ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF TUBER EXTRACTS OF GLOBBA BULBIFERA

Narasinga Rao V1,*, DSVGK Kaladhar1

1Dept. of Biochemistry, GIS, GITAM University, Visakhapatnam, AP, India.

ABSTRACT

Various Zingiberaceous plants show good phytocompounds in treatment of various human ailments. An experimentation on antioxidant and antimicrobial activity in tuber extracts of Globba bulbifera has been conducted. The Globba bulbifera tuber extracts shown antioxidant activity of the tested plant extracts (490 µg/ml to 680 µg/ml) is less compared to the standard (40 µg/ml). Methanol, ethanol, ethyl acetate and aqueous tuber extracts has shown good antimicrobial activity against tested microbes (9 to 32mm zone diameter). The extracts have shown good activity against fungi (10 to 23 mm) when compared with gram negative bacteria (10 to 21 mm) and gram positive bacteria (9 to 21mm). Hence Globba bulbifera tuber extracts has shown good antioxidant and antimicrobial activities.

Keywords : Globba bulbifera, solvent extraction, antimicrobial activity, antioxidant studies.

INTRODUCTION

The pharmacological companies produced a number of new antibiotics in the last four decades, resistance to these drugs by microorganisms has increased. The bacteria have the genetic ability to transmit and acquire resistance to antibiotics, which are utilized as therapeutic agents .The microbial resistance is developing and the use of antimicrobial drugs in the future is still uncertain. Hence, the actions must be taken to reduce these problems and to control the use of antibiotics, provide develop research and better understand the genetic mechanisms of resistance to continue studies to develop new drugs like natural or synthetic. The ultimate aim is produce appropriate and efficient antimicrobial drugs to the patients. The plants have a valuable source of natural products for maintaining good human health, in last decade, with more intensive studies for natural therapies. The use of plant metabolites for
pharmaceutical purposes has gradually increased in India. According to World Health Organization medicinal plants would be the best source to obtain a various varieties of drugs. About 75% of individuals from developed countries use traditional medicine, which are derived from medicinal plants. Therefore components from medicinal plants should be investigated to better understand their properties and efficiency[1].

The Reactive oxygen species (ROS) are free radicals like superoxide anion radicals (O$_2^-$), hydroxyl radicals (OH$^-$) and non-free-radical species such as H$_2$O$_2$ and singlet oxygen (1$^1$O$_2$), are various forms of activated oxygen. The importance of free radicals increasing attention over the decades. These molecules are exacerbate factors in cellular injury and in the aging process. ROS have significant interest among scientist and their broad range of effects on biological and medicinal systems studied in many experimental investigations. In living organisms, various ROS can form in different traditions. Normal aerobic respiration stimulates polymorphonuclear leukocytes, peroxisomes and macrophages appear to be the main endogenous sources of most of the oxidants produced by cells. The exogenous sources of ROS include tobacco smoke, certain pollutants, pesticides, and organic solvents [2]. Aging is one of the inimitable features in all organisms. The impaired function of many systems characterizes aging. When impairments occur in the brain, the susceptibility to neurodegenerative diseases amplifies considerably. The free radical presumption of aging posits that the functional impairments in brains are due to the attack on critical cellular components by free radicals, reactive nitrogen species and reactive oxygen species produced during normal metabolism [3].

Flavonoids are ubiquitous in photosynthesizing cells and are commonly present in fruits, vegetables, nuts, seeds, stems, tubers, flowers, tea, wine, propolis and honey. From centuries, these compounds have the principal physiologically active constituents have been used to treat human diseases. These class of natural products is becoming the subject for anti-infective research, and lots of groups have isolated and identified the structures of flavonoids possessing anti-fungal, anti-bacterial anti-viral activity and anti-oxidant properties[4]. The honey used as a medicine from ancient times in many cultures and is still used in ‘folk medicine’. The honey used as a therapeutic substance in the medical profession in recent times, and it is acceptance for the treatment of ulcers and bed sores, and other infections substantial from burns and wounds. So this indicates honey was used in infections not
responding to standard effective in rapidly clearing infection and promoting good healing. Honey has also been found to be effective in treating the bacterial gastro enteritis in infants[5]. The family Zingiberaceae consists of large number of medicinal plants and is well known for use in ethnomedicine. The study of this family indicates systematically analyse, and the use of Zingiberaceous plants for the treatment of various human ailments from NE India, in order to the valuation of biodiversity and its conservation and for future pharmacological studies[6]. The various medicinal plants are good sources for nutrient and non nutrient molecules, many of which have anti-oxidant and anti-bacterial, anti-viral and anti-fungal properties which can protect the human body against cellular oxidation reactions and pathogens[7]. The volatile oils of plants are generally isolated from plant material by steam distillation or hydro distillation and are variable mixtures of terpenoids, like monoterpenes[C_{10}], sesquiterpenes [C_{15}] and diterpenes [C_{20}] may also be found, and a mixture of low molecular weight aliphatic hydrocarbons (linear, saturated and unsaturated), alcohols, aldehydes, acids, acyclic esters or lactones and exceptionally nitrogen- and sulphur-containing compounds, coumarins and homologues of phenylpropanoids[8].

The extend of drug resistant pathogens is one of the serious threats to treatment of microbial diseases. Behind the ages essential oils and other extracts of plants have evoked as sources of natural products. They screened for their potential uses as alternative remedy for the treatment of many infectious diseases[9]. Essential oils possess good anti-bacterial, anti-fungal, anti-viral insecticidal and antioxidant properties. Some oils used in cancer treatment. Some other essential oils used in fragrance industries, food preservation and aromatherapy. The essential oils are a rich source of biologically active compounds which are increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils. So, it is reasonable to expect a variety of plant compounds in these oils with specific general antimicrobial activity and antibiotic potential [10].

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. The natural products, either as pure compounds or as standardize plant extracts; provide unlimited sources for new drug leads because of the availability of chemical diversity. There is a urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [11]. The severe infections caused by pathogenic microorganisms increased and an important cause of morbidity and mortality patients in worldwide[12]. In developing
countries, found that about three quarters of the population depends upon plant based preparations used in their traditional medicinal system and as the basic needs for human primary health care. Hence, several medicinal plants evaluated for possible antimicrobial activity and to get good remedy for a variety of ailments of microbial origin [13].

The natural antioxidants had shown a wide range of biochemical activities, like inhibition of ROS generation, through indirect or direct scavenging of free radicals, and change of intra cellular redox potential. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and the simultaneous protein damage, lipid per oxidation and DNA strand breakage. An antioxidant, which can quench reactive free radicals, can prevent the oxidation of various other molecules and may have health promoting effects in the prevention of degenerative diseases. In addition there is a converse relationship between dietary intake of antioxidant rich food and the incidence of human diseases [14]. Microbes shown the genetic ability to transmit and acquire resistance to antibiotics, which are utilized as therapeutic agents. Though pharmacological industries are producing a number of new antibiotics in the last two decades, resistance to these drugs by microorganisms increased to great extant. The new infections can occur in hospitals resulting in high mortality, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, these are multi-resistant [15]. The Nature has been a source of medicinal agents for including all types of living organisms for thousands of years with various energy sources. The herbal medicines used to control major diseases and the require to discover new molecular structures as lead compounds. The healing activity may be slow with the use of plant extracts but have permanent cure against various diseases [16].

MATERIALS AND METHODS
Collection of plant materials
Fresh plant consisting of leaves and rhizomes, were collected from Kerala during the July and August 2012 and fully matured tubers were washed thoroughly and dried in sunlight. The dried plant tubers were grinded to powder and 150gms of the dried plant material was used for the extraction.

Extraction Process
Freshly collected tubers of Globba bulbifera were dried under shade and powdered. The powdered material (150g) was extracted with ethanol, ethyl acetate, methanol and water.
separately using hot extraction with Soxlet Apparatus. The extracts obtained were concentrated to a small volume under Vacuum (50°C) and then dried in vacuum desiccators.

**Soxhlation process**

Soxhlation is an extraction process by which the plant material is going to be mixed with particular solvent and the plant extract is obtained by maintaining particular temperature. Weigh the plant material.

Nearly 150gms of weighing powder is taken and it is packed in a filter paper. Place the powder in a soxhalet apparatus. Take 200ml of solvent in a round bottomed flask and place it to the downside of the soxhlet, and top side of the soxhalet immerse. The condenser contains the outlet and inlet pipeline for continuous water supply to avoid evaporation of solvents. The total setup have to placed in water bath and allowed it to boil for 6 hours at desired temperature because each solvents having different boiling points.

In this apparatus the boiling of the plant material along with the solvents take place. Adjustment of the temperature is based on the boiling point of the solvents. While the process is going on the solvent gets dark brown color due to mixed up with plant material. The end point of the boiling solvent is noted based on the disappearance of the color solvents become a colorless solvents, may consider as an end point. In this process the solvent binds with the organic compound of the plant material is eluted out now that extract is called filtrate.

**Anti oxidant activity with DPPH**

Standard:  Ascorbic acid
Chemicals & Reagents
a. DPPH, Methanol

Nearly 100 µM DPPH (1,1-diphenyl-2-picrylhydrazyl): 3.9432 mg of DPPH was dissolved in methanol and made up to 100 ml to obtain a final concentration of 100 µM.

b. Stock solutions of test items: 3mg/ml stock solutions were prepared in DMSO.

c. Test items preparation: Appropriate dilutions (0.39 to 100µg/mL) of test items were prepared.

The reaction mixture was made by addition of 20ul of test items and 280ul of DPPH reagent to reach a final volume of 300ul and kept incubation in dark for 50 minutes and then
absorbance was read at 517 nm using spectrophotometer. An IC₅₀ value was determined as the concentration that elicits the half maximal response.

**Antimicrobial activity**

**Microorganisms**

Microbes from MTCC (Microbial Type Culture Collection) have been used in the present study. Various bacteria used in the present research work are Bacillus subtilis (MTCC 441) and Klebsiella pneumonia (MTCC 618) belongs to gram positive bacteria and Serratia marcescens MTCC 86, Pseudomonas aeruginosa MTCC 424, Enterobacter aerogenes MTCC 111 and Escherichia coli MTCC 443 belong to gram negative bacteria. Fungi used in the work are Aspergillus niger MTCC 282 and Candida albicans MTCC 227.

**Antimicrobial activity using Zone method**

The bacteria were grown in Muller-Hinton media (HiMedia Pvt. Ltd., Mumbai, India) at 37°C for 24 hours and fungi in Sabourand Dextrose Media (Himedia Pvt. Ltd., Mumbai, India) at 25°C for 72 hours, and were maintained on nutrient agar slants at -20°C. Inoculum of test organisms was prepared by growing pure isolate in nutrient broth for overnight. The overnight broth cultures were sub cultured in fresh nutrient broth and grown for 3 hours to obtain log phase culture. The agar plates were prepared by pour plate method using Muller-Hinton agar (MHA) medium for bacteria and Sabourand Dextrose agar (SDA) Media for fungi. The sterile MHA/SDA medium cooled to 45°C and mixed thoroughly with 1ml of growth culture of concerned test organism (1x10⁸ cells) and then poured into the sterile petri dishes and allowed to solidify. Wells of 8mm size were made with sterile borer and test extracts were added. The MHA plates were incubated at 37°C for 24 hrs for bacteria. The SDA plates were incubated at 25°C for 72 hrs for fungi. The diameter of zones of inhibition was measured in mm using HiMedia zone reader.

**RESULTS AND DISCUSSIONS**

India has about 45,000 plant species that has been claimed to possess medicinal properties [17]. Various parts of plants like leaves, roots, tubers, barks and seeds, are employed in ethanomedicine. A considerable part of this indigenous knowledge was documented from the past into the organize systems medicines such as Ayurveda, Yunani, sidha or other systems [18]. Caffeic acid (3,4-dihydroxycinnamic acid) is among the major hydroxyl cinnamic acids present in wine sinapic acid, which is a potent antioxidant. It also identified as one of the active antioxidant [19]
One such plant *Globba bulbifera* belongs to family Zingiberaceae shown to be having antimicrobial and antioxidant by present investigation. *Globba bulbifera* is a vulnerable medicinal plant source rarely available and becoming extinct due to climatic and other factors. These plant tubers shown good medicinal activities and secondary metabolite products have not been extensively documented. In the present days the whole plant *Globba bulbifera* are used for anti fungal, anti bacterial and anti oxidant properties for extracting plant metabolites against bacteria and fungi by using agar diffusion methods and the zones of inhibition, and DPPH radical activity were calculated.

The *Globba bulbifera* tuber extracts shown antioxidant activity with IC50 value for ethanol is 490 µg/ml, 560µg/ml for methanol, ethyl acetate is 590µg/ml and aqueous extract shown is 680 µg/ml. The IC50 value for standard (ascorbic acid) obtained in the experimentation is 40 µg/ml (Figure 1, Table 1). Though the antioxidant activity of the tested plant extract is less compared to the standard, the plant has shown good antioxidant activity.

![Figure 1: IC50 for tuber extracts of Globba bulbifera](image)

**Table 1: Spectrophotometric readings for antioxidant activity of Globba bulbifera**

<table>
<thead>
<tr>
<th>Log conc. (µg/ml)</th>
<th>Standard (Ascorbic acid)</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Ethyl acetate</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>31.25</td>
<td>40.13</td>
<td>5.55</td>
<td>7.33</td>
<td>5.42</td>
<td>5.02</td>
</tr>
<tr>
<td>62.5</td>
<td>75.63</td>
<td>11.21</td>
<td>15.43</td>
<td>10.43</td>
<td>9.22</td>
</tr>
<tr>
<td>125</td>
<td>80.75</td>
<td>22.45</td>
<td>26.32</td>
<td>21.25</td>
<td>19.41</td>
</tr>
<tr>
<td>250</td>
<td>100.23</td>
<td>33.23</td>
<td>37.32</td>
<td>31.32</td>
<td>29.85</td>
</tr>
<tr>
<td>500</td>
<td>125.43</td>
<td>44.53</td>
<td>49.65</td>
<td>42.15</td>
<td>40.21</td>
</tr>
<tr>
<td>1000</td>
<td>250.54</td>
<td>80.32</td>
<td>90.23</td>
<td>78.32</td>
<td>66.23</td>
</tr>
</tbody>
</table>
The plant tuber extracts were analyzed for antimicrobial activity against test microorganisms. All the prepared extracts have shown good antimicrobial activity. The results of antimicrobial activity of plant tuber extracts were represented in Table 2.

**Table 2: Antimicrobial activity of *Globba bulbifera***

<table>
<thead>
<tr>
<th>Organism</th>
<th>Zone of inhibition in mm (including well size of 8mm) at 100 µg/ml</th>
<th>Antibiotic at 10 µg/ml (Tetracycline)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol extract</td>
<td>Ethanol extract</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>32</td>
<td>21</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>19</td>
<td>20</td>
</tr>
</tbody>
</table>

Methanol, Ethanol, Ethyl acetate and aqueous tuber extracts has shown good antimicrobial activity against tested microbes (9 to 32mm zone diameter). The extracts have shown good activity against fungi (10 to 23) when compared with gram negative bacteria (10 to 21) and gram positive bacteria (9 to 21mm). The zone of inhibition for the bacterial standard, Tetracycline has shown inhibition zone from 10 to 14 mm at 10 µg/ml. The zone of inhibition for fungal standard, rifampin has shown inhibition zone for 13 and 16 mm for *Aspergillus niger* and *Candida albicans* respectively at 10 µg/ml. Antifungal medicinal plants belong to the Zingiberaceae family has also been reported in plants like *Curcuma longa* L., *Curcuma zedoaria* Rosc., *Curcuma malabarica* Vel., and *A. galanga* supports the use of their tubers in traditional medicine for the treatment of bacterial and fungal infections [20].

The potential compounds for developing antimicrobials from plants appear worthwhile, as it will lead to the development of a phytomedicine to act against microbes. The Plant based
antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials [21].

CONCLUSION
The *Globba bulbifera* has a wide spectrum of antimicrobial activity including antibacterial and antifungal activities. The *Globba bulbifera* tuber extracts has also shown antioxidant activity. With the ever increasing resistant strains of microorganisms to the already available and synthesized antibiotics, the naturally available *Globba bulbifera* could be potential alternative.

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REFERENCES


