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EFFECT OF AQUEOUS AND ETHANOL EXTRACTS OF *OCIMUM LAMIIFOLIUM* AND *AMARANTHUS DUBIUS* AGAINST BACTERIA ISOLATED FROM CLINICAL SPECIMEN

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Abstract

The aim of the study was to screen the antibacterial activities of aqueous and ethanol extracts of the two plants *Ocimum lamiifolium* and *Amaranthus dubius* available in Ethiopia. Crude extract of the leaves with aqueous and ethanol were screened for antibacterial activities against *S. aureus, Pseudomonas* spp. and *Escherichia coli*. The in vitro antibacterial activity was performed by agar disc diffusion method. Ethanol and aqueous extract of *Ocimum lamiifolium* and *Amaranthus dubius* revealed an elevated antimicrobial activity against *S. aureus, E. coli* and *Pseudomonas* spp. The present study shows that the maximum effect was exhibited by *Ocimum lamifolium.* The results obtained in the present study suggest that the ethanol and aqueous extracts of *Ocimum lamiifolium and Amaranthus dubius* revealed a significant scope to develop a novel antibacterial herbal formulation.

Keywords: *Ocimum lamiifolium*, *Amaranthus dubius*, Crude extracts, Antibacterial effects.

Introduction

Plants are a source of many potent and powerful drugs used medicinally in different countries. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries[1]. Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses[2].A wide range of medicinal plants parts is used to extract as raw drugs and they possess varied medicinal properties. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw materials for many herbal industries[3]. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Clinical microbiologists have great interest in screening of medicinal plants for new therapeutics[4]. The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds[5]. The development of drug resistance

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in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants[6]. Screening of medicinal plants for antimicrobial activities is important for finding potential new compounds for therapeutic use.

In recent years interest to evaluate plants possessing antibacterial activity for various diseases is growing[7]. Research has been done worldwide on the use of medicinal plants to cause human diseases and extracts of many plant species have been found to be active against many pathogenic bacteria. Aqueous extract of some plants have been reported from time to time to demonstrate antimicrobial activity. The efficacy of various species of medicinal plants against a variety of pathogens has been reported by a number of workers[8-17].

A wide variety of indigenous and minor crops have been utilized for daily consumption since ancient times. The family Labiateae is one of the largest families, which comprises the larger proportion of medicinal plant species. *Ocimum* is one of the important genera of family *Labiateae. Ocimum* species often referred toas the "king of the herb. *Ocimum lamiifolium* is an important medicinal herb belonging to family *Lamiaceae*. The plant extracts and isolated compounds of *Amaranthus* species have been tested for their bioactivity by various in vitro model systems[18].

Materials and Methods

Plant material

The *Ocimum lamiifolium* and *Amaranthus dubius* were collected from different area of Gondar region of Ethiopia in September, 2011. The species were botanically identified.

Preparation of the extracts

The leaves were cleaned with running water and dried first in sun light for seven days and then in an oven at 40ºC for about 24 hours. Finally, the dried materials were pulverized into fine powdered substance by a grinding machine. Twenty gram of powder of *Ocimum lamiifolium* and *Amaranthus dubius* were weighted with the electric balance and transferred into two separate 100 ml conical flasks. Then 40 ml of ethanol in one flask and 40 ml of distilled water in another was added. The conical flasks were closed by foil paper and put on dark place for maximum7 days. The crude ethanol and aqueous extracts were then filtered by passing the extracts through Whatmann No. 1 filter paper and then concentrated under vacuum at 40ºC by using a rotary evaporator. The residual extracts were stored in refrigerator at 4ºC in small and sterile plastic bottles.

Tested bacteria

Antibacterial activity of spices powder extracts was investigated against two gram-negative and two gram positive bacterial isolates, which were obtained from the Hospital Laboratory of Gondar University, Ethiopia. These include *Pseudomonas spp., Escherichia coli* and *S.aureus*. The tested bacteria were cultured on Nutrient agar at 37ºC for 24 h.

Inoculum preparation

Ten ml of distilled water was taken into the screw cap tube and pure colony of freshly cultured bacteria was added into the tube and vortex was done. The OD (optical density) was measured with the colorimeter and microbial population was confirmed to be within in 10^7 ml^{-1} to $10^8 ml^{-1}$. This suspension was used as inoculums.

Antimicrobial assay

The in vitro antibacterial activities of the test samples were carried out by disc diffusion method[19, 20]. In the disc diffusion method, Muller-Hintonagar was used as culture media and the discs were placed aseptically over the bacterial culture on nutrient agar plates and incubated at 37°C for 24 hours. After incubation for 24 hours, the zones of inhibition around the discs were measured by millimeter scale. Discs were impregnated with each treatment and control was assayed on agar medium plate for *Pseudomonas spp., Escherichia coli* and *S. aureus*. The diameter of zone of inhibition as indicated by clear area which was devoid of growth of microbes was measured to determine the antibacterial activity. The experiment was repeated two times to confirm the reproducible results. Sterile, blank paper discs impregnated with only sterile ethanol and distilled water served as negative control each time. Standard Gentamycin (10µg/disc) was used as positive control for comparison of the antibacterial activity. Minimum Inhibitory Concentration (MIC) value of the extracts of the *Ocimum lamiifolium* and

Amaranthus dubius were determined in present study following the serial dilution technique.

Results

The quantity of ethanol and aqueous extracts of *Ocimum lamiifolium* and *Amaranthus dubius* showed in table. 1. The maximum 10.7% and 12.9% was extracted from both the plant using aqueous extraction method. Whereas, least9.4% and 11% yield weighed from both the plants using ethanol as a solvent.

Table No. 01: Yield of plant extract using ethanol and aqueous extraction methods

Plant Species	Parts used	Extraction Type	% Yield (w/w)
Ocimum lamiifolium	Leaf	Ethanol	9.4
		Aqueous	10.7
Amaranthus dubius	Leaf	Ethanol	11
		Aqueous	12.9

Antimicrobial activities

Ethanol and aqueous extracts of *Ocimum lamifolium* were found sensitive to *S. aureus, E. coli* and *P. aeruginosa*. Crude ethanol extract produced zone of inhibition 12 mm and 13.5 mm against *S. aureus* and *E. coli* respectively and no zone of inhibition was observed for *P. aeruginosa*. Crude aqueous extract produced zone of inhibition of 13.5 mm, 15.5mm and 13 mm against *S. aureus, E. coli*and *P. aeruginosa* respectively (Table 2). However, the aqueous extract exhibited highest zone of inhibition (15.5 mm) against *E.coli*.

Ethanol and aqueous extracts of *Amaranthus dubius* were also found sensitive to *S. aureus, E. coli* and *P. aeruginosa*. Crude ethanol extract produced zone of inhibition 8.5 mm, 9 mm and 12 against *S. aureus, E. coli* and *P. aeruginosa* respectively.. Crude aqueous extract produced zone of inhibition of 10.5 mm against *S. aureus.* No zone of inhibition was observed for *E. coli* and *P. aeruginosa* (Table 2). However, the ethanol extract exhibited highest zone of inhibition (12 mm) against *P. aeruginosa*.

Table No. 02: Antibacterial activity of ethanol and aqueous extracts using agar well diffusion method

Micro organisms	Mean zone of incubation (mm)					
	Ocimum lamifolium		*Amaranthu sdubius*		Gentamycin	Dist. Water
	E	Aq.	E	Aq.		
S. aureus	12	13.5	8.5	10.5	26	-
E. coli	13.5	15.5	9	-	17	-
P. aeruginosa	-	13	12	-	31	-

E- Ethanol **Aq. -** Aqueous

The MIC value was also determined against the all tested bacteria. The MIC value of ethanol extract of *Ocimum lamifolium* was found to be 200mg and 100 mg against both *S. aureus* and *E. coli* respectively. Whereas, the MIC value of aqueous extract showed 200 mg against S. aureus and 100 mg against *E. coli* and *P. aeruginosa* each. The MIC value of ethanol extract of *Amaranthus dubius* was found to be 500mg against all tested bacteria and aqueous extract showed 1000mg against *S.aureus*(Table 3).

Table No. 03: MIC of plant extracts against bacterial isolates (mg/ml)

MO	*Ocimum lamiifolium*		*Amaranthus dubius*	
	E	Aq.	E	Aq.
S. aureus	200	200	500	1000
E. coli	100	100	500	-
P. aeruginosa	-	100	500	-

E- Ethanol **Aq. -** Aqueous

Discussion

The present study showed that most of the plant extract shave antibacterial activity against some of the common microorganisms of medical importance. This work could justify their traditional use in treatment of different diseases in human and animals. The fact that both ethanol and aqueous extracts of some plants are showing similar efficacy against some species of bacteria could be due to extraction ability of active ingredients responsible for antibacterial activity by the two extraction systems. On the other hand, some plants have shown variable activity by the two extraction methods presumably because of difference in extracting ability of specific active ingredient by the two extraction methods. Lino and Deogracious[21, 22] also reported difference in antibacterial activity when two extraction methods were used. The present study shows that the maximum effect was exhibited by *Ocimum lamifolium.* These results are in parallel with the previous report on *Ocimum spp*. by[23]. However, in the present study the maximum effect of tested plant extracts were showed on *E. coli* followed by *S. aureus* and *P. aeruginosa*. This result is in contradiction with the report by Pankaj and Puroshottam (2011), revealed that *S. aureus* (a Gram-positive bacterium) was observed as most susceptible bacterium as it was inhibited by extracts of *Ocimum spp*.. *Streptococcus pyogenes* and *Salmonella typhi* was found to be resistant to all the extracts tested[24]. The results of present study support the traditional usage of plant and *Ocimum lamifolium* and *Amaranthus dubius* plant extracts which possess compounds with antibacterial properties that can be used as antibacterial agents in new drugs for the therapy of infectious diseases caused by pathogens and further work may be carried out for pharmacological evaluation.

Conclusion

The present investigation revealed that the extract of *Ocimum lamiifolium* and *Amaranthus dubius* leaves have potent antimicrobial activity which explains its use in traditional system of medicines. Hence, *Ocimum lamiifolium* and *Amaranthus dubius* can be employed as a source of natural antimicrobials that can serve as an alternative to conventional medicines.

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