Research Article

Antimicrobial Activity of *Vernonia schimperi* and *Vernonia amygdalina* against Selected Clinical Pathogens

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ABSTRACT

In the present study the dried stem material of *Vernonia amygdalina* and the dried leaf, root and stem material of *Vernonia schimperi* was successively extracted with distilled water, ethanol 96%, and chloroform. These extracts were analyzed for their antimicrobial activity against the bacterial species *Staphylococcas aureus*, *Escherichia coli* and the fungi *Candida albicans*. The results of antimicrobial activity investigation showed that the both plant extracts had inhibitory effect on the growth of the selected pathogenic organisms. The leaf, root, and stem extracts of *V.schimperi* possess higher antimicrobial properties than the stem extract of *V.amygdalina*. When you compared the three parts leaf, stem and root of *V.schimperi* the leaf part of the plant shows highest sensitivity and the root extraction show very low sensitivity against the three selected pathogens. The chloroform and ethanol leaf extracts of *V.schimperi* possess higher antibacterial properties 38mm and 17mm against the selected microbial species *E.coli* and *C.albicans*. So the leaf of *V.schimperi* might have contained highest chemical constituent compared to the plant *V.amygdalina* and these chemical constituent is responsible for higher antimicrobial properties.

Keywords: *Vernonia schimper, Vernonia amygdalina,* Antimicrobial activity.

INTRODUCTION

The search for medicinal plants has been an integral part of the human society to cure the disorders associated with the human beings since the earliest time recorded. It is estimated that there are 250,000 to 500,000 species of plants on Earth[1]. A relatively small percentage (1 to 10%) of these is used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes[2]. The use of medicinal herbs and herbal preparations, including herbal extracts, can be found in the pharmacopoeias of numerous countries[3]. Nowadays, these plant based drugs have their existence as herbal drugs and symbolize safety in contrast to synthetic drugs that are considered as unsafe for human and environment.

Unlike modern drugs that invariably comprise a single active species, herbal extracts contain multiple active constituents. Interestingly, natural compounds contained in these "herbal cocktails" can act in a synergistic manner within the human body, and can provide unique therapeutic properties with minimal or no undesirable side effects[4]. Amongst the medicinal plants used in these herbal preparations for their therapeutic potential, some have been investigated methodically and some of are still left so there is need to explored such plants.

Standardization of plant materials or plant based drugs is the need of the day. In many pharmacopoeias the monographs of plant materials have been described only the physicochemical parameters. Hence for the proper standardization of herbal drugs and its formulations should be assayed to the modern methods describing the identification and quantification of active constituents in the plant material[5]. Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them.

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds[6]. Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes[7]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity[8].

Vernonia (Family: Astraceae) under the tribe of Vernoniae is the largest genus having about 1000 species. It is found mostly in tropical regions and have wide range of habitats of broad ecological diversity and climatic conditions included: tropical forests, marshes and wet areas, dry planes, tropical savannahs desert xeric or dry sites or even frosty regions of eastern North America. In Eritrea, plant remedies are still common therapeutic sources both for humans and animals. One of the common medicinal plants applied is the leaves of *Vernonia amygdalina* "GRAWA" to treat infectious diseases for washing "*sewa*" container (pot) and *Vernonia schimperi* "SGEMO" is used for treatment of general body weakness and muscular

pain by using traditional steam "*tish*" using stem and leaf. *V.amygdalina* is a member of Astraceae family. It is a shrub or small tree that grows in the tropical Africa. The leaves may be consumed as a vegetable (macerated leaves in soup) or aqueous extracts as tonics for the treatment of various illnesses. In the wild chimpanzees and man co- existing in the sub Saharan Africa, it has been observed to ingest the leaves when suffering from parasitic infection. *V.schimperi* is a less known species and widely distributed in the Arab region. The species is not explored for their phytoconstituents. In the present study efforts have been made for the preliminary phytochemical screening of the different extracts of the plant.

Many herbalist naturopathic doctors aqueous extract for their patient as the treatment for nausea, diabetes, dysentery and other gastrointestinal tract problem. Free living chimpanzees eat the leaves, if they are attacked by parasites. In Zimbabwe a root infusion is used to treat sexually transmitted disease. *V.amygdalina* has been in many homes in the eastern and western parts of Nigeria as food especially in the preparation of soups. The characteristic bitter taste is believed to have after taste of sweetness. The peeled stem is often used as chewing stick for cleaning the teeth and is very effective to prevent dental carries[9]. The bitterness of the leaves is also exploited by nursing mothers to assist in weaning their babies by rubbing the juice on their breast[10]. It is also suggested to be useful to nursing mother as it improves lactation. The plants leaves and other parts have been used solely or mixed with other plants for the treatment of various suspected illnesses. The Phytochemical investigation of *Vernonia schimperi* and *Vernonia amygdalina* were already done[11,12].

However, there was no previous research work on antimicrobial activities of solvent extracts of these two medicinal plants grown in Eritrea. Therefore, in this investigation has determined the antimicrobial activity of different plant parts of *Vernonia schimperi* and the stem part of *Vernonia amygdalina* using three different organic solvents. And compare antimicrobial activity of *V.schimperi* and *V.amygdalina* and to determine the effectiveness of antimicrobial within the *Vernonia schimperi* in leaf, stem, and root.

MATERIALS AND METHODS

Collection and identification of plant materials

The plant materials were collected from Mai-temenay and Mai nefhi area. The plant materials were identified and authenticated by Prof. Ghebrehiwet Medhanie, taxonomist in EIT. The plant materials were shed air dried at room temperature to a constant weight and pulverized into a coarse powder. The powder was sieved into plastic and stored away from direct sun light to prevent photolysis and decomposition.

Sample processing (solvent extraction)

The sample of *Vernonia schimperi* leaf, stem and root and *Vernonia amygdalina* stem is completely changed in to powder form. 2.5g of powder of the *Vernonia schimperi* leaves, stem, and root were dissolved to beakers containing each 10 ml of distilled water, ethanol 96%, and chloroform in order to obtain a concentration of 250 mg/ml. The extraction was done at room temperature for 24 hours. The extracts were stored in sterile capped reagent bottles and refrigerated at 4°C until when required for use. Then the powder mixed in containers provided with the above chemicals to prepare crude after stain for 24 to 72hr will be ready for antimicrobial sensitivity test during the procedure should have to prevent contamination.

Preparation of media

38 g of Muller-Hinton Agar (CM 0337) from OXOID was suspended in one litter of distilled water. The mixture was boiled to dissolve the medium completely. The solution was sterilized by autoclaving at 121°C for 15 minutes. The sterilized medium was allowed to cool to a temperature of 50°C in a water bath. The medium was dispensed into petridishes of 4 mm thickness in a safety cabinet. Finally, the medium was allowed to solidify in a safety cabinet and placed in a refrigerator at 5°C.

Microorganisms used

The antibacterial effect of this plant was evaluated on bacterial strains like *Escherichia coli ATCC25922, Staphylococcus aureus ATCC25923, and Candida albicans* were procured from Institute of American Type Culture Collection, 12301, Parklawn Drive, Rockville, Maryland, USA. Log phase cells were used for assays.

Preparation of inoculums

Three colonies for single species were selected. The organisms were inoculated in peptone water and incubated for 3 h at 35°C. Turbidity of the suspension was adjusted to match 0.5 McFarland and used for antibacterial sensitivity assay.

Assay of antimicrobial activity

Antibacterial assay was carried out by agar diffusion method[13] 0.1ml (contain 10^5 CFU/ml) of 24 h old bacterial culture was placed Muller-Hinton agar medium and spread throughout the plate by spread plate technique. A volume of 100 µl of the test extracts (250 mg/ml) were poured in the separate wells. Parallel to this, 100 µL of sterilized solvents were poured in wells (negative control) and the standard antibiotic discs were placed on the agar surface as positive control. The pert plates were incubated at 37°C and the zone of inhibition measured in mm after incubation for 24 h for bacteria and 48 h for *Candida albicans*.

RESULTS AND DISCUSSION

The present study carried out on the Antimicrobial activity of *Vernonia schimperi* and *Vernonia amygdalina* was analyzed against the bacterial species *Staphylococcas aureus*, *Escherichia coli* and the fungi *Candida albicans.* The plant extracts were prepared with water, chloroform and ethanol but tested with the some concentration 2.5 g per10ml solvent and tested over the following selected clenical pathogens S.*aureus, E.coli and C. albicans*. The results were read by measuring the inhibition zone in millimetre diameter around the wells. Presence of inhibition zone is regarded as the presence of antimicrobial activity. Below 6 mm inhibition zone were considered as poor or no effect (resistant). The solvent extracts (2.5g powder per10ml of solvent) were assessed for their antmicrobial activity against the microorganisms *S.aureus, E.coli* and *C.albicans*. The result can read by measuring the inhibition zone presence of inhibition regarded as the presence of antimicrobial activity. Below 6mm we can say poor or no effect. The inhibition zone is measured and the plates were incubated at 37 ± 2 ºC for 24 hours for bacterial and 25 ± 2 ºC for 48 hours for fungal activity, the results obtained are shown in tables 1-4.

Table 1: Stem of *Vernonia amygdalina* inhibition zone 250 mg powder per ml solvent

Microorganisms	Standard			Water	Chloroform	Ethanol
	Amp	Cipro	Genta			
E.coli	18mm	35mm	19mm	6mm	6mm	6mm
S.aureus	33mm	23mm	20 mm	6mm	6mm	6mm
C. albicans	-	-	-	6mm	11mm	16mm

Table 2: Leaf of *Vernonia schimperi* inhibition zone 250 mg powder per ml solvent

microorganisms	standard			Water	Chloroform	Ethanol
	Amp	Cipro	Genta			
E.coli	18mm	35mm	19mm	6mm	38mm	20mm
S.aureus	33mm	23mm	20	21mm	25mm	25mm
C. albicans	-	-	-	6mm	12mm	17mm

Table 3: Root of *Vernonia schimperi* inhibition zone 250 mg powder per ml solvent

microorganisms	Water	Chloroform	Ethanol
E.coli	9mm	18mm	15mm
S.aureus	6mm	6mm	6mm
C.albicans	6mm	13mm	12mm

Table 4: Stem of Vernonia *schimper i*nhibition zone of 250 mg stem powder per ml solvent

Microorganisms	Water	Chloroform	Ethanol
E.coli	6mm	35mm	22mm
S.aureus	6mm	15mm	13mm
C. albicans	6mm	6mm	6mm

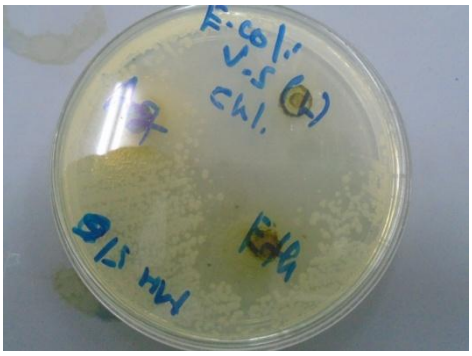

Figure 1: Leaf extraction result of *Vernonia schimperi* over *E.coli*

The results of the inhibition zones obtained the present study indicate that leaves have the potential to act as a source of useful drugs because of presence of various photochemical components such as phenols, flavonoids and tannin[11,12]. The results are very much encouraging but scientific validations necessary before being put into practice. The aqueous extracts showed minimum sensitivity 6mm and 9mm against all the pathogens under this investigation. This is because the diffusivity of water is very low as compared to the other solvent it is also possible that the active chemical constituents were not soluble in water. The drying process may have caused conformational changes to occur in some of the chemical constituents found in these plants.

The secondary plant metabolites (phytochemicals) with antimicrobial potency have been actively investigated as alternatives to and/or in combination with antibiotics in the therapy of different microbial infections[14,15]. In this study, the leaf, root and stem extract of *V.schimperi* were most effective against *S.aureus, E.coli,* and *C.albicans* that of *V.amygdalina* tables 2-4. The root extraction show very low sensitivity against *S.aureus* table 3. When compared the three parts leaf, stem and root of *V.schimperi* the leaf part of the plant shows highest sensitivity against the three microbial constituent table 2 and fig 1. So the leaf of *V.schimperi* might have contained highest chemical constituent[11, 12] compared to the plant *V.amygdalina*

Frequent uses of antibiotics make the organisms to become resistant to such antibiotics[16]. Because of this reason new antibiotics are discovered to control the infections disease causing pathogens. In this regard higher plants play an important role by providing antibiotic compounds. Higher plants are rich in active principles, which are used as therapeutic drugs[17].

CONCLUSION

Finally the present investigation concluded that the medicinal plants studied here can be seen as a potential source of useful drugs. This plants extract used as antibacterial agent for destroying the pathogenic organisms and also used curing number of diseases. Further studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds.

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