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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 $B.\ cerus$, $S.\ aureus$, 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
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-allylesters), 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 antiparasitic 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 diallysulfdie, 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). 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 Na2CO3) 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. About 18 h broth culture of the test bacteria isolates (B. cereus, S. aureus, Micrococcus sp., E. coli, Klebsella 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 borner. 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...
results indicate 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 have a 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 equimixture 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

<table>
<thead>
<tr>
<th>Name of plants</th>
<th>Enzymatic antioxidants</th>
<th>Non-enzymatic antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOD (unit/mg protein)</td>
<td>CAT (unit/mg protein)</td>
</tr>
<tr>
<td>Garlic</td>
<td>17.51 ± 0.05</td>
<td>259 ± 0.04</td>
</tr>
<tr>
<td>Onion</td>
<td>3.74 ± 0.04</td>
<td>26.62 ± 0.11</td>
</tr>
</tbody>
</table>

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

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

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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