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RESEARCH PAPER

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *ALLIUM SATIVUM* AND *ALLIUM CEPA*

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Garlic and onion have been used widely as common household spices from the ancient period and have also been regarded as traditional healers. This study was designed to evaluate the antioxidant and antibacterial activities of fresh extracts of garlic and onion. Activities of enzymatic antioxidants (superoxide dismutase and catalase) and non-enzymatic antioxidant (ascorbic acid content) activities were measured and compared in between garlic and onion extracts. Superoxide dismutase and catalase activities in garlic were found noticeably high ($p < 0.05$) compared to onion but significantly reverse in case of the ascorbic acid content ($p < 0.05$). Likewise, six bacteria were chosen to study antibacterial activities of garlic and onion. The zones of inhibitions exhibited by the extracts against *B. cereus, S. aureus, Micrococcus* sp., *E. coli, Klebsiella* sp. and *Proteus* sp. were compared with the reference antibiotic chloramphenicol (1%). Antibacterial activity of the garlic extract singly and its mixture with onion extract in the ratio 1:1 against the tested bacteria were found significantly higher ($p < 0.05$) than the onion extract.

Key words: Garlic, Onion, Antibacterial activity, Antioxidant activity, *Allium cepa*, *Allium sativum*.

INTRODUCTION

Antioxidant compounds in food are found to have a health-protecting factor (Rahman *et al* 2012). Various enzymatic and non-enzymatic constituents present in foods show antioxidant activities.Enzymatic antioxidant, *i.e.* superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Bray and Bettger, 1990; Powell, 2000), peroxidase (POD), glutathione reductase (GR) and glutathione S-transferase (GST) (Csiszár *et al* 2007), operate in concert together with several non-enzymatic molecules such as glutathione, α-tocopherol, ascorbic acid and β-carotene (Mahadik and Scheffer, 1996) to contrast the reactive oxygen species action and to avoid oxidative damage (Bray and Bettger, 1990; Powell, 2000) and thereby prevent the propagation of free radical chain reactions (Pavlović *et al* 2002; Mahadik and Scheffer, 1996).

Similarly, due to the ability to donate two hydrogen atoms, ascorbic acid can react with many different free radicals and has an antioxidative effect (Belitz and Grosch, 1992; Packer *et al* 2002).

Allium species such as onions *(Allium cepa)* and garlic *(Allium sativum)* are used as foodstuff, condiment, flavouring, and folk medicine (**Figure 1**). Onion has been revered throughout the time not only for its culinary use, but also for its therapeutic properties (Skrinjar and Nemet, 2009).

Onion bulbs contain a good number of phytochemicals, most of which are hydrocarbons and their derivatives (Griffiths *et al* 2002). Several authors have reported pharmaceutical

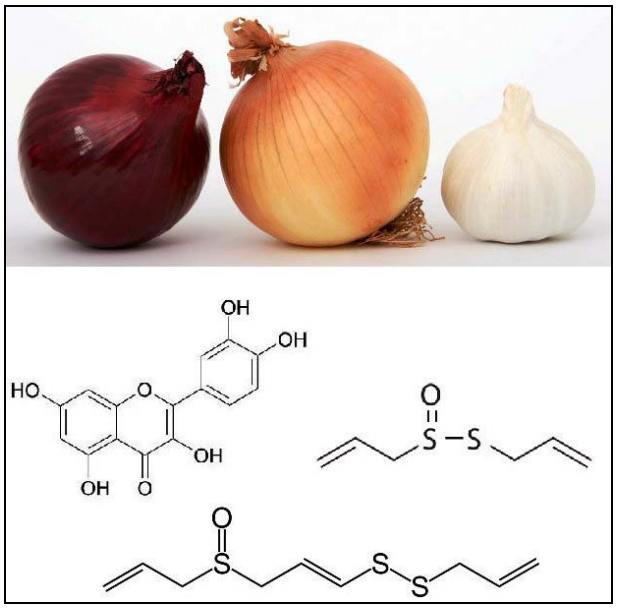

Figure 1. Garlic & onion with active constituents

activity of extracts of onion including antitumor, antidiabetic, antioxidant, antibacterial, antiallergic and molluscicidal activity (Helen *et al* 2000; Lampe, 1999). *In vitro* studies have shown onion to possess antibacterial, antiparasitic, and antifungal activity (Rose *et al* 2005; Zohri *et al* 1995), which includes dipropyl disulphide; flavour compound, allicin; antioxidant, antidiabetic, antihypertensive, antibiotic and antithrombotic activities, diethyl sulphide; insecticidal property, dimethyl disulphide; gas odorant, chemical synthesis, mercaptopropane/ propylmercaptan; flavour compound (Augusti, 1996; Rahman *et al* 2012; Skrinjar and Nemet, 2009). The main antibacterial agent in onion is quercetin and allicin (thio-2-propene-1-sulfinic acid-5-allyl-esters), quercetin binds to the bacteria DNA gyrase while allicin inhibits certain thiol containing enzymes in the microorganisms by the rapid reaction of thiosulfinates (Ankri and Mirelman, 1999).

On the other hand, garlic can be used as herbal medicine and has attracted particular attention of modern medicine because of its widespread health use around the world, and the cherished belief that it helps in maintaining good health, warding off illnesses; and providing more vigour (Banerjee and Maulik, 2002) and modern scientific research has revealed that garlic possesses numerous therapeutic properties that are similar to onion, including antioxidant effects, an ability to inhibit the formation of inflammatory compounds, and direct anti-inflammatory effects.

The allicin is considered to be most potent antibacterial agent and also exhibits anti-parasitic activity against major human intestinal parasites. Another constituents of garlic known as ajoene may play a great role as topical antifungal agent. The sulfur constituents in garlic have been verified for antiviral activity (Gebreyohannes and Gebreyohannes, 2013). Garlic derived organo sulfur compounds such as diallylsulfide, diallyldisulfide and diallyltrisulfide provide significant protection against carcinogenesis (Das *et al* 2007; Ziu *et al* 1994). Similarly, other pharmaceutical effects of garlic include antiulcer (Jiang *et al* 2008), antidiabetic (Sheela *et al* 1995), antihypertensive (Caro, 1978), lipid lowering agents (Cerella *et al*, 2011; Kim and Kwon, 2009), neuro-regenerative (Borek, 2006; Mathew and Biju, 2008), enhances male reproduction (Makar and Toth, 2002; Wilkes *et al* 2009), topical application in warts (Dehghani *et al* 2005) and probiotic effects (Jaime *et al* 2001). Also, extensive research reports have been published showing huge scope in area of biological and pharmacological activities of herbal extracts (Jain *et al* 2011; Srividya *et al* 2012; Sadanand and Palanivelu, 2015; Agarwal *et al* 2015; Avula *et al* 2015). Therefore, keeping in view the pharmaceutical potential of onion and garlic, present study was undertaken to evaluate antioxidant and antibacterial activities of fresh extracts of these two household species.

EXPERIMENTAL

Extract preparation

Fresh garlic and onion bulbs were purchased from the local market, Kathmandu, Nepal and were verified from National Agricultural Research Council (NARC), Kathmandu. Skins of garlic and the onion bulbs were peeled out, washed with sterilized water and air dried for about 2 h and sliced. Then the garlic and onion pieces were ground in an electric blender (Greenline) separately and filtered using the clean and dry muslin cloths. The crude juices were squeezed out, then they were further filtered through Whatmann filter paper No. 1 under vacuum pressure. The extracts were kept in a refrigerator at 4°C for further analysis. These extracts were used for enzyme activities, ascorbic acid content and antibacterial activities.

Determination of total protein

To calculate enzyme activities, total protein content of garlic and onion were estimated

which was helpful to calculate the enzyme activities. Protein of garlic and onion were determined by the Bradford method with bovine serum albumin (10-100 μg/ml) as standard (Bradford, 1976).

Determination of SOD activity

SOD activity was determined spectrophotometrically (Elico SL150 UV-VIS Spectrophotometer) by measuring the ability of the enzyme to inhibit photochemical reduction of Nitro blue tetrazolium (NBT) in the presence of riboflavin in light. For this, modified protocol was followed in which 1.3 ml of solution A (0.1 mM EDTA containing 50 mM Na_2CO_3) and 0.5 ml Solution B (90 μM NBT in Solution A). Then, 0.1 ml of Solution C (0.6% Triton X in Solution A) and 0.1 ml of solution D (20 mM Hydroxylamine.HCl, pH=6) were added to test tube and mixed well. After that, 5 μl of extract (garlic and onion) were added to the reagent mixture and immediately absorbance at 560 nm at an interval of 20 s was taken. One unit (U) of SOD was the amount that causes 50% inhibition of NBT reduction in light. The enzyme activity was expressed in terms of specific activity (U/mg protein) (Dhindsa *et al* 1981).

$$\% \text{ inhibition} = \frac{\Delta E/\min (NE) - \Delta E/\min (E)}{\Delta E/\min (NE)}$$

where:

$\Delta E/\min$ (NE) = Activity change (Non-enzyme source)

$\Delta E/\min$ (E) = Activity change (Enzyme source)

Determination of CAT activity

CAT activity was determined by decomposition of hydrogen peroxide (H_2O_2) which, in turn, was measured by the decrease in absorbance at 240 nm. For this, modified protocol was followed in which 13 ml phosphate buffer (50 mM, pH 7), 250 μl 0.75 M H_2O_2 were added, so that absorbance at 240 nm was approximately 0.5. Then, 10 μl of extracts (garlic and onion), 3 ml of H_2O_2 containing a phosphate buffer was added. The absorbance was read at 240 nm at the time interval of 20 s for 2 min. One unit (U) equals to the amount of H_2O_2 (in μmol) decomposed in 1 min (Upadhyaya *et al* 1985).

$$\text{Specific activity} = \frac{\Delta A240/\min \times \text{Vol. of mixture}}{0.071 \times \text{g of protein}}$$

Determination of ascorbic acid

For the determination of ascorbic acid, the extracts (garlic and onion) were diluted 10 folds with 6% meta-phosphoric acid. Firstly, 20 ml standard ascorbic acid (10 mg of ascorbic acid in 6% (*w/v*) meta-phosphoric acid) solution was transferred in an Erlenmeyer flask and titrated against the dye solution (2, 6-dichlorophenol-indophenol) till the appearance of a light pink colour. Similarly, 20 ml of respective extracts were also titrated against the dye solution and volume of dye used were recorded. Ascorbic acid was calculated as amount of ascorbic acid in 1 g of sample (mg/g) (CS, 2010).

Preparation of inoculums

About 18 h broth culture of the test bacteria isolates (*B. cereus, S. aureus*, *Micrococcus* sp., *E. coli*, *Kleibsella* sp. and *Proteus* sp.) were suspended into sterile nutrient broth. They were standardized according to the National Committee for Clinical Laboratory Standards (NCCLS, 2002) by gradually adding normal saline to compare their turbidity to McFarland standard of 0.5 which is expressed in colony forming unit (CFU) per millilitre (ml) and is approximately 1.0×10^6 CFU/ml.

Antibacterial activity

The antibacterial activity of the crude extract was determined in accordance with the agar-well diffusion method described by (Irobi *et al* 1994). The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (10^6 CFU/ml). Two hundred microliter of the standardized cell suspensions were spread on a Mueller-Hinton agar (Hi-media). Wells were then bored into the agar using a sterile 6 mm diameter cork borer. Approximately 50 μl extracts of the garlic, onion and 1:1 garlic and onion mixture was introduced separately into wells, allowed to stand at room temperature for about 2 h and then incubated at 37°C. Controls were set up in the parallel using 1% chloramphenicol and sterile distilled water were used to reconstitute the extract. After 24 h, the plates were observed for zones of inhibition.

Statistics

The obtained data were analyzed statistically in Microsoft Excel (window10) and SPSS (version 21). The two tailed type student's t-test was applied to find out significant difference in SOD and CAT activity and ascorbic acid content between garlic and onion extracts, as well as the zone of inhibition shown by garlic and onion, separately and in an equi-mixture with the

reference antibiotic against each selected bacteria.

RESULTS AND DISCUSSION

The present study investigated antioxidant activities of fresh extracts of garlic and onion, and antibacterial activities of fresh extracts of garlic, onion and 1 : 1 garlic and onion mixture. The six bacteria, suchas *B. cereus, S. aureus*, *Micrococcus* sp, *E. coli*, *Kleibsella* sp. and *Proteus* sp. were taken to study antibacterial activities.

In this study, the specific activities of enzymatic antioxidants SOD as well as CAT of garlic against onion were found to be knowingly high ($p < 0.05$) but the ascorbic acid content of onion against garlic was found to be suggestively high ($p < 0.05$) (**Table 1**).

Extracts obtained from the garlic and onion were assessed as singly and in combination for the inhibitory effects against the selected six bacteria and 1% chloramphenicol was taken as a reference. From the observation, garlic extracts singly produced the highest susceptibility (mm) toward *B. cereus* (51 ± 0.19). So, it was set up as the most sensitive bacteria, followed by *S. aureus* (37 ± 0.17) and *E. coli* (34 ± 0.48). All these three bacteria showed greater potency than 1% chloramphenicol with high significances ($p < 0.05$). Likewise, onion extracts susceptible only to *B. cereus* (20 ± 0.22) was too less susceptible compared to the reference, while other bacteria did not show any susceptibility to it. In the case of garlic and onion mixture, higher susceptibility was observed for *B. cereus* (36 ± 0.79), *S. aureus* (27 ± 0.25), *E. coli* (22 ± 0.21) than the reference with high significances ($p < 0.05$) and reverse with other bacteria. These observations suggested that garlic and onion mixture inhibited *Micrococcus* spp more strongly than the *B. cereus,* although *B. cereus* was inhibited strongly by garlic when applied singly (**Table 2**).

Experimental evaluation done by few researchers (Bray and Bettger, 1990) suggested that the SOD activity in onion was very high *i.e.* 13.24 unit/mg protein. Similarly, other researcher reported that the garlic had been effective against diseases, in the pathophysiology of which oxygen free radicals (OFRs) have been implicated. Effectiveness of garlic could be due to its ability to scavenge OFRs. The decrease in –OH adduct products was due to scavenging of hydroxyl (–OH) and not by scavenging of formed –OH adduct products. These results suggest that allicin scavenges –OH and has antioxidant activity (Skrinjar and Nemet, 2009). A few authors also suggested that onions contain sulfur and quercetin both being strong antioxidants. They both have shown to help neutralize the free radicals in the body and protect the membranes of the body's cells from damage (El-Meleigy *et al* 2010). This finding of ascorbic acid also agrees with other authors who reported that the onion has a high level of ascorbic acid content that helps to improve resistance to infection, making it valuable for colds and flu. It is taken as preventive for many conditions including stomach infection, circulatory problems and arteriosclerosis (Powell, 2000).

The extent of the bacterial inhibitory effect of the onion could be attributed by their mixture. The antibacterial activity of onion extract can be attributed to the presence of flavonoids and polyphenols which has been reported to a have broad spectrum of antibacterial activity (Hendrich, 2006). Polyphenols from plants have been reported to have antibacterial activity (Ani *et al* 2006). The same observations were reported where bacterial effect of onion extract against *E. coli, S. aureus* and *S. entritidis* by using the agar diffusion method (Skrinjar and Nemet, 2009). Moderate antibacterial activity of onion against *E. coli* and *S. Typhimuium* were also observed in few data as well as they demonstrated that various mixture of *S. entritidis* and *S. Typhimuium* (Skrinjar and Nemet, 2009; Zohri *et al* 1995). The major antibacterial effect of garlic is due to allicin (Abdou *et al* 2001) so that, garlic extracts have been found to possess antibacterial property against *S. typhimuium*, *E. coli* No 1, *S. epidermidis*, *S. Aureus* (Ani *et al* 2006; Ankri and Mirelman, 1999). Some of the advantages that herbal preparations have over the synthetic ones are that they do not act directly on bacteria, but create an adverse environment for them, thus threatening their survival and they have also been found to deter the development of resistant strains of microorganisms (Irobi *et al* 1994).

CONCLUSION

The SOD and CAT activities in present study revealed very high potential in garlic as compared to that of onion but reverse was true in case of ascorbic acid. In case of selected bacterial species, onion singly showed the least antibacterial activities whereas Garlic singly was found to be more potent compared to the equi-mixture of garlic and onion. Thus, it can be concluded that the antioxidant activities of garlic are higher than onion and also the antibacterial

Table 1. Specific activities of enzymatic antioxidants (SOD and CAT) and non-enzymatic antioxidants (ascorbic acid) in the garlic and onion extracts

Name of plants	Enzymatic antioxidants		Non-enzymatic antioxidants
	SOD (unit/mg protein)	CAT (unit/mg protein)	Ascorbic acid (mg/g)
Garlic	17.51 ± 0.05	259 ± 0.04	0.46 ± 0.04
Onion	3.74 ± 0.04	26.62 ± 0.11	0.54 ± 0.04

SOD: Superoxide dismutase, CAT: Catalase

Table 2. Zone of inhibition produced by garlic, onion bulbs and equal mixture of garlic and onion against the bacteria

Organisms	Zone of inhibition (mm)			
	Garlic	Onion	Garlic : Onion	1% Chloramphenicol
B. cereus	51 ± 0.19	20 ± 0.22	36 ± 0.79	32 ± 0.12
S. aureus	37 ± 0.17	ND	27 ± 0.25	12 ± 0.49
Micrococcus sp.	42 ± 0.05	ND	40 ± 0.19	52 ± 0.45
E. coli	34 ± 0.48	ND	22 ± 0.21	21 ± 0.48
Kleibsella sp.	22 ± 0.44	ND	18 ± 0.19	26 ± 0.70
Proteus sp.	18 ± 0.49	ND	17 ± 0.61	21 ± 0.39

ND: Not detected, 2-tailed t-test: Significance ($p<0.05$)

activities of garlic and its mixture are more potent than onion alone.

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