Antimicrobial activity of wild olive crude extracts *in vitro*

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ABSTRACT

Wild olive crude extracts were screened for antibacterial activity against five different bacterial human pathogens. Four different solvents were used for the extraction. Antibacterial activities of plant extracts were compared with those of standard antibiotics. The capacity of the extracts and antibiotics were evaluated on the basis of their capacity to inhibit the growth of pathogenic bacteria measured as zone of inhibition (ZOI, expressed in mm). Almost all bacteria showed to be sensible against the antibiotics with the value of ZOI ranging from 10-32 mm, while the effectiveness of olive extracts varied from one species to other with ZOI values of 7-12 mm. Extract obtained with methanol appeared to be the most effective against all pathogenic bacteria compared to those obtained with other solvents.

Keywords: Bacterial pathogen, *Olea cuspidata* fruits, antibacterial activitiy

INTRODUCTION

Olive belonging to *Olea europaea* L. is a typical Mediterranean species where more than 95% of the world total production is localized (1). Besides a variety of beneficial health effect of extra virgin olive oil, antimicrobial properties of the flavor compounds present in olive fruit and leaves against a broad range of saprophytic and plant pathogenic fungi, certain species of lactic acid and other human and plant pathogenic bacteria have widely been studied by several authors (2, 3, 4, 5, 6). Phenolic compounds have been shown to inhibit the growth of *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* (7, 8). Oleuropein, has been shown to inhibit sporulation of *Bacillus cereus* (9). Hydroxytyrosol resulted effective against clinical human pathogenic strains of *Haemophilus influenzae*, *Moraxella catarrhalis*, *Salmonella typhi*, *Vibrio parahaemolyticus* and *S. aureus* (10). Increasing resistance to antibiotics, wide-spread use of immune-suppressing drugs and a rise in bacterial infections emphasize the necessity to find and develop new antimicrobial agents.

In addition to cultivated *O. europaea* which includes both the cultivated olive and the oleaster, there are several other wild olive species. *O. cuspidata* Wall., is one of them which is endemic to Nepal (11). Although there are many investigations on the antimicrobial activities of olive oil and leaves of *O. europaea*, no study is available on those of *O. cuspidata*. In our study, we focused to evaluate and describe for the first time the antimicrobial activity of wild olive fruits against a wide spectrum of human pathogenic bacteria.

MATERIAL AND METHODS

Plant materials

Fresh green fruits (0.5 kg) of *O. cuspidata* were collected from the Northwestern district of Bajura, Nepal in September, 2010. Fruits were washed under sterile distilled water (SDW), blotted with paper towels and dried in open air in the shadow. Once completely dried it was ground to a powder.

Solvents

Petroleum ether (P), Chloroform(C), Methanol (M), and Water (W) were the solvents used in the extraction process.

Preparation of extracts

Ground powdered fruits were extracted in four different solvents at a room temperature 40% (w/v) concentration (40-g dried fruits for each solvent) and autoclaved at 121 °C for 20 min. (12). The mixture was left for 24 hours and filtered by using using Whatman filter paper no.1. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Same process was repeated to obtain extract from others solvents. Dried extracts from each solvent were stored in labeled sterile screw capped bottles at -20 °C.

Strains, media and growth conditions

All microorganisms used for the assay were obtained from the laboratory of Western Regional Hospital Limited (WRHL), Pokhara. All the organisms were standardized by the laboratory themselves. They were *Bacillus subtilis (WRHL BS011), Escherichia coli (WRHL EC 007), Pseudomonas aeruginosa (WRHL PA 003), Shigella flexineri (WRHL SF 009) and Staphylococcus aureus (WRHL SA 011).* The first and the last are Gram positive while the others are Gram negative bacteria.

The culture medium for the bacteria consisted of 0.8% nutrient broth (NB, BBL), 0.5% yeast extract (Difco), and 0.1% glucose. All stock strains were kept at 4 °C.

Screening for antimicrobial activities

The dried plant extracts were dissolved in aqueous dimethylsulfoxide (DMSO) to a final concentration of 250 mg/ml and sterilized by filtration through a 0.45 µm membrane filter. Empty sterilized antibiotic discs (6 mm diameter) were each impregnated with 100 µL of extract. All the bacteria mentioned above were incubated at 35±0.1 °C for 24 h by inoculation into Nutrient Broth. Bacterial suspensions (10^6 CFU/ml) were spread on Mueller-Hinton Agar (Oxoid Ltd., Hampshire, England) plates (1 ml inoculum/plate). The discs injected with extracts were placed on the inoculated agar by pressing slightly. Petri dishes were placed at 4 °C for 2 h, plaques injected with bacteria and incubated at 35±0.1 °C for 24 h (13,14). Besides extracts, five different antibiotics (Amikacin, Azithromycin, Ciprofloxacin, Nitrofurantoin and Norfloxacin) were used (each 50 µg of concentration) to evaluate their antimicrobial effects. SDW was used as negative control. At the end of the period, inhibition zones formed on the medium were evaluated in mm. Studies were performed in triplicate.

RESULTS AND DISCUSSION

The antimicrobial activities of the plant extracts and antibiotics were clearly observed against the tested bacterial species. The zones of inhibition (ZOI) formed both by the plant extracts and antibiotics are reported in table 1. Almost all antibiotics were found to be effective against the tested bacterial species in different level except ciprofloxacin and norfloxacin which did not inhibited the growth of *E. coli* and nitrofurantion which was not effective against *P. aeruginosa*. On the other hand, antimicrobial activities of plant extracts obtained by using different solvents were appeared to be very different in terms of effectiveness since some bacterial species are more resistant and some other more susceptible to the extracts. In particular, extracts obtained with petrolium ether and chlotoform only inhibited the growth of Gram positive bacteria while those obtained with water inhibited only the growth of *P. aeruginosa*. Moreover, an homogeneous antimicrobial activities were observed by extracts obtained with methanol against all bacterial species although at lower level (Tab. 1).

Concerning the zone of inhibition, the values were merely higher for those caused by antibiotics respect to those caused by the plant extracts. ZOI values ranged from 10-32 mm for antibiotics while they were much more lower ranging from 7-12 mm for the plant extracts. In addition, extracts obtained by water is not effective against the tested bacterial cultures except a poor effect seen against *P. aeruginosa* with ZOI of 7 mm. All the bacterial species are less susceptible to the plant extracts as compared to standard antibiotics. It is likely that both the antibiotic and plant extracts have higher antibacterial effects to Gram positive bacteria compare to those Gram negative (Tab. 1).

Antibiotics & fruit extracts	Zone of Inhibition (in mm)									
	S. aureus		*E. coli*		*S. flexinerai*		*P. aeruginosa*		*B. subtilis*	
Azithromycin	32[I]	c [II]	13	a	32	c	10	a	30	c
Amikacin	25	c	18	b	25	c	19	b	16	b
Ciprofloxacin	21	b	0		11	a	25	c	29	c
Norfloxacin	22	b	0		22	b	20	b	12	a
Nitrofurantoin	20	b	19	b	20	b	0		18	b
E 1	8	a	0		0		0		9	a
E 2	7	a	0		0		0		12	a
E 3	10	a	10	a	10	a	9	a	12	a
E 4	0		0		0		7	a	0	
SDW	0		0		0		0		0	
E 1	Extract obtained with Petroleum ether (P)									
E 2	Extract obtained with chloroform(C)									
E 3	Extract obtained with methanol (M)									
E 4	Extract obtained with water (W)									
SDW	Sterile distilled water									
I	Values are the mean of three replicates									
II	Means followed by the same letter are not significantly different at P=0.05.									

Table 1: Antimicrobial activities of olive fruits and antibiotics

Finding new antimicrobial agents with wide spectrum and effective mode of action constitute a priority in pharmaceutical industries in order to control the increasing resistance of pathogen infections. In our study, we found wild olive fruits having an antibacterial activity against the tested bacteria although their effectiveness is lower compared to that of standard antibiotics.

In spite of being a small country, Nepal possesses around 7000 species of vascular plants with more than 2000 medicinal species. Most of the wild floras of Nepal are rich in medicinal and aromatic properties like antibacterial, antiviral, antihelminitic, anticancer, sedative, laxative, cardiotonic, diuretic and others. They are important sources of bio-molecules, with application for the manufacture of pharmaceuticals and cosmetics (15). Further studies are necessary to carry out a detail antimicrobial screening also of other pathogens to establish a database which allow to manage and conserve these plant species many of which are recognized also in the traditional medicine.

Studies carried out in Pakistan showed that oil obtained from wild olive (*O. cuspidata*) is almost comparable to edible oil of *O. europaea* and have all physical and chemical properties required for any seed oil used for edible purpose (16). Since the environmental condition of the Himalayan regions of Nepal, India and Pakistan is very similar, we suppose that oils obtained from wild olive from Pakistan and Nepal have a very analogous properties. We tested the antibacterial properties of *O. cuspidata* fruits for the first time since previous studies have focused exclusively on *O. europaea* fruits and leaves from Mediterranean regions.

In our study, extracts obtained with methanol showed a good effectiveness against the tested bacteria compare to those obtained with other solvents. This is probably because, methanol, compare to other solvents, allows to extract all the phenolic compounds from the olive fruits. Nevertheless, a thorough investigation is needed to compare phenolic compounds of *O. cuspidata* fruit from Nepal with those from other countries since environmental condition found to be widely influential on the percentage of acid and phenolic compounds of the fruits. Study carried out in Kenya on *O. cuspidata* showed that oil extracted from olive fruits has lower content of oleic acid which is found to be associated to the high average temperature of the summer and lack of the winter cold temperature of the country (17). In addition, further study is to be hoped also to perform a thorough analysis among the phenolic compounds of *O. cuspidata* and those of *O. europaea*.

CONCLUSIONS

Various plant extracts were prepared by using different solvents with the objective of evaluating better antibacterial activities in comparison with some standard antibiotics. Among these, the extract obtained with

methanol was found to have a better effectiveness against the tested bacteria while those obtained with petroleum ether, chloroform and water showed the antibacterial activities only against certain bacteria. The antibiotics used in this study appeared to be more effective than the plant extracts. Our study showed that besides fruits and leaves of *O. europaea* already tested, also the fruits of *O. cuspidata* have antibacterial functions which can be attributed to the phenolic compounds.

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