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Chemical Constituents Screening and *in Vitro* Antibacterial Assessment of *Prunus Africana* Bark Hydromethanolic Extract

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Abstract
Worldwide many people use plants as a source of medicine in the treatment of various diseases. Plants have been used since the origin of man in the treatment of diseases even before the invention of allopathic drugs. Plants' originating drugs are believed to be safe, readily available and affordable especially to people living in developing countries. The current study was done to analyze the antibacterial activity and perform phytochemical screening of the plant *Prunus africana* bark. The plant samples were extracted using methanol and water in the ratio 9:1. The antibacterial activity was done using well diffusion method and the chemical constituents screening was done using standard procedures. From the results the plant was found to contain all the phytochemicals tested except steroids. The plant inhibited the growth of all the microorganisms tested. *Salmonella typhi* had the highest zone of inhibition of 17.33±0.882, then *Proteus vulgaris* with a zone of inhibition of 16.67±0.333, *Serratia marscecens* 16.67±0.333, *Escherichia coli* 12.33±0.333 and *Bacillus cereus* 11.67±0.333 zone of inhibition. Penicillin which was used as the positive control inhibited the growth of all the microorganisms tested while the negative control did not inhibit any of the microorganisms. The inhibition of the plant against all the microorganisms is a clear indication that if further study is done the plant can be used in treatment against infections caused by the microorganisms tested. Further research needs to be done to isolate the active compounds and analyze their structural composition, their mode of action and their effect in the *in vivo* environment.
Keywords: Phytochemical, Antibacterial, Plants, Prunus africana, Medicinal

INTRODUCTION

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. Studies by WHO have shown that a large number of individuals are using medicinal plants for treatment even today (WHO 1988). Globally it is estimated that 80% of the population in the developing countries and 40% of those from developed countries use natural medicines which mainly consists of plants for their basic health care (Prabakaran et al, 2011). This could be attributed to poverty in these countries which makes many people unable to access modern hospitals and purchase allopathic drugs for treatment. It is due to this reason that many people turn to plants which are believed to be non-toxic, readily available and affordable to the local population (Ngule et al, 2013).

The continued emergence of drug resistant microorganisms has always been a concern to scientists and pharmaceutical companies. Drug resistant microorganisms have also been an economic concern with impacts of them being felt by pharmaceutical companies, patients, medical practioners and the public (Gowan 2001) however plants have provided an alternative source of active compounds which can be used as drugs. The invention of active antibiotic compounds from plants has increased the interest on the study of plants as a source of new antibiotics (Cowan 1999 and Charindy et al, 1999).

The plant *Prunus Africana* is used traditionally in the treatment of chest pain, malaria and fever (Bii at al, 2010). Ethnobotanically the plant is also used by the Nandi community in Kenya in the treatment of diabetes, stomach sproblems and high blood pressure. The current study was done to determine the antibacterial activity of hydromethanolic extracts of *Prunus africana* bark and the screening of the phytochemicals present in the plant.

MATERIALS AND METHODOLOGY

Sample Collection and Preparation:

The plants bark was randomly harvested in the natural forest around University of Eastern Africa, Baraton. The plant samples were identified by a taxonomist in the University of Eastern Africa, Baraton. They were then thoroughly mixed and spread to dry at room temperature in the chemistry laboratory for about three weeks and ground into fine powder. The samples were stored in transparent polythene bags.

Extraction procedure:
Using electric analytical beam balance 100g of the powdered samples were placed in 500 ml conical flask, methanol and water were then added in the ratio of 9:1 respectively until the samples were completely submerged in the solvent. The mixture was then agitated for thorough mixing and kept for 24 hours with frequent shaking 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^oC. The residue was obtained and stored at 4^0C for the study.

Qualitative phytochemical analysis:
The extracts phytochemical analysis for identification of chemical constituents was done using standard procedures with minor adjustments (Trease 1989, Harbone 1973 and Sofowara 1993).
1. 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 $FeCl_3$ was added to the filtrate and observations made. A brownish green color or a blue-black coloration indicated the presence of tannins.
2. Saponins: About 0.1g of the extract was mixed with 5 ml of water and vigorously shaken. The formation of stable form indicated the presence of saponins.
3. Flavonoids: About 0.1g of the extract was added in to a test tube. To the test tube 5ml of dilute ammonia and 2ml of concentrated sulphuric acid was added and heated for about 2 minutes. The appearance of a yellow color indicated the presence of flavonoids.
4. Terpenoids: About 0.1g of the extract was taken in 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 tarpenoids.
5. 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 indicate the presence of steroidal ring (glycone portion of glycoside)
6. 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.
7. 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.
8**. 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
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 in to blood agar plates. The plates were then incubated at 37^0C for 24 hours. The blood agar media was prepared according to the manufacturers instructions. The plates were sterilized by the use of an autoclave at 121 ^0C. Approximately 20ml of the prepared media was poured in to the sterilized plates and then the surface of the media was flamed using a Bunsen burner flame to remove air bubbles and sterilize the media surface. 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 (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 were taken from a blood agar plate (Himedia) culture using a sterile swab in to Mueller Hinton broth (Himedia). The broth culture was incubated at 37^0C 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 $0.132A^0$ 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:
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 instructions. The media and the plates were sterilized in an autoclave at 121^0C for 15 minutes. The plates were flamed on the surface using a non luminous flame to remove air bubble and also ensure sterility of the surface. 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 in to a germicidal wood. The germicidal lamp was put on for 30minutes to sterilize the surface of the plates and other equipments. The bacterial suspension was smeared on the media and five wells with a diameter of 6cm each were drilled in each agar plate using a cork borer. Three of the wells were filled with 0.1ml of the 500mg/ml of the extract. The other wells were filled with 0.1ml 0f 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 37^0C for between 24 to 48 hours and the zones of inhibition measured in millimeters with the aid of a ruler.

RESULTS AND DISCUSSION

Table 1: Phytochemical results

Phytochemical	Inference
Tannins	++
Saponins	++
Flavonoids	++
Terpenoids	++
Glycosides	++
Alkaloids	++
Steroids	--
Phenols	++

Bioassay results:

Table 2: Antibacterial activity of *Prunus Africana* against selected pathogenic microorganisms

Microorganisms	Extract mean ± S.E (mm)	Penicillin mean ± S.E (mm)	DMSO mean ± S.E (mm)
Bacillus cereus	11.67±0.333	25.67±0.333	0.00±0.000
Proteus vulgaris	16.67±0.333	21.33±0.333	0.00±0.000
Salmonella typhi	17.33±0.882	27.67±1.202	0.00±0.000
Serratia marcescens	16.67±0.333	32.00±0.577	0.00±0.000
Escherichia coli	12.33±0.333	20.33±0.333	0.00±0.000

Key: S.E = Standard Error

Table 3: Turkey's honestly significant difference among microorganisms using 500mg/ml of *Prunus africana* extract

Comparison	P-value	Significance
Bacillus cereus vs *P. vulgaris*	0.000	S
Bacillus cereus vs *S. typhi*	0.000	S
Bacillus cereus vs *S. marcescens*	0.000	S
Bacillus cereus vs *E. coli*	0.997	NS
B. cereus vs *B. cereus* control	0.000	S
Proteus vulgaris vs *S. typhi*	0.997	NS
Proteus vulgaris vs *S. marcescens*	1.000	NS
Proteus vulgaris vs *E. coli*	0.000	S
P. vulgaris vs *P. vulgaris* control	0.000	S
Salmonella typhi vs *S. marscecens*	0.997	NS
Salmonella typhi vs *E. coli*	0.000	S
Salmonella typhi vs *S. typhi* control	0.000	S
Serratia marscecens vs *E. coli*	0.001	S
Serratia marscecens vs *S. marscecens* control	0.000	S
Escherichia coli vs *E. coli* control	0.000	S

Key: S= Significant, N.S = Not Significant

From the results (Table 1) the plant was found to contain all the phytochemicals tested except steroids. The plant inhibited the growth of all the microorganisms tested (Table 2). There was significance difference in

the zones of inhibition amongst the microorganisms. The tukey's pair wise comparison (Table 3) showed that the zones of inhibition of *Bacillus cereus* were significantly lower as compared to those of *Salmonella typhi, Proteus vulgaris* and *Serratia marcescens* ($P<0.001$), however there was no significant difference between the zones of inhibition of *Bacillus cereus* and *Escherichia coli* ($p>0.05$). The inhibition zones of *Escherichia coli* were significantly lower as compared to that of *Salmonella typhi* ($p<0.001$) but not significantly different to that of *Serratia marscecens* ($p>0.05$). *Proteus vulgaris* was significantly inhibited as compared to *Escherichia coli* while *Salmonella typhi* zones of inhibition were significantly higher than those of *Proteus vulgaris*. The zones of inhibition caused by the positive control were significantly higher as compared to those caused by the plant extract.

The presence of tannins in this plant may enable it to have astringent property which makes it useful in preventing diarrhea and controlling hemorrhage due to the ability of tannins to precipitate proteins, mucus and constrict blood vessels (Kokwaro 2009). This is the reason why traditional healers used plants rich in tannins to treat wounds and burns since they are able to cause blood clotting. Some tannins have been reported to inhibit HIV replication selectively besides the use of diuretics (Argal 2006).This shows how traditionally used medicinal plants rich in tannins can be used to control this dangerous disease. Tannins have also shown antiparasitic effects (Akiyama et al, 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 growths of many fungi, yeast, bacteria and viruses have been proven to be inhibited by tannins (Chung et al, 1998).

Terpenoids have shown great potency in the treatment against microorganisms. According to Andrew (1980), terpenoids have been studied in the *in vivo* environment and found to inhibit the growth of various bacteria. They have also shown potency in the treatment against *Plasmodium falciparum* which is the causative agent of malaria. Terpenoids have been found to inhibit the growth of fungi *Candida albicans* (Murata, et al., 2008).

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 et al, 2004, Doye 1983 and Karou et al, 2006).

The results obtained in this research are in conformity with those obtained by Bii (2010), in which the plants methanolic extract was found to inhibit the growth of most of the microorganism, however, different since in the current study the plant was found to inhibit the growth of all the microorganisms tested. The current study is also in conformity with previous studies in which the plant was found to contain flavonoids (Dixon et al, 1983). However apart from flavonoids the plant was also found to have diverse spectrum of phytochemicals which previous studies have shown them to have great medicinal value (Ghasemzadeh 2011). In this study we also demonstrate the use of a solvent system, methanol-water to extract the compounds from the plant. The presence of these important pharmacologically important compounds and the bacterial inhibition of the plants may be a scientific justification of the plants' ethnobotanical use in the treatment of various diseases.

CONCLUSION

From this study it may be concluded that the antibacterial activity of the plant is closely attributed to the presence of the important pharmacological compounds found in the plant. The antibacterial activity of the plant could be due to synergistic effect of two or more compounds in the plant. The data obtained in this research may be a scientific justification the plant's bark use in the treatment of various diseases affecting human beings. In future, if more research could be conducted, the plant extract could be useful in the treatment of 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 could also be also be used to treat immunologically compromised patients including AIDS and malignant disease victims (Cotton 1987 and Tuazon 1979). The plant's ability to inhibit the growth of *E. coli* is a scientific justification that the plant could be used to treat against enteric infections caused by the bacteria. The plants extract could 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).

Salmonella sp. makes one of the most common food poisoning forms all over the world (Bakers 2007). The data obtained shows that the plant's bark extract could be used to treat against food poisoning caused by *Salmonella typhi*. The plant could also be used to treat against typhoid, paratyphoid fever, traveler's diarrhea, gastroenteritis in adults and gastroenteritis in children (Hallstrom 2011). The plant's ability to inhibit the growth of *Serratia marcescens* shows how the plant could be important to treat against the bacteria which according to

Okunda (1984) cause nosocomial urinary tract infections. The inhibition of the plant against these bacteria is therefore note worthy since the microorganisms have been found to have resistance against most of the currently used antibiotics. *Prunus africana* can *be* a good source of active compounds for a variety of diseases affecting human beings in the world today. However, further research needs to be done to isolate the active compounds and analyze their structural composition, their mode of action and their effect in the *in vivo* environment.

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