Study on antioxidant and antimicrobial activities of methanolic leaf extract of *Glycosmis pentaphylla* against various microbial strains

Israt Jahan Bulbul and Nisrat Jahan

Abstract

The purpose of the present study was to investigate the antioxidant and antimicrobial potential of methanolic extract of *Glycosmis pentaphylla* (family: Rutaceae) leaf which possesses significant drug value. *In vitro* antioxidant activity was performed using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity and total phenol content by Folin–Ciocalteu Reagent. The antioxidant activity of different concentrations of plant extracts were found to increase in a concentration dependent manner where the IC₅₀ value was 69.64 μg/ml compared to the ascorbic acid (27.02 μg/ml). The total phenol content was found in plant extract was 25.28 mg/g GAE. The antimicrobial assay was performed by disc diffusion method using kanamycin and nystatin as the standard. The highest antimicrobial action was found to be against *Salmonella paratyphi* (zone of inhibition with 22 mm). The MIC was carried out by the serial dilution technique where it showed highest inhibitory effect with minimum concentration of 16 μg against *Salmonella paratyphi* and *Escherichia coli*. The obtained results indicate that investigated plant could be potential sources of natural antioxidants and antimicrobial agents and can be used in the treatment of oxidative disorders and many infectious diseases caused by microorganisms.

Keywords: *Glycosmis pentaphylla*, Antioxidant activity, Antimicrobial activity, Minimum Inhibitory Concentration (MIC)

1. Introduction

Medicinal plants impart an effective place by reducing mortality on participation in the cure of various severe diseases and ensure human wellbeing. According to the recent estimation of WHO more than 80 people of developed country rely on traditional medicines for the health need and 60 people use vitamin and phytotherapy for the health benefits at some points [1]. Many phytochemical compounds like phenolics, polyphenols, terpenoids, quinones act as antioxidants and antimicrobial agents. Antioxidant protects the body cells from highly reactive oxygen species (ROS) called free radicals including superoxide, hydrogen peroxide, hydroxyl (HO), peroxyl (ROO) and alkoxyl (RO) are produced *in vivo* during oxidation [2,3]. These antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS, and therefore can enhance the immune defense and lower the risk of health complications caused by oxidative stress [4]. They have effects on atherosclerosis, ischemic heart disease, cancer, Alzheimer’s disease, in the aging process, ischemia-reperfusion injury in hind limb, DNA damage, liver damage.

Phenolic compounds, tannins, flavonoid and alkaloids are the most important antimicrobial agent and bioactive constituents in plant [5]. Tannin and flavonoid compounds inhibit the microbial growth by causing the bacterial colonies to disintegrate which probably results from their interference with the bacterial cell wall [6]. They also hasten the healing of wounds, inflamed mucous membrane and arrest bleeding [7, 8]. Multiple drug resistance in human pathogenic microorganism has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. So this work was carried out to determine the antimicrobial and antioxidant potentials of *G. pentaphylla* in advert to search for new source of medicine.

*Glycosmis pentaphylla* (Bengali name- Ashsaoara, Kawatuti, Matmati, Ban Jamir) belongs to the family Rutaceae. It is a shrub or small (1.5–5.0 m) tree widely distributed from India, Malaysia and Southern China to the Philippine Islands and represented by nearly 11 species [9]. Traditionally the leaf is used as juice and paste in the treatment of fever, liver complaints, cough [10], rheumatism, anemia, jaundice, eczema, ascariasis, skin infections bone fracture, fracture induced pain and certain other diseases.
Stem is used as toothbrushes for its fibrous nature [11]. The plant G. pentaphylla have some phytochemical constituents like flavonoids, phenols, steroid, tannins, saponin, protein, glycoside [12], glyco-quinone (1) and glycocitrine-III [13] terpenoid [14], amides, imides, coumarins, arborine, glycozolinic, 3- formylcarbazole, glycosinine, mupamine, varbazole, 3- methyl carbazole, glycolone, glycosolidol, glycozolinic, glyrophymoline, glycomide, glycozoline, noracrone, des- N-methylacronym, des-N-methylnoracronyince, furoquinoline bases (kokusaginine and skimminianie), alkaldoids (glycosine, arborine, glycosminine, glycosamine, glycine, glycine, arbinol and isorbinol), arborine, two isomeric terpene alcohols, myrcyl alcohol, stigmaster, S-sitosterol, arborine, and furthermore glyoric acid, glycozolidine, dictamine [15]. So guided by the literature review of G. pentaphylla the present study has been carried out to determine antioxidant and antimicrobial activity of this plant.

2. Materials and methods
2.1 Sample Collection & Preparation
Sample leaves of G. pentaphylla were collected from Gazipur, Bangladesh in January 2014 and authentication of the plant sample has been done by the taxonomist and a voucher specimen (DACB-35914) was submitted to the national herbarium center Mirpur, Dhaka, Bangladesh. After proper identification, leaf samples were placed for sun drying for several days, then oven dried for 24 hours at considerably low temperature (40 °C) for better grinding and the dried samples were ground into coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Dept. of Pharmacy, Southeast University and stored in an airtight container for further study. The leaves were dried at room temperature, ground into uniform powder to increase the surface area of the sample for extraction. 500g powder of leaf was weighed, poured a sterile flat bottomed flask containing 1000 ml of methanol with vigorous shaking for 7 days to enhance proper dissolution of the bioactive compounds in the samples and then filtered by cotton plug followed by Whatmann filter paper (125mm) at room temperature. The filtrates were concentrated at 45 °C under reduced pressure using vacuum pump rotary evaporator (Stuart rotary evaporator, RE3022,UK) until the extracts became completely dry. The extracts were stored at 4°C in a refrigerator until required for further analyses [5].

2.2 Microorganisms
Antibacterial activity was determined by using fourteen microorganisms including four Gram positive bacteria (Bacillus cereus, Bacillus subtilis, Sarcina lutea and Staphylococcus aureus), seven Gram negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, Shigella dysenteriae, Shigella boydii, Vibrio parahaemolyticus, Vibrio mimicus) and three Fungi (Candida albicans, Aspergillus niger, Saccharomyces cerevisiae). The microorganisms were obtained from the Institute of Nutrition and Food Sciences (INFS), Dhaka University The bacterial strains were first subcultured in nutrient agar (NA) medium & incubated at 37 °C for 18 h while the fungal strains were subcultured for 72 h at 25 °C [10].

2.3 In vitro Antioxidant Activity
2.3.1 DPPH radical scavenging activity
DPPH radical scavenging activity of the methanolic extract of G. pentaphylla extracts was determined on the stable radical 1, 1-diphenyl-2- picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams et al. [17], 2 ml methanol solution of plant extract or standard ascorbic acid at different concentrations were mixed with 3 ml methanol solution of DPPH in the test tube. The test tube was incubated at room temperature for 30 min in a dark place to complete the reaction. The absorbance of the solution was measured at 517 nm by UV spectrophotometer (UV–1800PC SHIMADZU, Japan) The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extracts as compared to that of ascorbic acid. DPPH free radical scavenging ability (%) was calculated with the following formula:

\[ \text{IC}_{50} = \frac{A_0 - A}{A_0} \times 100 \]

Where A0 is the absorbance of the control and A is the absorbance of the test or standard. Then % inhibitions were plotted against respective concentrations used and from the graph IC50 was calculated. The lower IC50 indicates higher radical scavenging activity and vice versa [19].

2.3.2 Total phenol content
Total phenolic content in the extracts was determined by the Folin-Ciocalteu reagent method described by Demiray et al. [19], 1 ml of extract solution (200μg/ml) extracts was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 (v/v) and 4 ml (75g/L) of sodium carbonate. A series of gallic acid solutions were prepared in the range of 1-100 mg. The tubes were vortexed for few seconds and allowed to stand for 30 min at 26 °C for color development. Absorbance of samples and standard were measured at 765 nm. All the sample analyses were carried out in triplicates (n=3). Total phenol content was expressed as gallic acid equivalents (mg GAE) per gram of dry weight [20].

2.4 In vitro Antibacterial activity
2.4.1 Disc diffusion Method
Antibacterial activity of methanolic extract of G. pentaphylla was carried out by disc diffusion method. To prepare agar media solution 28 grams of agar was mixed with 1L of water followed by autoclaving for sterilization for 20 minutes at 121 °C. With the help of an inoculating loop, the test organisms were transferred from the pure culture to the agar slants under a laminar air flow unit. The inoculated slants were then incubated at 37 °C for 18-24 hours to ensure the growth of the test organisms. The red heat of loop is required before each and every bacteria inoculation. The agar media was taken in test tube after autoclaving and the organism was transferred from the subculture to the test tube containing 10 ml of autoclaved melted medium with the help of an inoculating loop in an aseptic area. The test tubes were shook by rotation to get a uniform suspension of the organism. The bacterial suspensions were immediately transferred to the sterile petri dishes (120 mm diameters) with and rotated clockwise and anticlockwise for the homogenous distribution of organism in media (depth 4 mm). Dried and sterilized filter paper discs (5 mm diameter) were then impregnated with 10 μl (500 μg/disc) of the test substances using micropipette. Standard antibiotic discs (Kanamycin 30μg/disc), standard antifungal discs (Nystatin 30 μg/disc) and blank disc (impregnated with solvent) were used as a positive and control. Positive controls were used to ensure the activity of standard antibiotic against the test organisms along with the comparison of the response between antimicrobial agent and the test samples [21] & negative controls was used to ensure the residual solvent and the filter paper not active themselves [22].
After incorporation petri dishes were transferred to the refrigerator for 1-2 hours at inverted position for the diffusion of drug. After 1-2 hours the petri dishes were transferred to the incubator at 37 °C for 12-18 hours. The test material having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antibacterial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The measurements were carried out in triplicate to have average zone of inhibition [23].

2.4.2 Minimum Inhibitory Concentration (MIC)
Minimum inhibitory concentration (MIC) is the lowest concentration which resulted in maintenance or reduction of inoculums viability Serial tube dilution technique was used to determine MIC of the extract against these bacteria. Stock solution was prepared by dissolving the plant extract of 1.024 mg/2 ml distilled water (512 µg/ml) (3 drops Tween 80 was added to facilitate dissolution). In serial dilution technique, 1 ml prepared stock solution was transferred to test tube containing 1 ml nutrient broth medium (256 µg/ml). Serial dilution was carried out to get 128, 64, 32, 16, 8, 4, 2 µg/ml of concentration. After preparation of suspensions of test organisms (107 organisms per ml) 1 drop of suspension (0.02 ml) was added to each broth dilution. Distilled water with 3 drops of Tween 80 and kanamycin were used as negative and positive control respectively. After 18 h incubation at 37 °C, the tubes were then examined for the growth. The MIC of the extract was taken as the lowest concentration that showed no growth. Where the concentration of the extract was below the inhibitory level, growth was observed in those tubes with turbid (cloudy) broth medium [19].

3. Result
3.1 DPPH radical scavenging activity
DPPH radical scavenging activity of methanol extract of G. pentaphylla is shown in Figure 1 where increasing the concentration of the extract, activity was found to increase remarkably and the inhibitory capacity of the plant extract was found good in comparison to the reference antioxidant ascorbic acid. The percentage of inhibition of methanolic extract of G. pentaphylla was 58.33 at concentration of 100 µg/ml. In case of DPPH method for the studying of the antioxidant potential the methanolic extract of G. pentaphylla provided IC₅₀ value 69.64 µg/ml where IC₅₀ value for ascorbic acid was 27.02±4.52 µg/ml.

3.2 Total phenolic content
The methanolic extract of G. pentaphylla leafs was subjected for the determination of the total phenol content. Total phenol content of the samples was expressed as mg/g GAE (gallic acid equivalent). The amount of total phenol content in G. pentaphylla was 25.28 mg/g GAE. Different studies suggest that different types of polyphenolic compounds such as flavonoids, phenolic acids were found in plants have multiple biological effects, including antioxidant activity [24].

3.3 Determination of Zone of inhibition
The search for compounds with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistance microorganisms. The antibacterial activity of the methanolic extract of G. pentaphylla was studied against gram positive, gram negative bacteria and fungi shown in Table 1

Table 1: Antimicrobial activity of test samples

<table>
<thead>
<tr>
<th>Test microorganisms</th>
<th>G. pentaphylla (mg/disc)</th>
<th>Kanamycin (30 µg/disc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>17</td>
<td>42</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>9</td>
<td>42</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td>Sarcina lutea</td>
<td>15</td>
<td>42</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>Salmonella paratyphi</td>
<td>22</td>
<td>42</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>Shigella boydii</td>
<td>12</td>
<td>42</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus</td>
<td>12</td>
<td>42</td>
</tr>
<tr>
<td>Vibrio mimicus</td>
<td>9</td>
<td>42</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>17</td>
<td>42</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>17</td>
<td>40</td>
</tr>
</tbody>
</table>

G. pentaphylla provided a significant antimicrobial effect, producing a diverse range of zone of inhibition where the average diameter is 8-22 mm. The maximum zone of inhibition was 22 mm against S. paratyphi and the minimum zone of inhibition is 8 mm against C. albicans. It has also provided the zone of inhibition 9 mm against B. subtilis & V. mimicus; 12 mm against V. parahaemolyticus, S. dysenteriae, S. boydii, P. aeruginosa; 15 mm against S. aureus, S. lutea 17 mm against B. cereus, S. cerevisiae, A. niger and 20 mm against E. coli.

3.4 Minimum Inhibitory Concentration (MIC)
According to the obtaining result from Antimicrobial Activity the minimum inhibitory concentration (MIC) of methanolic extract of G. pentaphylla leaf was determined by serial dilution technique against 14 microorganisms. The highest MIC of G. pentaphylla was found against S. paratyphi & E. coli with 16 µg of MIC.
4. Discussion

Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. In this study, antioxidant potential of methanolic extract of G. pentaphylla leaf was evaluated based on the ability to scavenge the DPPH. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule and is usually used as a substrate to evaluate the antioxidant activity of a compound [25]. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet color). As the electron became paired of in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stoichiometrically coincides with the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the test drugs to scavenge the free radicals independently [26]. Based on the data obtained from this study, DPPH radical scavenging activity of G. pentaphylla leaf extract was higher compared to that of ascorbic acid. It was revealed that G. pentaphylla leaf extract did show the proton donating ability served as free radical inhibitor or scavenger, converted them into more stable products and terminate the radical chain reaction. This may be important in protecting cellular DNA, lipids and proteins from free radical damage [27]. Several studies have also revealed that the phenolic content in the plants are associated with their antioxidant activities for their redox properties that allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. Phenol constituents are well known for their high antioxidant activity based on the absorbance values for the various extract solution, reacted with Folin-Ciocalteau reagent and compared with the standard solutions of gallic acid equivalents. It is understood that the high phenolic content may be responsible for the free radical scavenging activity of the plant extracts [28].

The zone of inhibition obtained of methanolic extract of G. pentaphylla and the standard drug for the antibacterial activity were in the range of 8-22 mm against all tested bacteria and fungi. This inhibitory activity may be caused by the presence of some active principles in them which are able to restrict the growth of bacteria by inhibiting protein synthesis of bacterial cell wall or alter the membrane function, inhibit protein synthesis or synthesis of purine and pyrimidines, hinder respiration or antagonize the metabolic pathways of microorganism leading to retardation of growth of bacteria [29]. The highest zone of inhibition found in methanolic extract of G. pentaphylla was found against S. paratyphi and the lowest zone of inhibition against C. albicans. It was found at the antimicrobial activity of the extracts correlated strongly with the DPPH activity due to the availability of the antioxidative compounds to exert different inhibitory effect against tested organisms [30].

The serial dilution assay quantifies the antibacterial activity of pure compound by providing the MIC value of the compound for specific susceptible test organisms and important consideration in the further development of bioactive compounds. No inhibition was observed in the test tube containing sample lower than the above concentrations. Methanolic extract of G. pentaphylla revealed the inhibition activity with minimum concentration against S. paratyphi & E. coli among other microorganisms.

5. Conclusion

The present study exposes G. pentaphylla as a promising source of natural antimicrobial and antioxidants agents on various infectious diseases and the aging process of human being to discover of natural-product pharmaceuticals. On the basis of encouraging results of antimicrobial and antioxidant potentials it can be suggested that further studies should be carried out on purifying and isolating the phytochemicals along with evaluation of the antioxidant, antibacterial and antifungal properties in vivo.

6. Reference

10. Rajib Ahsan, Km Monirul Islam, Musaddik A, Haque E. Activity of Methanol Extract of Some Medicinal Plants Against Carbon Tetrachloride Induced Hepatotoxicity in

Table 2: Minimum Inhibitory Concentration of plant extract against several organisms.

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Name of the organisms</th>
<th>G. Pentaphylla (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bacillus cereus</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>Bacillus subtilis</td>
<td>256</td>
</tr>
<tr>
<td>3</td>
<td>Staphylococcus aureus</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>Sarcina lutea</td>
<td>64</td>
</tr>
<tr>
<td>5</td>
<td>Escherichia coli</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>P. aeruginosa</td>
<td>128</td>
</tr>
<tr>
<td>7</td>
<td>Salmonella paratyphi</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>Shigella dysenteriae</td>
<td>128</td>
</tr>
<tr>
<td>9</td>
<td>Shigella boydii</td>
<td>128</td>
</tr>
<tr>
<td>10</td>
<td>Vibrio parahaemolyticus</td>
<td>128</td>
</tr>
<tr>
<td>11</td>
<td>Vibrio mimicus</td>
<td>256</td>
</tr>
<tr>
<td>12</td>
<td>Candida albicans</td>
<td>256</td>
</tr>
<tr>
<td>13</td>
<td>Aspergillus niger</td>
<td>32</td>
</tr>
<tr>
<td>14</td>
<td>Saccharomyces cerevisiae</td>
<td>32</td>
</tr>
</tbody>
</table>


12. ebenezer T, kadamban d, ms shekhwat s. justin packia jacob, phytochemical evaluation and antioxidant activity of glycosmis mauritiana (lam.) tanaka leaf extract; international journal of pharma research & review. 2013; 2(9):16-23


