INTRODUCTION

Plants have formed the basis of traditional medicine systems that have been in existence for thousands of years. Even in modern times, plant-based systems continue to play an essential role in health care. It has been estimated by the World Health Organization that approximately 80% of the world’s population from developing countries rely mainly on traditional medicines (mostly derived from plants) for their primary health care [1]. The plant-based chemical compounds are classified into two classes; primary and secondary metabolites based on their chemical, biosynthetic origin, and functional groups. Primary metabolites are involved in growth and development, and secondary metabolites are in defense mechanism against harmful pests and infectious agents. Plant derived chemicals such as terpenoids, phenolics, alkaloids, flavonoids, glycosides, diterpenes, triterpenes etc., which shows better compatibility with the human body to resist against harmful diseases [2].

Most of the aromatic and medicinal plants contain chemical compounds with antioxidant properties. Several studies carried out on medicinal plants led to the development of natural antioxidant formulations for food, cosmetic, and other applications [3]. There is an increasing interest in natural antioxidants, for example, polyphenols present in medicinal and dietary plants, which might help to prevent oxidative damage. Naturally occurring antioxidants increase the antioxidant capacity of the plasma and dietary plants, which might help to prevent oxidative damage. Naturally occurring antioxidants increase the antioxidant capacity of the plasma and dietary plants, which might help to prevent oxidative damage. Naturally occurring antioxidants increase the antioxidant capacity of the plasma and dietary plants, which might help to prevent oxidative damage.

Most of the aromatic and medicinal plants contain chemical compounds with antioxidant properties. Several studies carried out on medicinal plants led to the development of natural antioxidant formulations for food, cosmetic, and other applications [3]. There is an increasing interest in natural antioxidants, for example, polyphenols present in medicinal and dietary plants, which might help to prevent oxidative damage. Naturally occurring antioxidants increase the antioxidant capacity of the plasma and reduce the risk of disease [4]. At present, keen interests and widespread researches on exogenous antioxidants from natural sources perhaps, due to the fact that they are less expensive, readily available and believed to have lesser side effects when compared to their synthetic counterparts [5].

**Baliospermum montanum** is commonly known as Danti, which belongs to the family Euphorbiaceae. It is found in tropical and subtropical Himalayas from Kashmir eastwards to Arunachal Pradesh. Root, leaf, and seeds of the plants are used medicinally. The root contains phorbol ester belonging to diterpene hydrocarbon, namely, montanin, baliosperm, 12-deoxyphorbal-13-palmitate, 12-deoxy-5β-hydroxyphorbal-13-myristate, and 12-deoxy-16-hydroxyphorbal 13-palmitate. Leaves contain β-sitosterol, β-D-glucoside, and hexacosanol. Steroids, terpenoids, and flavonoids are also reported in this plant [6]. The root is acrid, thermogenic, purgative, anti-helminthic, carminative, and anti-inflammatory. Seeds, roots, and leaves are used to treat abdominal pain, constipation, piles, hemithinic manifestations, skin disorders, wound, jaundice, asthma, bronchitis, and also used in snakebite [7,8]. Thus, the aim of this study was to evaluate the phytochemical and antioxidant activity of methanol, aqueous, and chloroform extracts of *B. montanum* leaf, stem, root, and stem-derived callus.

METHODS

**Collection and sterilization of seeds**

*B. montanum* and seedlings collected from wild plants grown in Western Ghats (Karnataka), India. The plant material was authenticated at the Botanical Survey of India, Western Regional Centre, Pune, and the voucher specimen was deposited in the No. BSI/WRC/100-1/ADEN.CER./018/77. The seeds will be washed thoroughly under running tap water for 20 min and rinsed with Teepol solution 5% (v/v) and washing with sterile double distilled water 3–4 times and the sterilized seeds will be transferred to nursery trays, then pots containing a mixture of sterile soil, sand, and manure (2:1:1) and maintained in the greenhouse condition.

**Callus induction**

Nodal explants were sterilized with different sterilants (Teepol and bavistin), followed by 0.1 % HgCl₂ for 2 min under aseptic condition and then washed with sterile water for 3–5 times to remove the traces of sterilants. Finally, sterilized explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations and
Results and Discussion

Preparation of extracts

The extracts were prepared according to the procedure of Samydurai and Saradha [9]. Briefly, 10 g of dried leaf, stem, root, and stem–derived callus were finely powdered using blender and extracted with 100 ml of different solvents (methanol, aqueous, and chloroform) for 24 h. Then, the extracts were concentrated by evaporation. The dried extract was stored at 4°C until further analysis.

Preparation of callus

Callus induction

Stem explants were inoculated on MS medium supplemented with different concentrations of auxins and cytokinins (Table 1). Among the various concentrations and combinations, maximum creamish compact callus was achieved on MS medium supplemented with 0.5 mg/L of KIN + 3.0 mg/L of BAP (17.33±1.15) and green semi-friable callus was recorded in 0.5 mg/L of BAP + 0.5 mg/L of NAA (16.80±0.34) compared to other concentrations (Fig. 1). The previous studies also reported that maximum callus induction was observed in combination of NAA (4.0 mg/L) + BAP (0.5 mg/L) from intermodal region of Orthosiphon stamineus [15]. Studies by Nathar and Yatoo [16] noticed highest callus induction on combination of 2, 4-D (2.0 mg/L) + KIN (1.0 mg/L) from the Artemisia pallens shoot tip region compared to other concentrations and combination of growth hormones.

Total phenolic content

Phenolic compounds are ubiquitous secondary metabolites in plants. They have multiple biological effects such as prevention of platelet aggregation and damage of red blood cells [17,18]. They play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [19]. The results were determined using the linear regression equation (y = 0.058x + 3.00).

Table 1: Effect of auxins and cytokinins on callus induction from stem explants of Baliospernum montanum

<table>
<thead>
<tr>
<th>Growth hormones</th>
<th>Concentration (mg/L)</th>
<th>Number of explants</th>
<th>Number of explants responded</th>
<th>Nature of callus</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP+NAA</td>
<td>0.5+0.5</td>
<td>20</td>
<td>16.80±0.34</td>
<td>Green semi-friable callus</td>
</tr>
<tr>
<td></td>
<td>0.5+1.0</td>
<td>20</td>
<td>15.60±1.21</td>
<td>Green semi-friable callus</td>
</tr>
<tr>
<td></td>
<td>3.0+1.0</td>
<td>20</td>
<td>13.66±2.08</td>
<td>Creamish compact callus</td>
</tr>
<tr>
<td>6-furfurylaminopurine+BAP</td>
<td>0.5+1.0</td>
<td>20</td>
<td>12.66±2.08</td>
<td>Creamish compact callus</td>
</tr>
<tr>
<td></td>
<td>0.5+2.0</td>
<td>20</td>
<td>13.66±2.51</td>
<td>Creamish compact callus</td>
</tr>
<tr>
<td></td>
<td>0.5+3.0</td>
<td>20</td>
<td>17.33±1.15</td>
<td>Creamish compact callus</td>
</tr>
<tr>
<td>2, 4-dichlorophenoxyacetic acid+NAA</td>
<td>0.5+0.5</td>
<td>20</td>
<td>09.33±1.52</td>
<td>Yellowish friable callus</td>
</tr>
<tr>
<td></td>
<td>0.5+1.0</td>
<td>20</td>
<td>14.33±1.52</td>
<td>Yellowish friable callus</td>
</tr>
<tr>
<td></td>
<td>0.5+2.0</td>
<td>20</td>
<td>16.20±1.70</td>
<td>Yellowish friable callus</td>
</tr>
</tbody>
</table>

Values represent the Mean±SD in triplicates. One-way analysis of variance followed by Duncan’s multiple range tests using SPSS software. p<0.05 was considered statistically significant. BAP: 6-benzylaminopurine, NAA: 1-naphthaleneacetic acid.
Total flavonoid content

Flavonoids are the most common group of polyphenolic compounds which provide health benefits through cell signaling pathways and antioxidant effects [23]. They act as chemical messengers, physiological regulators, and cell cycle inhibitors [24]. These metabolites are mostly used in plants to produce yellow and other pigments which play an important role in the colors of plants. In addition, flavonoids are readily ingested by humans and they display anti-inflammatory, antiallergic, and anticancer properties [25]. The results were determined using the linear regression equation \( (y = 0.047x + 0.0879) \ r^2=0.9939 \). The flavonoid content of different extracts was varying widely between 1.22 and 3.94 mg QE/g. The methanolic extract of leaf recorded maximum flavonoid content (3.94±0.07 mg/g) followed by aqueous leaf extract (3.18±0.77 mg/g). Studies by Awah and Verla [26] revealed maximum flavonoid content in the methanolic leaf extract of *Ocimum gratissimum* compared to other phytochemical constituents. According to Basma et al. [27], the methanolic extract of *Euphorbia hirta* leaves exhibited a significant amount of flavonoid content followed by the flowers, roots, and stem, respectively. Ruwali et al. [28] also reported that methanolic extract of *Michelia champaca* leaf exhibited higher flavonoid content compared to other solvent extracts.

**Table 2: Total phenolics, flavonoids, and tannin content of different extracts of *Balsispermum montanum***

<table>
<thead>
<tr>
<th>Plant samples</th>
<th>Solvents (mg/g)</th>
<th>Total phenolics (mg GAE/g)</th>
<th>Total flavonoids (mg QE/g)</th>
<th>Total tannins (mg CE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>Methanol</td>
<td>3.22±0.034</td>
<td>3.94±0.077</td>
<td>6.95±0.38</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>4.17±0.02</td>
<td>3.18±0.77</td>
<td>6.25±1.89</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>0.04±0.06</td>
<td>1.89±0.35</td>
<td>5.65±0.27</td>
</tr>
<tr>
<td>Stem</td>
<td>Methanol</td>
<td>3.13±0.10</td>
<td>2.17±0.03</td>
<td>3.77±0.51</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>2.30±0.13</td>
<td>2.00±0.04</td>
<td>3.34±0.11</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>0.31±0.09</td>
<td>1.52±0.10</td>
<td>2.67±0.32</td>
</tr>
<tr>
<td>Root</td>
<td>Methanol</td>
<td>4.49±0.15</td>
<td>2.11±0.20</td>
<td>6.66±0.06</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>3.77±0.11</td>
<td>1.59±0.02</td>
<td>2.86±0.23</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>3.66±0.23</td>
<td>1.22±0.03</td>
<td>1.99±0.12</td>
</tr>
<tr>
<td>Stem callus</td>
<td>Methanol</td>
<td>1.62±0.05</td>
<td>1.42±0.03</td>
<td>1.61±0.24</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>2.05±0.03</td>
<td>2.21±0.01</td>
<td>1.39±0.76</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>0.48±0.01</td>
<td>1.28±0.02</td>
<td>1.16±0.27</td>
</tr>
</tbody>
</table>

Values represent the Mean±SD in triplicates. One-way analysis of variance followed by Duncan’s multiple range tests using SPSS software. p<0.05 was considered statistically significant. GAE: Gallic acid equivalent.
relationship as represented in Fig. 3. The leaf extract exhibited higher reducing power activity, whereas methanolic extract of root recorded lower in methanolic extract of leaf in *Withania somnifera* compared to other extracts. Simur [35] reported that the antioxidant activity was higher in methanolic extract of leaf in *Withania somnifera* compared to other extracts.

**CONCLUSION**

In the present investigation, leaf extract of *B. montanum* exhibited higher phenolic, flavonoid, and tannin content compared to other extracts. The DPPH and reducing power activity were found to be higher in methanolic leaf and root extract which can correlate to higher scavenging activity. Further investigations are required to identify their active metabolites and antiproliferation activity.

**ACKNOWLEDGMENT**

The authors are thankful to the Department of Botany, Bangalore University, Bengaluru.

**AUTHORS’ CONTRIBUTIONS**

Sushma has done a phytochemical and antioxidant activity. Raveesha coordinated the work and writing of the manuscript.

**COMPETING INTEREST**

The authors declared that they have no conflicts of interest.

**REFERENCES**


