoclave at 121°C. Approximately 20ml of the prepared media was poured into the sterilized plates and the surface of the media was flamed using a Bunsen burner flame to remove air bubbles. The Mueller Hinton broth was prepared according to the manufacturer's instructions. About 5ml of the broth was transferred in to sterile test tubes. The transfer of the media to the plates and test tubes was done under sterile germicidal wood.

Preparation of the Bacterial Suspension: The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard [17&18]. The McFarland standard was prepared by dissolving 0.5 g of BaCl₂ in 50 ml of water to obtain a 1% solution of Barium chloride (w/v). This was mixed with 99.5 ml of 1% sulphuric acid solution. Three – five identical colonies of each bacterium were taken from a blood agar plate (Himedia) culture using a sterile swab into 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 1x10⁸ CFU/ml.

Preparation of the Extract Concentrations and Antibiotic: Extracts stoke solutions were prepared by dissolving 500 mg in 1 ml of dimethylsulfoxide (DMSO). An antibiotic control was made by dissolving 500 mg of penicillin in 1 ml of sterile distilled water. DMSO served as a negative control.

Determination of the bioactivity of the Extract: Mueller Hinton agar plates were prepared as per the manufacturer's (Himedia, India) instructions. The media and the plates were sterilized in an autoclave at 121°C for 15 minutes. The media was poured on the plates. The plates were flamed on the surface using a non-luminous flame to remove air bubble. The cork borer was sterilized using a non-luminous flame. The plates and all the equipment's to be used for the experiment were then transferred into a germicidal wood. The germicidal lamp was put on for 30minutes to sterilize the surface of the plates and other equipment. The bacterial suspension was smeared on the media and six wells with a diameter of 6cm each were drilled in each agar plate using a cork borer. Four of the wells were filled with 0.1ml of the 500mg/ml of the extract. The other wells were filled with 0.1ml of 500mg/ml of penicillin and 0.1ml of 100% DMSO positive and negative controls respectively. Three plates were made for each bacterial organism and extract giving a triplicate reading for each microorganism and extract. The plates were labeled on the underside and incubated at 37°C for between 24 to 48 hours and the zones of inhibition measured in millimeters with the aid of a ruler.

RESULTS AND DISCUSSION

The fresh leaves water extract of the plant inhibited the growth of all the microorganisms used. The inhibition zones caused by the plant extract were high in *Salmonella typhi* (21.00±0.000), followed by

Staphylococcus epidermidis, *Proteus vulgaris* and *Bacillus cereus* with inhibition zones of 18.67±0.577, 18.00±0.333 and 18.00±0.577 respectively. *Serratia marcescens* and *Serratia liquefaciens* had inhibition zones of 13.00±0.333 and 13.33±0.577 respectively. *Escherichia coli* had the least inhibition zone of 9.67±0.577. Penicillin which was used as the positive control inhibited the growth of all the microorganisms tested while DMSO (negative control) did not show any inhibition zones against any of the microorganisms tested. The inhibition of the plant against the microorganisms is noteworthy since the selected microorganisms have been found to have great clinical importance. The inhibition of the plant against the microorganisms could be attributed to the presence of the important pharmacological compounds found in the plant.

The presence of Saponins (Table 1 and 2) 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].

Table 1: Phytochemical examinations of *Tetradenia riparia*

Phytochemical	Inferences
Tannins	Present
Saponins	Present
Terpenoids	Absent
Flavonoids	Present
Cardiac glycosides	Present
Phenols	Present
Alkaloids	Present
Steroids	Absent

Table 2: Antibacterial activity of Infused *Tetradenia riparia* against selected pathogenic microorganisms.

Microorganisms	Extract mean ± mean S.E (mm)	Penicillin mean ± S.E (mm)	DMSO mean ± S.E (mm)
<i>Bacillus cereus</i>	18.00±0.577	23.67±0.882	0.00±0.000
<i>Serratia liquefaciens</i>	13.33±0.577	29.00±0.577	0.00±0.000
<i>Serratia marcescens</i>	13.00±0.333	26.33±0.333	0.00±0.000
<i>Salmonella typhi</i>	21.00±0.000	22.67±0.667	0.00±0.000
<i>Escherichia coli</i>	9.67±0.577	31.33±0.333	0.00±0.000
<i>Proteus vulgaris</i>	18.00±0.333	24.33±0.577	0.00±0.000
<i>Staphylococcus epidermidis</i>	18.67±0.577	26.67±0.333	0.00±0.000

S.E = Standard Error

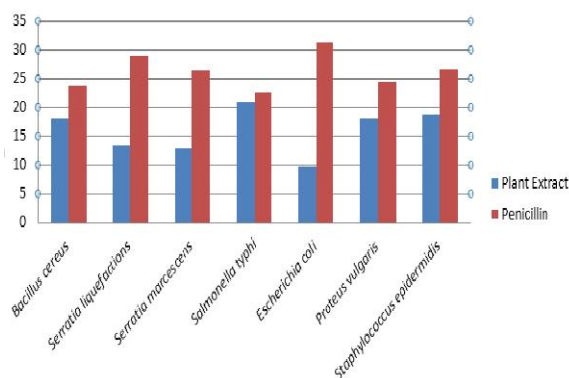


Figure 1: Comparison on the zones of inhibition caused by the plant extract and Penicillin

Alkaloids which are secondary metabolites, they can be defined as a cyclic compound which has nitrogen in a negative oxidation state. They affect the chemical transmitter's action of the nervous system. They also have other pharmacological activities such as analgesic, antispasmodic, antihypertensive effects and antiarrhythmic effects and antibacterial. Cryptolepine a major alkaloid in *S. acuta* was found to be an antimalarial agent [23]. Cryptolepine has also been used clinically to treat malaria, colic and stomach ulcers and also used in anticancer drugs [24]. Studies have been done on pharmacological properties of alkaloids on antiprotozoal, cytotoxic and anti-inflammatory properties [25].

Tannins are also secondary metabolites in plants. They are glycosides of gallic or Protocatechuic acid. 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 rich in tannins to treat wounds and burns since they are able to cause blood clotting. Some tannin have been reported to inhibit HIV replication selectively besides the use of diuretics [26]. This shows how traditional medicinal plants rich in tannins can be used to control this dangerous disease. Tannins have also shown antiparasitic effects [27]. Tannins can also be used to protect the kidney since when taken the poliovirus, herpes complex virus and various enteric viruses are inactivated [28]. 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 [29].

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 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 modulate immune responses. The dosage and kind of tannins are critical to these effects [29].

Flavonoids are secondary metabolites with polyphenolic structure and synthesized in plants, through polypropanoid pathway [14]. Flavonoids have been classified into six sub-groups which include flavones, flavanol, flavanone, flavan-3-ols, isoflavone and anthocyanidin. 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 been 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 [30]. 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 been 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 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 [31]. The unexpected results relating cardiac glycosides with anticancer properties have created a great interest in this secondary metabolite. This has led to clinical trial of cardiac glycosides based drugs in clinics [32].

The current research is inconformity with previous studies in which the plant was found to have antibacterial activity. According to Njau et al [33], the plant was found to inhibit the growth of *Escherichia coli*, *Staphylococcus* sp. and *Enterobacter faecalis*. The dried leaves of the plant were also found to inhibit the growth of all the microorganisms tested [34], however the current study is different since the fresh leaves were found to inhibit the growth *Salmonella typhi* much higher as compared to the dried leaves.

The presence of important pharmacological phytoconstituents and the antibacterial activity observed in the plant fresh leaves water extract indicates the medicinal importance of *Tetradenia riparia*. The current study may be a partial scientific justification of the plants use in the treatment against the infections caused by all the tested microorganisms. The isolation of active ingredients, identification of their structural make up, mode of action and their effect in the *in vivo* environment remains inevitable in order to provide the safety precautions (if any) which are needed when using this plant. Further research on the isolation of the active ingredients needs to be done in the future.

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