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Antibacterial activity and Preliminary phytochemical screening of hot- aqua extract of *Tetradenia riparia* leaves

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Abstract

Nature is a paradise of medicinal solutions to all ailments affecting human beings. Medicinal plants have been used widely to treat against the currently widespread strains of drug resistant bacteria. Green medicine has attracted great interest due to the belief that it is safe, cheap and more dependable than aliphatic drugs, which have adverse side effects. The current study was done to analyze the Phytochemical and antibacterial activity of hot – aqua extract of *Tetradenia riparia* leaves. The Phytochemical results showed the plant to contain very important compounds (Table 1). The infused plant extract (Table 2) inhibited the growth of all the microorganisms tested. *Bacillus cereus* was the microorganism which had the highest zone of inhibition of 22.67 ± 0.453 , followed by *Serratia liquefaciens* with an inhibition zone of 21.00 ± 0.577 , *Enterobacter aerogenes* inhibition zone of 14.33 ± 0.333 , *Salmonella typhi*, 13.33 ± 0.333 and *Escherichia coli*, 13.33 ± 0.887 . Penicillin which was used as the positive control inhibited all the microorganisms while dimethyl sulfoxide (DMSO) which was used as the negative control did not show any zones of inhibition against the microorganisms used in the study. The results obtained in this study shows that the plant's potency in the treatment against all the bacterial organisms tested. However further research needs to be done to isolate the active compounds, determine their structure, their mode of action and their effect in the *in vivo* environment.

Key-Words: Plants, Antibacterial, Phytochemical, *Tetradenia riparia*, Ethnobotany

Introduction

Nature is a paradise of medicinal solutions to all ailments affecting human beings through medicinal plants. Medicinal plants have been used widely to treat against the currently widespread strains of drug resistant bacteria. Scientists all over the world are working hard to provide scientific justification on the traditional use of medicinal plants to treat almost all the ailments affecting human beings. Green medicine has attracted great interest due to the belief that it is safe, cheap and more dependable than aliphatic drugs, which have adverse side effects (Anthoney, 2014).

Microbial resistance to the currently used antibiotics has greatly increased in the last four decades despite efforts by the pharmaceutical industries to produce new antibiotics.

Several measures have been put in place in various countries all over the world to control the spreading of drug resistant microorganisms, however, the microorganisms have continued to develop new ways to mutate and acquire resistance to drugs (Nascimento, 2000). According to Montellia and Levy (1991), data collected on resistant microorganisms shows that the period ranging from 1980-1990 recorded the highest number of microbial drug resistance. The increase on the number of drug resistance microorganisms calls for quick action to control the situation.

Plants have been used since time immemorial to treat most of the diseases affecting human kind. According to Ngule (2013), about 80% of the individuals from developing countries are using traditionally known plants as medicine. The world health organization (WHO), recommends medicinal plants to be the best source of a variety of drugs (Santos, 1990). Botanical medicine is the oldest known type of medicine. The use of plants as source of medicine is as old as the origin of man himself. Medicinal plants have been used widely over all the cultures as sources of drugs for treatment

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of various ailments affecting human beings and animals (Sigh, 2010).

The medicinal values of plants are attributed to pharmacologically active compounds which have no direct impact on the plants main processes but research has proven them to have great medicinal value. These compounds that the plant uses to protect itself against predators are called secondary metabolites or phytochemicals. Over the recent decades, scientist have developed great interest on botanicals to isolate these compounds through various methods such as column chromatography and thin layer chromatography in order to purify them and study their structural elucidation. Studies have shown that plants have great potential in treatment against drug resistant microorganisms (Muroi, 1996).

Medicinal plants have been tested extensively and found to have great pharmacological uses such as anti-inflammatory activity, antibacterial activity, anti-diabetic activity, anti-fungal activity, anticancer activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity, anthelmintic activity, pain relief activity, central nervous system activity, sexual impotence and erectile dysfunction (Hosahally, 2012, Farook, 2011., Kisangau, 2007., Deepa, 2007., Joshi, 2012., Arivoli, 2012., Adu, 2011 and Kamatenesi-Mugisha, 2005).

The plant *Tetradenia riparia* is a highly branched soft 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 the 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.

Material and Methods

Sample Collection and Preparation

The leaves of the plant were randomly harvested in the month of October 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 biology department 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 were 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 (Ngule, 2013).

Qualitative phytochemical analysis

The extracts phytochemical analysis for identification of chemical constituents was done using standard procedures with minor adjustments (Trease, 1989; Harborne, 1973 and Sofowara, 1993).

Tannins: About 0.1 g of the extract 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 FeCl₃ was added to the filtrate and observations made. A brownish green color or a blue-black coloration indicated the presence of tannins.

Saponins: About 0.1g of the extract was mixed with 5 ml of water and vigorously shaken. The formation of stable foam indicated the presence of saponins.

Flavonoids: About 0.1g of the 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 the presence of flavonoids.

Terpenoids: About 0.1g of the extract was taken in to 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.

Glycosides: About 0.1g of the extract 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 color indicated the presence of steroidal ring (glycone portion of glycoside)

Alkaloids: About 0.1g of the 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 Mayers and Wagner's reagents were added by the side of the test tube. A resulting precipitate confirmed the presence of alkaloids.

Steroids: About 0.1g of the extract was put in a test tube and 10 ml of chloroform added and filtered. Then 2 ml of the filtrate was mixed with 2ml of a mixture of acetic acid and concentrated sulphuric acid. Bluish green ring indicated the presence of steroids.

Phenols: About 0.1g of the 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.

Bioassay study

Preparation of the Bacterial Suspension

The turbidity of every bacterial suspension was prepared to match to a 0.5 McFarland standard, a procedure similar to that used by Biruhalem (2011) and Donay et al., (2007). The McFarland standard was prepared by dissolving 0.5 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% sulphuric acid solution. Three – five 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 standard. The culture that exceeded the 0.5 McFarland standard were each adjusted with the aid of a UV spectrophotometer to 0.132A^0 at a wavelength of 600 nm in order to obtain an approximate cell density of 1×10^8 CFU/ml (Ngule, 2013).

Preparation of the Extract Concentrations and Antibiotic

Extracts stock 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 by the manufacturer's instructions. The bacterial suspension smeared on the media and 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 penicillin and DMSO control 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 (Ngule, 2013).

Results and Discussion

From the phytochemical analysis of the plant (Table 1) it was found to contain tannins, saponins, flavonoids, phenols, and alkaloids. The presence of these compounds in the plant could directly be associated with medicinal value of the plant. Previous studies have shown that the presence of tannins in a plant may enable it to have astringent property which makes it 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 rich in tannins to treat wounds and burns since they are able to cause blood clotting.

Tannins have been reported to inhibit HIV replication selectively besides the use of diuretics (Argal, 2006). Tannins have also shown antiparasitic effects (Akiyama, 2001). 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 (Chung, 1998). 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 (Sharma, 2006). Alkaloids on the other hand have been found to have analgesic, antispasmodic activity, antihypertensive effects, anti-malarial activity, anticancer and anti-inflammatory activities (Banzouzi, 2004, Boye, 1983 and Karou, 2006). The presence of these phytochemicals in the plant leaves and based on the data provided in the literature about them gives the plant its great pharmacological value. The infused plant's extract (Table 2) inhibited the growth of all the microorganisms tested. *Bacillus cereus* was the most inhibited microorganism, followed by *Serratia liquefaciens*, *Enterobacter aerogenes*, *Salmonella typhi* and *Escherichia coli*. Penicillin which was used as the positive control inhibited all the microorganisms while DMSO which was used as the negative control did not show any zones of inhibition against the microorganisms used in the study. One –way analysis of Variance showed that there was significant difference in the zones of inhibition between the microorganisms.

The Turkey's pair wise comparison (Table 3) showed that the zones of inhibition of *Serratia liquefaciens* were significantly higher as compared to those of *Salmonella typhi*, *Proteus vulgaris*, *Escherichia coli*, and *Enterobacter aerogenes* ($P < 0.05$), however, there was no significance difference in the zones of inhibition of *Serratia liquefaciens* and those of *Bacillus cereus* ($P > 0.05$).

The zones inhibition of *Salmonella typhi* were insignificant as compared to those of *Proteus vulgaris*, *Escherichia coli*, and *Enterobacter aerogenes* ($P > 0.05$), but significantly lower as compared to those

of *Bacillus cereus*. *Proteus vulgaris* zones of inhibition were found to be insignificant as compared to those of *Escherichia coli* and *Enterobacter aerogenes* ($P > 0.05$), but significantly higher compared to those of *Bacillus cereus* ($P < 0.001$). Zones of inhibition of *Escherichia coli* were significantly lower than those of *Bacillus cereus* ($P < 0.001$), but insignificant as compared to those of *Enterobacter aerogenes* ($P > 0.05$). *Bacillus cereus* zones of inhibition were significantly higher than those of all the other microorganisms ($P < 0.001$) except for *Serratia liquefaciens*. The zones of inhibition caused by penicillin were significantly higher as compared to those caused by the plant extract against the microorganisms.

The current study is in conformity with the previous studies in which the plant ethanolic-aqua extract was found to have great antibacterial and antifungal activity against *Staphylococcus aureus*, *Candida albicans*, *Mycobacterium smegmatis*, *Microsporum canis*, *Trichophyton mentagrophytes* and *Bacillus subtilis* (Vlietinck, 1995). However the current study is different in that the plant leaves samples were extracted using decoction method. The study therefore proves that, when the plant is infused as the trend is traditionally, it does not lose its activity and therefore a scientific prove that the active compounds in the plant remain undenatured even at high temperatures.

After *in vivo* experiments are done to prove its safety and mode of action the plant's extract can be used to treat infections caused by *Bacillus cereus* viz posttraumatic wounds, self-limited gastroenteritis, burns, surgical wounds infections, and ocular infections such as endophthalmitis, corneal abscess and panophthalmitis (Garcia-Arribas, 1988 and Sankararaman, 2013).

The plant extract can also be used to treat immunologically compromised patients including AIDS and malignant disease victims (Cotton, 1987 and Tazon, 1979). The plant's ability to inhibit the growth of *E. coli* is a scientific justification that the plant can be used to treat against enteric infections caused by the bacteria. The plants extract can also be used to treat against gastro-intestinal diseases, ear infections, urinary tract infections and wounds infections caused by *Proteus vulgaris* (Goodwin, 1971 and Neter, 1943).

The plants ability to inhibit the growth of *Serratia liquefaciens* shows that the plant can be important in the treatment against the infections caused by the bacteria which according to Okunda (1984), cause nosocomial urinary tract infections. The inhibition of the plant against this bacteria is therefore note worthy since the microorganisms have been found to have

resistance against most of the currently used antibiotics. *Enterobacter aerogenes* is major cause of a wide variety of nosocomial infections viz, pneumonia, urinary tract infections, meningitis, wound infections and intravascular and prosthetic devices infections (Santos, 1990, blot, 2003 and Donnenbery, 2005). *Salmonella* sp. makes one of the most common food poisoning forms all over the world (Baker, 2007). The data obtained shows that the plant leaves extract can be used to treat against food poisoning caused by *Salmonella typhi*. According to Su (2006), *Salmonella typhi* causes a wide spectrum of diseases which include typhoid fever and non-typhoid salmonellosis which are of great public concern. Despite diversified research on antibiotics against the bacteria, the bacteria still remains obstinate with the treatment against the microorganism taking several weeks. This creates the need to search for more effective antibiotics against the bacteria which are quick to suppress the bacterial effects and eradicate the microorganisms within a shorter period of time. The plant *Tetradenia riparia* has shown great potency in the action against the microorganisms, therefore, demonstrating high potency in the fight against *Salmonella typhi* and other *Salmonella* species.

Conclusion

The plants *Tetradenia riparia* has shown great potency as a source of new antibiotics in the treatment against all the infections caused by the microorganisms tested. The results in this research are a clear indication that the plant extracted in the traditional way can be used for the treatment of various diseases affecting human beings today. It is worthy to mention that the action of the plant against the microorganisms might be due to a synergistic effect of two or more compounds found in the plant. From the results obtained in this research we can recommend the plant for the treatment of all the ailments caused by the bacteria used. However, further research needs to be done in order to isolate the active compounds, determine their structure, their mode of action and their effect in the *in vivo* environment.

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Table 1: Phytochemical results

Phytochemical	Observation	Inference
Tannins	Brown green color	Present
Saponins	Stable form	Present
Terpenoids	No grey coloration	Absent
Flavonoids	Yellow color	Present
Phenols	Blue-black color	Present
Alkaloids	precipitate	Present

Table 2: Antimicrobial activity of methanolic-aqua extract against selected pathogenic microorganisms

Microorganisms	Extract mean \pm S.E (mm)	Penicillin mean \pm S.E (mm)	DMSO Mean \pm S.E (mm)
<i>Serratia liquefaciens</i>	21.00 \pm 0.577	47.67 \pm 0.577	0.00 \pm 0.000
<i>Salmonella typhi</i>	13.33 \pm 0.333	35.33 \pm 0.333	0.00 \pm 0.000
<i>Proteus vulgaris</i>	12.33 \pm 0.667	37.67 \pm 0.577	0.00 \pm 0.000
<i>Escherichia coli</i>	13.33 \pm 0.887	46.67 \pm 0.577	0.00 \pm 0.000
<i>Enterobacteraerogenes</i>	14.33 \pm 0.333	40.00 \pm 1.000	0.00 \pm 0.000
<i>Bacillus cereus</i>	22.67 \pm 0.453	45.67 \pm 0.577	0.00 \pm 0.000

Key: S.E. = Standard error

Table 3: Tukey's honestly significant difference among micro-organisms using 500mg/l of methanolic-aqua extract

Comparison	P-value	Significance
<i>S. liquefaciens</i> vs <i>S. typhi</i>	0.003	S
<i>S. liquefaciens</i> vs <i>P. vulgaris</i>	0.001	S
<i>S. liquefaciens</i> vs <i>E. coli</i>	0.003	S
<i>S. liquefaciens</i> vs <i>E. aerogenes</i>	0.015	S
<i>S. liquefaciens</i> vs <i>B. cereus</i>	0.511	NS
<i>S. liquefaciens</i> vs <i>S. liquefaciens control</i>	0.000	S
<i>S. typhi</i> vs <i>P. vulgaris</i>	1.000	NS
<i>S. typhi</i> vs <i>E. coli</i>	1.000	NS
<i>S. typhi</i> vs <i>E. aerogenes</i>	1.000	NS
<i>S. typhi</i> vs <i>B. cereus</i>	0.000	S
<i>S. typhi</i> vs <i>S. typhi control</i>	0.000	S
<i>P. vulgaris</i> vs <i>E. coli</i>	1.000	NS
<i>P. vulgaris</i> vs <i>E. aerogenes</i>	0.979	NS
<i>P. vulgaris</i> vs <i>B. cereus</i>	0.000	S
<i>P. vulgaris</i> vs <i>P. vulgaris control</i>	0.000	S
<i>E. coli</i> vs <i>E. aerogenes</i>	1.000	NS
<i>E. coli</i> vs <i>B. cereus</i>	0.000	S
<i>E. coli</i> vs <i>E. coli control</i>	0.000	S
<i>E. aerogenes</i> vs <i>B. cereus</i>	0.000	S
<i>E. aerogenes</i> vs <i>E. aerogenes control</i>	0.000	S
<i>B. cereus</i> vs <i>B. cereus control</i>	0.000	S

Key: S= significance, NS= no significance

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