

Full Length Research Paper

Study of antibacterial and antioxidant activities of four common Nepalese kitchen spices

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Given the alarming incidence of antibiotic resistance in bacteria of medical importance and multiple side effects associated with the modern day chemotherapeutics, there is a constant need for new and effective therapeutic agents that could be easily extracted from our daily used Nepalese culinary. To study the antibacterial and antioxidant activity of common spices, locally available Clove (*Eugenia caryophyllus*), Cinnamon (*Cinnamomum zylancium*), Cumin (*Cumin cyminum*) and Timur (*Zanthoxylum alatum*) were subjected to cold extraction using ethanol and was assayed through agar well diffusion method and DPPH radical scavenging activity for different concentration gradient (100 to 500 µg). *Zanthoxylum* and *Eugenia* have showed potent antimicrobial activity against *Proteus vulgaris* and *Pseudomonas aeruginosa* followed by *Cinnamomum* and *Cumin* against *Pseudomonas aeruginosa*. All the extracts showed effective antimicrobial activity against gram positive and gram negative bacteria, however *Escherichia coli* remains ineffective towards any of the concentration of spices. DPPH radical scavenging activity showed effective antioxidant activity of the spices in the following order: *Eugenia* (93.84%) > *Cumin* (90.4%), *Zanthoxylum* (88.73%) > *Cinnamomum* (87.23%). Hence, our present study demonstrated that the ethanolic extract of different spices, *Eugenia* being the most effective, possess potent antibacterial and antioxidant activity and can be further analyzed for antimicrobial therapeutics and pharmacological evaluation.

Key words: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, *Eugenia*, *Zanthoxylum*, *Cinnamon*, *Cumin*.

INTRODUCTION

The alarming rise of antibiotic resistance has been a global concern. The reason behind such situation has been notified as the haphazard use of the antimicrobial drugs which are excessively used during therapeutic treatment of infections. Additionally, the problems associated with modern day chemotherapeutics has a

gamut of adverse effect from hypersensitivity, immune-suppression to allergic reactions (Agrawal et al., 1996).

Natural products from plants and its significant parts has been proved to have lesser side effects in comparison to chemotherapeutics, hence they possess potential in being used as an 'alternative remedy' (Tepe

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et al., 2004). In developing countries modern-day antibiotics remains out of reach and 'plant-derived' drugs are their vital resources for treating infections (Thippeswamy et al., 2005). Plants products such as essential oil have been corroborated to have efficient effect against bacteria, virus, fungi and insects in addition to antioxidants properties. The active compounds cognate to these beneficial effects are most essentially secondary plant metabolites like glycosides, alkaloids and more and are profusely present in plant genre like spices and herbs (Pangsatabam et al., 2015; Saranraj et al., 2014).

With the most relevant notion, 'Kitchen spices' has been defines as the "vegetative products or mixture free from extraneous matter used for flavoring or imparting aroma in food". These spices are usually derivatives from different parts of plants like barks, leaves, fruits, buds, rhizomes and resins (Green et al., 1999).

The present research has mainly focused on four different spices used on daily basis in Nepalese Kitchen. The common spices like Clove (*Eugenia caryophyllus*), Cinnamon (*Cinnamomum zylancium*), Cumin (*Cumin cyminum*) and Timur (*Zanthoxylum alatum*) are used for the purpose of seeking its antimicrobial and antioxidant properties in the present study.

Cinnamon, also referred as *C. zylancium*, possess several potential health benefits. Antimicrobial activity, anti-inflammatory properties, controlled blood sugar, depletion of cardiovascular and colonic cancer along with the boosted cognitive function are reported in several scholars for cinnamon (Khan et al., 2003; Mata et al., 2007; Shen et al., 2010).

Another effective spice is clove, which is mostly used as agent for flavoring food items or herbal supplement. Clove has also been found to be utilized as a topical treatment in case of pain in tooth. Other usage of clove comprises the treatment for oral cavity inflammation, supplement during common cold or cough (Consumer information from USDA, 1997). Cumin also exhibits potential activity against microbes and fungi along with effective antioxidants activity. These properties are mainly found to be correlated with the content of phenol in cumin (Taira et al., 1992; Shetty et al., 1994; Zhang, 2004).

Lastly, extensive application of Timur has been seen in home-remedy for its carminative, antihelmintic and stomachic properties. The plant parts mainly fruits and seeds are widely linked with home-remedy as aromatic tonic during dyspepsia or fever. In addition, properties like anti-odor, disinfectant and antiseptic has enlarged its spectrum of application from dental troubles to lotion for skin problems like scabies (Green et al., 1999).

MATERIALS AND METHODS

Species collection

All the spices namely Clove (*E. caryophyllus*, family Myrtaceae),

Cinnamon (*C. zylancium*, family Lauraceae), Cumin (*C. cyminum*, family Apicaceae) and Timur (*Z. alatum*, family Rutaceae) were collected from local market of Kathmandu Nepal.

Chemicals and standards

The chemicals used were ethanol (Thermo-Fisher Scientific, India), DPPH and ascorbic acid (Sigma Aldrich, USA). All other chemicals used were of the highest commercially available grade. For absorption measurement, double beam U-2800 UV-visible spectrometer, HITACHI, Japan, was used.

Preparation of extracts

The spices were ground in clean and sterilized grinder and the fine powder was taken. 20 g of sample was weighed and soaked in 200 ml ethanol (for 48 h). After that it was stirred in a magnetic stirrer for 30 min and filtered by using Whatman No. 1 filter paper. The filtrate was taken and concentrated to dryness in rotatory evaporator at reduced pressure. Dry extract was dissolved in mother solvent to make the stock solution of 10 mg/ml.

Collection and maintenance of culture

All the pure cultures of the test organisms were collected from National Public Health Laboratory, Kathmandu, Nepal. Six different bacterial strains, *Staphylococcus aureus* (ATCC 25923), *Proteus vulgaris* (ATCC 15028), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 700603), *Salmonella typhi* (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 27853) were taken. These organisms were then sub cultured in a sterile nutrient agar and incubated at 37°C for 24 h to obtain fresh culture.

Assay of antibacterial activity

Spices are believed to possess antibacterial activity and thus its assay was done by well diffusion method. At first the 24 h culture of six bacterial strains *S. aureus* (ATCC 25923), *P. vulgaris* (ATCC 15028), *E. coli* (ATCC 25922), *K. pneumonia* (ATCC 700603), *S. typhi* (ATCC 14028) and *P. aeruginosa* (ATCC 27853) were taken and cultured in nutrient broth. The sterile plates of Mueller-Hinton Agar (MHA) were prepared. A McFarland 0.5 standard was prepared and the bacterial suspension was compared to the 0.5 McFarland standards to adjust the turbidity of the inoculums for the susceptibility test. The bacteria were transferred into Petri-plates containing solidified agar using sterile cotton swab. The swab was used to spread the bacteria on the media in a confluent lawn. Wells were prepared by punching the agar plate already inoculated with a pure culture of the test organism with the help of sterile borer of 6 mm. Wells were made at the equidistance on the Petri plate. Then 40, 50, 60 and 70 µl of samples were added in wells from the stock solution of 10 mg/ml. In the two wells, 50 µl of ethanol (as negative control) and 50 µl of reference antibiotic solution (as positive control) were added. Gentamycin (1%) and Vancomycin (1%) were used as reference antibiotic for Gram-negative bacteria and Gram-positive bacteria, respectively. Diffusion of extracts, antibiotics and methanol were allowed at room temperature for 1 h. All the plates were then covered with lids and incubated at 37°C for 24 h and zone of inhibitions (ZOIs) were measured. For each sample all the experiments were performed thrice and obtained results were averaged.

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity

1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) radical scavenging

Table 1. Antibacterial activity of Nepalese kitchen spices extract.

Sample	Amount of sample (µg)	Zone of Inhibition (mm)					
		<i>S. typhi</i> (ATCC 4028)	<i>E. coli</i> (ATCC 5922)	<i>P. vulgaris</i> (ATCC 15028)	<i>K. pneumonia</i> (ATCC 700603)	<i>P. aeruginosa</i> (ATCC 27853)	<i>S. aureus</i> (ATCC 25923)
<i>Zanthoxylum</i>	100	8±1	-	12±2	9±1	6±1	7±1
	200	12±1	-	12±1	11±1	7±1	10±1
	300	14±2	-	16±2	12±1	12±1	14±2
	400	14±2	6±1	20±3	12±1	12±1	14±2
	500	16±1	7±1	24±3	16±3	16±2	16±1
<i>Eugenia</i>	100	10±1	8±1	9±1	10±1	10±1	10±1
	200	12±2	8±1	9±1	11±1	14±2	11±2
	300	14±1	10±1	10±1	14±1	26±3	14±2
	400	14±2	14±2	12±2	16±2	26±3	16±2
	500	16±2	14±1	14±2	20±3	26±3	20±3
<i>Cinnamomum</i>	100	-	-	-	-	-	8±1
	200	-	-	-	-	10±1	10±1
	300	8±1	-	6±1	7±1	12±2	11±2
	400	10±1	7±1	8±1	9±1	14±2	11±2
	500	10±1	8±1	8±1	10±1	16±2	14±2
<i>Cumin</i>	100	-	-	-	-	10±1	6±1
	200	8±1	-	-	-	12±2	6±1
	300	12±2	-	8±1	-	14±2	10±2
	400	12±2	6±1	10±1	7±1	14±2	11±2
	500	12±2	6±1	11±2	8±1	20±3	14±1
Gentamycin (1%)		16±2	18±2	19±1	18±2	22±2	-
Vancomycin (1%)		-	-	-	-	-	20±3

activity was measured according to the method of Ilhami et al. (2005). Extract solutions were prepared by dissolving of different dry extract in methanol to produce a solution of 10 mg/ml. 600 µM DPPH was dissolved in 300 ml methanol and used as stock solution. The plant extract in methanol was taken in various concentrations, that is, 10, 20, 30, 40 and 50 µl from stock of 10 mg/ml (100, 200, 300, 400, and 500 µg) whose final volume was maintained 1 ml and were mixed with an aliquot of 2 ml of 600 µM DPPH solution in methanol and incubated at 25°C for 30 min. Absorbance of the test mixture was read at 517 nm using a spectrophotometer against a DPPH control containing only 1 ml of methanol in place of the extract. All experiments were performed thrice and the results were averaged. Ascorbic acid was used as a standard. Percentage inhibition was calculated using the following expression

$$\% \text{ Inhibition} = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

Where, A_{control} and A_{sample} stand for absorbance of the control and absorbance of tested extract solution respectively.

RESULTS

Antibacterial activity

The result of antibacterial activity of spices is shown in

Table 1. The antibacterial potential may be due to various phytochemicals present in them. *Zanthoxylum* was most effective against *Proteus vulgaris* showing maximum zone of inhibition of 24±3 mm when 500 µg extract was used. *Eugenia* was most effective against *P. aeruginosa* showing maximum zone of inhibition of 26±3 when 300 to 500 µg extract was used. Similarly, *Cinnamomum* and *Cumin* were most effective against *P. aeruginosa* showing maximum zone of inhibition of 16±2 and 20±3 mm respectively when 500 µg extract was used. All the extracts were least effective against *E. coli* than other strains used.

Evaluation of antioxidant assay

DPPH radical scavenging activity

DPPH radical scavenging activity of Nepalese kitchen spices extracts at different concentrations (100 to 500 µg) was measured, with ascorbic acid as the standard. The DPPH radical scavenging activity is shown in Figure 1 and extent of radical scavenging was found to increase generally with increasing concentrations for all samples.

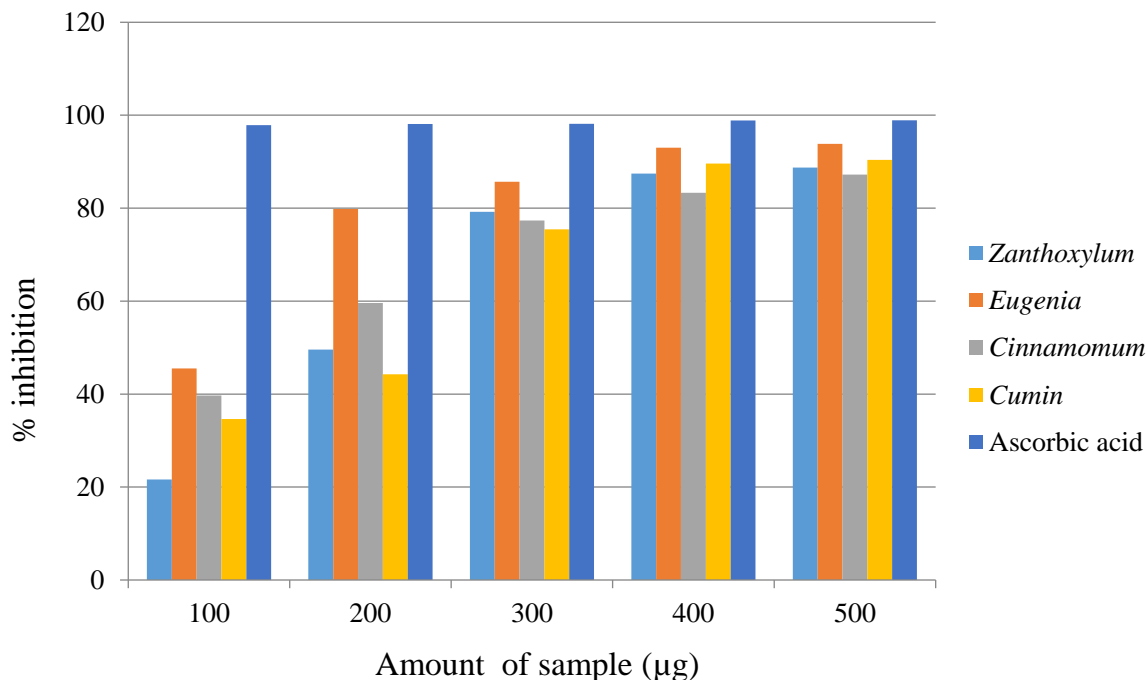


Figure 1. Antioxidant activity of Nepalese kitchen spices extract.

The results shows *Eugenia* (500 µg) exhibited the highest radical scavenging activity with 93.84%±4.51 followed by the *Cumin* (500 µg) extract with 90.4%±5.87, *Zanthoxylum* (500 µg) extract with 88.73%±5.64 and *Cinnamomum* (500 µg) extract with 87.23%±7.23.

Statistic

The results are expressed as mean ± standard deviation (SD).

DISCUSSION

Spices are believed to possess antibacterial activity and antioxidant activity and thus it was assayed through well diffusion method and DPPH radical scavenging activity. It was found that the ethanolic extract of different spices possess efficient antibacterial and antioxidant activity in a concentration dependent manner.

The beneficial medicinal effects of plant materials typically results from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (Shen et al., 2010). Spices and their phytochemicals have become an exciting research topic because of their important and observed biological

activities and because they are used extensively in the culinary, industrial and pharmacological fields. These biological activities are mainly attributed to their antioxidant and antibacterial properties due to their high polyphenol content (Gaur, 1990; Mata et al., 2007).

The growth of new infectious disease and emergence of several infections appears to have been controlled, but the increase in multidrug resistant bacteria is followed which poses a serious threat to human and animal and creates the necessity for studies directed towards the development of new antimicrobials.. Plants can be the best source for such bioactive compounds (Rasooli, 2007). So, since both gram positive as well as gram negative bacteria are found to be sensitive against spice extract (Table 1), spices can be efficient source for deriving those compounds. Antioxidants are capable of protecting cells from free radical damage, act as chemo preventive agents by inhibiting the generation of free radicals and play an important role in neutralizing oxidative damage (Ouattara et al., 1997; Pokhrel et al., 2015). DPPH assay is well known method for the evaluation of free radical scavenging activity (Rasooli, 2007). Antioxidant activity may be due to various phytochemicals such as phenols and flavonoids which are well known for their antioxidant potential due to hydrogen donating property of their hydroxyl groups. Strong DPPH activity of clove might be related to phenolic antioxidant components such as eugenol, isoeugenol, etc. (Tepe et al., 2004). Dorman et al. (2000) identified as much as 94% phenyl propanols in clove oil of which eugenol was 91% of phenyl propanols (Duke et

al., 2003).

Conclusion

The study conducted has shown that crude ethanolic extracts of multiple kitchen spices found in Nepal possess effective antibacterial activity against both Gram positive and Gram negative bacteria. Also the extract has been found to contain effective antioxidant capacity. On further exploration of the bioactive constituents present in those plants development of novel antibacterial and antioxidant compounds could be possible.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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