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Profiling of antimicrobial properties of *Rubus keniensis* Standl. Crude methanol root bark extracts against selected human pathogenic bacteria

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ABSTRACT

Rubus keniensis is a rare plant species and is used by the Ogiek community who dwell in the Mau Forest Complex in Kenya. Fruits of the plant are eaten by human when ripe while the roots are chewed against stomach ailments. Dry root bark methanol extracts was initially screened against various strains *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Staphylococcus aureus* and the extract showed reasonable activities against the test organisms with some giving exhibiting zones of that are good enough to advocate for further researches on plant parts. Further studies on the MIC/MBC are promising to the extent of recommending the plant for further consideration as an alternative source of treatment of wounds in Diabetics and other ailments. The involvement of pharmaceutical industry in the study has been suggested for purposes of new drugs development.

Keywords: *Rubus keniensis,* Methanol root extract, Antimicrobial activity.

1. Introduction

Use of plants, both higher and lower, for therapeutic purposes against ailments is as old as human kind [1]. Early humans must have learnt and made their choices through careful observation and error. Penicillin which was an accidental discovery in the last half of the 20th century revolutionized the regimes towards the tackling of bacterial infections with lots of ramifying results most of which were positive [2]. This meant that the negative results could possibly present challenges to humankind. Such solution could immediately be found in higher plants nearly all of which produce metabolites which posses' different levels of biological activities against pathogenic microorganisms and parasitic flora and fauna.

In the past three decades, traditional medicine has been accepted as an alternative form of healthcare [3]. The development of microbial resistance to the available antibiotics has led several people to investigate the antimicrobial activities of higher medicinal plants [4, 5]. There is also increased use of plants extracts as cosmetics and pharmaceutical products, something which has generated a lot of interest in identification of active compounds [5]. In doing so, it is imperative to carry out systematic studies on plants. This therefore means that, plants being sessile are capable of synthesizing a vast array of secondary metabolites as defence mechanisms for protecting themselves against pathogenic infections [6]. Despite the fact that there are several natural and synthetic products available to ameliorate fungal and bacterial maladies, it is recognized that both resistant fungi and bacteria are on the increase [7]. Consequently, there is a need to detect new sources of antifungal compounds with potential application in medicine and also as additives in food and food preservatives.

There have been reports of systematic biological investigations of the *in vitro* antimicrobial activities of extracts of some plant extracts from all over the world that are used in traditional medicine[8]. The exploration of new antimicrobial compounds has led to collaborative interests in natural products chemistry, agriculture and medicine in fundamental research. Isolation and identification of compounds which have antibiotic effects without being cytotoxic to cells of higher plants and animals could be beneficial.

One of the serious indicators of activity is to establish the minimum concentration of the bioactive crude extracts of the plant that could inhibit microbial growth and the concentration that could kill the microbes completely referred to as minimal bactericidal concentration (MBC).

After carrying out studies on the effects of various concentrations and zones of inhibitions it was necessary to assay the crude extracts for purposes of obtaining their bacteriostatic or bactericidal effects or both Virtually, all the antibiotics that are currently in use in allopathic medicine are subject to challenges in that they are subject to effective resistances by pathogenic microorganisms to the extent that they are rendered ineffective within a short duration of their introduction into the regimen. However, one way of verifying their efficacy is by carrying out studies to determine their MIC, MBC and MFC's.

Antibiotics are complex compounds, natural or synthetic products, that inhibit the growth of other organisms particularly bacteria and fungi [9]. To date the term antibiotics often includes synthetically produced antibacterial and anti-fungal substances. The first major classes of antibiotics were introduced in the 1940s and 1950s. In bacterial infections they were hailed as miracle drugs that eliminated bacteria without doing much harm to cells of the treated individuals [10]. Soon there was a glut of varieties of the drugs being introduced into the market which became saturated. Many pharmaceutical firms have since then shied away from the development of new antibiotics have instead focused on combating chronic diseases [11]. The problem of antibiotic resistance is aggravated by two factors. One is overuse of antibiotics both in humans and animals and second non-compliance of patients to the courses of treatment. Both the long-term exposure to low doses and the failure to finish a prescription encourage more resistant bacterial strains to thrive [12]. The universal problem of pathogen resistances to antibiotics is compounded by the emergence of opportunistic infections both fungal and bacterial infections [13]. The infectious diseases such as fungal dermatitis have become more frequent due to immunocompromised and immunosuppressed conditions arising from HIV conditions. The management of such cases is more often resistant to known antimycotic drug than before [14].

Natural and synthetic products available to ameliorate fungal and bacterial maladies, it is recognized that both resistant fungi and bacteria are on the increase [7]. Consequently, there is a need to detect new sources of antifungal compounds with potential application in medicine and also as additives in food and food preservatives. One of the serious indicators of activity is to establish the minimum concentration of the bioactive crude extracts of the plant that could inhibit microbial growth and the concentration that could kill the microbes completely referred to as minimal bactericidal concentration (MBC).

In the current study the antimicrobial activities of crude methanol root extracts by disc diffusion method and agar serial dilution methods have been carried out by *in vitro* assays. The phytochemical evaluation has been done through standard wet bench methods.

Rubus keniensis Standl (Rosaceae) is prickly scrambling, plants whose fruits are gathered and decanted as wild fruit [15]. The roots are washed and boiled and the resultant decoction taken as remedy against various stomach ailments by the Ogiek people of Kenya. Species of this genus are used in East Africa for various ailments [16].

2. Material and Methods

2.1 Plants Material

Roots parts were collected from Mau summit forest of the Western Highlands of the Rift Valley in Kenya. Voucher specimen was prepared and deposited at the National Museums of Kenya, Nairobi and held for future reference number AO49/NMK/04/07/2008. Chopped and air dried bark peeled off and dried in lab at room temperature till full dryness then ground into powder to pass through a mesh of 0.5 mm diameter then hermetically sealed and refrigerated for future use. Preparation of the Methanolic Extract 50 gm of the air dried powder sample was extracted with 400 ml of 98.9% methanol for 48 hours and the same process repeated once more. The supernatant extract was decanted filtered evaporated in a water bath rotor evaporator to yield a thick semisolid dark substance amounting to 5.0 gm. The extract was kept in a vial and refrigerated at 4 ^{0}C for future use.

2.2 Microbial strains and Pathogens

The following microbial strains and pathogens obtained from the National Public Health Laboratory Bank in Nairobi were used in the *in vitro* studies on the methanolic extract of the plant: *Staphylococcus aureus* (Haemolytic), *S. aureus* (Oxford strain), *S. aureus* (Candida strains), *S. aureus* (Pigmented), *S. aureus* (Pigment with staphylokinase), *S aureus* (ATCC 20591), *S. aureus* (Hospital Strain), *Pseudomonas aeruginosa* (MDR), *P. aeruginosa* (KEMRI), *P. enteritidis* and *P. aeruginosa* (ATCC 29212), *Enterococcus faecalis* (Hospital Strain) *E.* spp, *Klebsiella pneumoniae* (Hospital strain), *K. pneumonia* (Belgium Strain), *K. oxytoca*, and *K. pneumoniae*, (WHO Collection), *Candida albicans,* locally isolated strains at the National Public Health Laboratory, *Escherichia coli* [Std. 259222, *E. coli* 35218 and *E. coli* (0,25/B15)].

Known weights of individual test plant extracts which had previously shown bioactivity during the bioassay tests were weighed out into sterile test tubes and then reconstituted with four droplets of Dimethyl Sulfoxide just to dissolve it and then topped with sterile distilled water to the required volume of 10 ml. Further, serial dilutions were made to give a total multiple of four concentrations 10, 20, 30 and 40 µml, respectively. Each dilution was impregnated onto paper discs each measuring 6 mm in diameter. The discs were desiccated before being placed onto pathogen-seeded Mueller – Hinton agar. Similarly, various concentrations of the essential oils were also prepared to give 10, 20, 30 and 40 µml, respectively. The discs were then dipped into the oil and then placed onto the dishes using the same procedure as in the case of the reconstituted methanol extracts. Each of the agar materials was autoclaved for 15 minutes at 121 ºC and 1.5 kg f/cm³ before being poured onto sterile plates; each plate holding 20 ml of it. Each plate was then seeded with the organism separately using nutrient broth no. 2 as diluting agent of the organisms previously prepared by scooping the organism with a sterile wire loop then dipping into the broth.

The same process was repeated with every selected strain or species. The discs previously treated with the drugs were carefully placed on the clearly marked portions of the plates and then incubated for 24 hours at relative humidity of 98% and temperature of 37 ^{0}C. The records of zones of inhibitions of the diameter with no growth of each species of micro-organism or strains were taken individually based on the concentrations. Gentamycin was used as control and for comparative purpose. The active extracts from the antimicrobial screening were tested for minimum inhibitory

concentrations (MICs) and minimum bactericidal/fungicidal concentrations (MBsC/MFCs). The MICs were determined using doubling serial dilution method in a peptone water solution for bacteria and PDA broth for fungi for the active extracts to give a final extract concentration of between 1.95 and 800 µg/ml. Each tube was then inoculated with 0.1 ml of standardized bacterial suspension (1×10^8 CFU/ml) and fungal suspension (1×10^8 spores/ml). The cultures were incubated at 37 ºC for 24 hr for bacteria, 48 hr for yeast and at 25 ºC for 5 days for moulds. The first tube showing no growth was taken as the MIC. MBC and MFC were determined by sub-culturing 0.1 ml of all the tubes showing no growth on Nutrient Agar (NA) for bacteria and PDA plates for yeast and moulds. After 24 h incubation at 37 ºC for bacteria, the first plate showing no growth was considered as the MBC, while after 48 hr at 37 ºC for *Candida* and 5 days at 25 ºC for fungi, respectively, the first plate showing no growth was taken as the MFC (Michael *et al.*, 2003; Barry, 1986).

2.3 Wet Bench Analyses of the Extracts

Using standard wet Bench methods the extract were investigated to establish various classes' chemical compounds in the extract as given in Ikan [18].

3. Results

The following compounds found to be present in the wet bench analysis: flavonoids, anthroquinones, saponins, steroidals, reducing sugars and polyoses. In the *in vitro* studies, there were zones of inhibitions as shown the subsequent Tables below using the following pathogenic bacteria and some of their stains: *S. aureus, K. pneumoniae, P. aeruginosa,* and *E. faecalis.*

Table 1: Zones of inhibition (mm) by *R. keniensis r*oot bark methanol extracts

Pathogens	Conc in mg/ml			
	20	40	80	160
P. aeruginosa (Clinical isolates)	10	16	20	22
P. aeruginosa ATCC 27853	10	15	20	22
Control	6	6	6	6

The strains used included: *E. faecalis* (hospital strain) and *E. faecalis* (ATCC 29212) (Table 2)

Table 2: Zones of inhibition (mm) by *R keniensis* root bark against *E. faecalis* and its strains

Conc. in mg/ml	Zones of inhibition (mm)
20	6
40	6
80	7
160	10
Control	6

Other strains used were: (clinical strain) *K. pneumoniae* (Belgium strain) *K. pneumoniae* (WHO Std and MDRS): There was similar activity in all the strains (Table 3).

Table 3: Zones of inhibition (mm) by *R. keniensis* root bark against *K. pneumoniae oxytoca*

Conc. in mg/ml	Zones of inhibition (mm)
20	7
40	10
80	12
160	14
Control	6

S. aureus (B. haemolytic), S. aureus (Oxford strains) *S. aureus* (Danida strain) *S. aureus* (Pigmented), *S. aureus* (Pigmented with staphylokinase) *S. aureus* (ATCC 20591) and *S. aureus* (Clinical isolates): There was similar activity in all of them (Table 4).

Table 4: Zones of inhibition (in mm) by *R. keniensis* root bark methanol extracts against *S. aureus*

Conc. in mg/ml	Zones of inhibition (mm)
20	7
40	12
80	13
160	15
Control	6

There was similar activity in all the strains and (ORSA). The positive control had no growth (Table 5).

Table 5: Zones of inhibition (mm) by *R. keniensis* on *S. aureus* oxacillin resistant (ORSA)

Conc. in mg/ml	Zones of inhibition (mm)
20	14
50	14
100	16
200	20
Control	6

There was similar activity in all the strains and (ORSA). The positive control had no growth (Table 6).

Table 6: Zones of inhibition (mm) by *R. keniensis* on *S. aureus* oxacillin resistant (ORSA)

Conc. in mg/ml	Zones of inhibition (mm)
20	14
50	14
100	16
200	20
Control	6

All the strains of *P. aeruginosa* showed similar response to the drug (Tabl 7).

Table 7: Zones of inhibition (in mm) by methanol extracts of root bark of *C. abyssinica* against *Pseudomonas* spp.

Pathogens	Conc in mg/ml			
	20	40	80	160
P. aeruginosa (Clinical isolates)	7	9	10	11
P. aeruginosa ATCC 27853	7	9	10	11
Control	6	6	6	6

Table 8: MIC/MBC of various plants extracts in µg/ml

pathogen	Minimal inhibitory concentration (MIC)			Minimal bactericidal concentration (MBC)		
S. aureus and ORSA	125	250	500	300	600	1200
E. faecalis and its strains	310	625	1250	315	625	1250
K. pneumoniae	250	500	1000	315	625	1250
P. aeruginosa	250	500	1000	250	500	5000

4. Discussions

Root bark methanol extracts of *Rubus keniensis* were active with varied efficacies against several microorganisms. There was significant effect $P<0.05$ might help give actual efficacies with change of the extracts concentrations on inhibition of the growths

of all the species and strains of *Pseudomonas*. Higher concentrations of the extract was more efficient as compared to the lower ones with pooled StDev=0.258. The results were the same as in the cases of *E. faecalis*, *K. pneumoniae* and *S. aureus*. This is demonstrated in the Tables 1 to 8.

This was verifiable by the fact that the methanol extracts was effective, with an MBC, against *E. faecalis* at 1250 µg/ml. There were significant P≥1 antibacterial activities of the extracts, except for *Salmonella typhi, E. coli* and their stains that are resistant. *Pseudomonas* and its strains had MIC of 1000 µg/ml and MBC of 2000 µg/ml as the highest and 500 µg/ml, MIC and MBC in the case of *S. aureus* and its strains. From this genus, there are *R. rigidus* which is used as remedy against coughs and colds in children, *R. steudneri* for digestion and *Rubus sp* is used against abdominal complaints [16]. However, there is not any report in literature, of the plant's use as medicine anywhere in the World. The screening of *Rubus keniensis* of the family Rosaceae revealed the presence of flavonoids, anthroquinones and saponins but only a very small presence of steroidols and reducing sugars. The extracts showed good activities against both Gram –ve and +ve bacteria. This is possibly due to the presence coumarins and alkaloids are reported to be present in this family [19]. The family is known for its several genera which are of medicinal importance as in the case of hyperplasia conditions in where *Prunus africana* (Hook.f.) Kalkm.

5. Conclusion and Recommendation

The plant has reasonable antimicrobial activities against some Gram Negative bacteria and this explains its use as a remedy for stomach ailments by the community. This is also good activity against *Pseudomonas* spp. currently there are high incidences of Diabetes Mellitus amongst middle-aged people all over World. In such cases secondary infections by the pathogene are usually wounds which are resistant to the antibiotics in the market. Unfortunately the challenges facing the medical Personnel are the rates at which the pathogens develop resistance to the current antibiotics in market. Nosocomial infections in such age related cases are commonplace. It is recommended that further and intensive research be done with the aim of discovering and developing new pharmaceuticals that could be in the management of such maladies. After all the cost of the allopathic drugs is beyond the affordability of majority of the victims, a large number of whom live in the Developing World without medical Insurance covers.

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