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Potential *In vitro* Anti-Bacterial Action of Selected Medicinal Plants Against *Escherichia coli* and Three *Salmonella* Species

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Abstract: A case control experimental study design was conducted to determine *in vitro* antibacterial activity of selected plants in Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia. Methanol extract of traditionally used plants collected from different parts of the country to treat diarrhea in animals and human were considered. *Calpurnia aurea*, *Salivia schimperi, Verbasicum sinaticum, Hypericum revolutum and Petrolobium stelatum* at a concentration of 250mg/ml were evaluated for their antibacterial activity using agar well diffusion test method. The results obtained show that methanol extract of *Calpurnia aurea and Salivia schimperi* are the most active plants against all the bacteria species tested. The highest zone of inhibition (15.6 mm) was recorded for *Petrolobium stelatum* against *E. coli*, *Verbasicum sinaticum* and *Hypericum revolutum* against *S. typhmurium* and *S.paratyphi* while, *Petrolobium stelatum* did not induce inhibition zones against *S. typhmurium*. Generally, most of the extracts have shown considerable activities against *E. coli and Salmonella* species but further study is required to dissect the active ingredients responsible for this effect at *in vitro* and *in vivo* levels.

Key words: Antibacterial Activity · *E. coli* · Medicinal Plant · *Salmonella*

INTRODUCTION

Various important phytoconstituents like alkaloids, phenolic compounds, triterpenoids, coumarins, tannins, steroids etc. have been isolated. But only few pharmacological activities like antimicrobial, antiviral, antitumour and antifungal activities have been scientifically reported [1]. In deed the use of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tee tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents [2].

Despite the impressive scientific progress in vaccination and chemotherapy, infectious diseases remain a serious health issue [3]. Due to the rapid spread of multi-drug resistant strains of bacteria, the occurrence of bacterial infections that cannot be treated with conventional antimicrobial agents is increasing [4]. Moreover the new generation antibiotics are less available and are expensive for resource poor communities. Because of the aforementioned reasons, lots of efforts have been made to discover new antimicrobial agents from various sources such as micro-organisms, animals and plants [5].

Antimicrobial compounds of medicinal plants differ from antibiotics as they have fewer side effects, better

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patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature [6, 7]. Essential oils of these plants rather than their extracts have used in the treatment of infectious pathologies [2, 8, 9].

In Ethiopia, traditional remedies represent not only part of the struggle of the people to fulfill their essential drug needs but also they are integral components of the cultural beliefs and attitudes [11]. The Ethiopian flora is estimated to contain between 6500 and 7000 species of higher plants of which about 12% are endemic. More than 95% of traditional preparations in the country are of plant origin [12-14]. Some of the common uses of the medicinal plants sold in markets include fumigation, vermifuge, pain relief and treating skin infections. Antimicrobial and wound healing plants are among some of the major medicinal plants that are commonly available in the Ethiopian markets [15].

A total of 315 extracts/ fractions from 63 traditionally used Ethopian plants were screened for their antimicrobial activity against known strains of *Staphylococcus aureus, Salmonella gallinarum, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Candida albicans*. It was found that 63 plants showed activity against one or more microorganisms. The aqueous extracts from six plants (*Cucummin prophetarum, Calpurnea aurea, Rosa aeruginosa, Clematis sinensis, Calotropis procera, Rumex steudelly*) were found to be active against all the test microorganisms [16].

Even though, in Ethiopia, ethnoveterinary surveys conducted in different parts of the country showed the use of different medicinal plants for treatment of various infectious diseases of livestock by traditional healers [17-21], the efficacy of most of these plants has not been investigated scientifically. Therefore, the objective of this study is to evaluate the antibacterial activity of leaves of *Calpurnia aurea*, *Salivia schimperi, Verbasicum sinaticum, Hypericum revolutum and Petrolobium stelatum* against four bacterial strains at *in vitro*.

MATERIALS AND METHODS

Study Area of Medicinal Plant Collection: The plant material was collected and identified in Addis Ababa University, Faculty of Natural and Computation Sciences, Biology Department. The leaves of *Verbasicum sinaticum* were collected from Northern Shoa, Lallo Mama Woreda. While the leaves of *Calpurnia aurea* were collected from around Debre Zeit, East Shewa. *Hypericum revolutum* and *Petrolobium stelatum* were collected from (Wello) Lalibela-Asheten RI and Rama Ras Alula respectively. The leaves of *Salivia schimperi* were collected from Gondar, near Tara Gedam Monastery. Table 1 shows the herb type, local name, part of the plant used and medicinal uses traditionally as main concerns. The *in vitro* antimicrobial activity trial on selected plants was conducted at the laboratory of the Aklilu Lemma-Institute of Pathobiology (Addis Ababa University).

Study Methodology: The study design was a case-control *in vitro* experimental study with five plant extracts, one positive control (gentamicin) and one negative control (distilled water). The experiments were repeated three times and the results were expressed as average value of zone of bacterial growth inhibition by each plant extract.

Preparation of Plant Materials

Plant Material Extraction: The test plant selected based on traditional medicinal uses. Dried and powdered leaves specimens of the test plants were subject to alcoholic extraction by 80% methanol. Briefly, the garbled plants were dried in air at room temperature, powdered using pestle and mortar and kept in amber colored bottle for further use. Alcoholic (methanol) extraction was conducted by percolating 200-300 g of the powdered plant materials using 80% methanol for 24 hours, which is then filtered through Whatman filter paper Number1 (Basic Model, Buchi, UK). The solvent was evaporated using a Rota vapor and the extract was kept in a stoppered sample vial at 4°C until used.

Bacterial Preparation for the Experiment

Culturing Bacterial Colonies: Bacterial species used in this study were obtained from Bacteriology Laboratory of Aklilu Lemma-Institute of Pathobiology. The specimens were originally isolated from animals and humans during disease investigation and kept lyophilized. These are *Salmonella typhimurium*, *Salmonella typhi, Salmonella paratyphi and Escherichia coli.* Each bacterial isolate was homogenized with 3 mL of nutrient broth and a loopful of broth containing the bacteria was inoculated on blood agar. It was then incubated at 37°C for 24 h. Purity and viability of the organisms was checked by plating, Gram staining and conducting primary and secondary biochemical tests. The test bacteria were suspended separately into sterile universal bottles containing nutrient broth and incubated at 37°C for 18 hours. Normal saline was added gradually to adjust the culture turbidity to that of McFarland turbidity standard, which corresponds to approximately ($1.5X10^8$ CFU/mL).

Table 1: Considered medicinal plant species, local name, part extracted and traditional medicinal use

Herb used	Local name	Part used	Traditional medicinal use
Calpurnia aurea	Digita	Leaf	Against amoebic dysentery and diarrhea in animals, wound
Salvia schimperi	Dibirik	Leaf	Against Syphilis and venereal disease
Verbasicum sinaticum	Ketetina	Leaf	Against anthrax, superficial fungal infections and wounds
Hypericum revolutum	Avetia	Leaf	Anti-inflammatory and healing agent
Petrolobium stelatum	Kentefa	Leaf	Against tuberculosis and related respiratory diseases

Table 2: Effect of methanol extract of five medicinal plants against *E. coli* and *Salmonella* species Gm=Gentamicin DW=Distelled Water

	Mean zone of inhibition (mm) to each plant extract						
Bacteria	*Calpurnia aurea*	*Salivia schimperi*	*Verbasicum sinaticum*	*Hypericum revolutum*	*Petrolobium Stelatum*	Gm	Dw
E. coli	13.3	13.6	6.3	11.3	15.6	14	0
S. typhi	10.33	9.6	5.6	5.6	9.3	18	0
S. typhmurium	8.6	6.0	0	0	0	15	0
S. paratyphi	10.0	11.3	0	0	7.3	13	0

On the other hand, the antibacterial activity of most of the herbal extracts against the three species of *Salmonella* was less than that of the positive control (gentamicin 10µg) except for *Calpurnia aurea* against (*S. typhi* and *S. paratyphi*) and *Salivia schimperi* against *S. paratyphi*.

Determination of Antibacterial Activities: The antibacterial activity test was undertaken using agar well diffusion method as previously described [1]. Briefly, 1 mL of the test culture ($1.5X10^8$ CFU/mL) was spread into a sterile plate with 20 ml Muller Hinton molten agar. Six wells of approximately 8 mm in diameter and 7 mm depth were made on the surface of the Muller Hinton molten agar plates using a sterile borer. Stock solution of each plant extract was prepared at concentration of 250 mg/mL in distilled water. Each five wells of the six wells were filled with 0.35 mL of the plant extracts. The sixth well was filled with 0.35 mL of distilled water served as a negative control and a disc of gentamicin (10µg) served as a positive control. The plates were then incubated at 37°C for 24hours and zone of inhibition was measured in millimeter with a ruler. The experiments were repeated three times and the result were expressed as average value of zone of inhibition of each plant.

RESULTS

The findings of this *in vitro* antimicrobial activity show that *Calpurnia aurea and Salivia schimperi* were effective on all the bacteria species tested (Table 2). The antibacterial activities of most of the herbal drugs (*Calpurnia aurea*, *Salivia schimperi, Verbasicum sinaticum, Hypericum revolutum and Petrolobium stelatum* at a concentration of 250 mg/ml against *E. coli* were found to be almost comparable to that of the positive control (Gentamicin 10µg).

DISSCUSION

The findings of this study demonstrated the methanol extract of *Petrolobium stelatum* has antimicrobial activity comparable to gentamicin against *E. coli* isolates. Moreover, except *Verbasicum sinaticum* all the other plants also demonstrated promising activities against the bacteria. Gram-negative bacteria including *E. coli* are frequently reported to have developed multidrug resistance to many of the antibiotics currently available in the market [22, 23]. Therefore, it is interesting that *E. coli* are susceptible to the tested plant extracts on the present study.

A previous study has also reported that extracts of *Calpurnia aurea* showed stronger activity against *E. coli* at a concentration of (100 mg/mL) [24]. However, further investigation is needed to ascertain if the most pathogenic strains of this organism are also sensitive to the extract. Of course, *in vitro* finding is not always dependable, plants which are effective *in vitro* might not work when used *in vivo* and some plants which showed little or no effect *in vitro* study might also be effective when evaluated in animals due to various factors that affect or favor the release of active ingredients in human and animal bodies and immunomodulating actions [4]. Since methanol extracts of selected plants leaves have age and concentration dependent antibacterial activity against some of the tested organisms [25], it should also be advisable to consider the growth stage of plant parts used.

The preliminary results in the present study, therefore, not only confirm the justifiable use of some of the plants against these microorganisms in the traditional health care system but also reflects the hope for development of effective chemotherapeutic agents in the future from same or similar plants. That could be a good solution since; there are very few, if any, antibiotics to which these microorganisms have not developed resistance [26].

In this study *Calpurnia aurea and Salivia schimperi* are effective to all the bacteria species considered, the highest zone of inhibition was recorded for *Petrolobium stelatum* against *E. coli. Verbasicum sinaticum* and *Hypericum revolutum* did not induce inhibition zone against *S. typhmurium* and *S. paratyphi* while *Petrolobium stelatum* extract failed to inhibit the growth of *S. typhmurium*. Similar to this study, previous report by Geyid [27] also showed that *Verbasicum sinaticum* methanol extract has no activity against the tested microbes.

CONCLUSIONS

Under the existing modern public and animal health coverage and underlying socio-economic condition, there exists no justification to ignore the role of ethno medicine and choose a handicapped approach of promoting modern veterinary medicine alone. Integration between the traditional healers and standard professional could make more effective delivery of health care service in human and animals. Methanol extract of *Calpurnia aurea and Salivia schimperi* are the most active plants against all the bacteria species tested in this study. The highest zone of inhibition was recorded for *Petrolobium stelatum* against *E. coli*. *Verbasicum sinaticum* and *Hypericum revolutum* against *S. typhmurium* and *S. paratyphi* were found to have large inhibition zones respectively. Generally, most of the extracts have shown considerable activities against *E. coli and Salmonella* species but further study is required to dissect the active ingredients responsible for this effect at *in vitro* and *in vivo* levels.

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