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Biochemical Profile, *In vitro* Toxicity, and Cytotoxic activity of *Eragrostis amabilis* (L.) Wight. Arn. and *Eragrostis pilosa* (L.) Beauve

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Abstract: The present study is intended to reveal the biochemical profile and assess the toxicity and cytotoxic activity of aerial and underground parts of *Eragrostis amabilis* (L.) Wight. Arn. and *Eragrostis pilosa* (L.) Beauv. extracts using Brine shrimp lethality bioassay and Dalton's lymphoma ascites cell (DLA). The maximum amount of protein 31.2 mg/g, amino acid 50.5 mg/g, glucose 62.8 mg/g and Indole acetic acid 29.5 mg/g were found in *E. amabilis* aerial parts. The maximum amount of total phenolics (218.21 mg/g) and tannins (88.5 mg/g) was observed in ethanolic extracts of *E. amabilis* aerial parts. The highest value of flavonoids was stated in chloroform extracts of *E. amabilis* underground parts (544 mg/g). The maximum amount of total phenolics and total tannin was observed in ethyl acetate extracts of *E. pilosa* aerial parts. The highest amount of flavonoids was found in ethyl acetate extracts of *E. pilosa* underground parts. The toxicity (LC_{50} values) of *E. amabilis* aerial and underground parts were ranged from 0.48 - 1.52 mg/ mL and 0.012 - 1.154 mg/ mL respectively. The LC_{50} value of *E. pilosa* aerial and underground parts extract was ranged from 1.089 - 1.904 mg/ mL and 0.038 -1.726 mg/ mL. The high cytotoxicity (ICT_{50}) was observed in ethyl acetate extracts of *E. amabilis* and *E. pilosa*.

Keywords: phytoprofile; flavonoids; phenols; toxicity; cytotoxicity.

1. Introduction

The grasses possess primary (crude proteins, carbohydrates, and fats) and secondary metabolites (β-sitosterol, flavanoids, alkaloids, glycosides, triterpenoids) for their sustainable establishment in the ecosystem. In addition, they possess the mineral constituents' viz., oxides of magnesium, phosphorous, calcium, sodium, and potassium, and other compounds like vitamin C, carotene, etc. Green grass contain 10.47% crude protein, 28.17% fiber, and 11.75% of total ash [1].

Some monocots like *Allium cepa*, *Allium sativum,* and *Zea mays* showed important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins,

terpenoids etc. [1]. Coffie *et al*. (2014) studied the phytochemical composition of wet and dry states of *Bambusa vulgaris, Bambusa ventricos,* and *Oxytenanthera abyssinica* leaves confirmed the existence of saponins, general glycosides, coumarins, and cyanogenic glycosides [2]. Megha *et al*. (2016) revealed carbohydrates, tannins, steroids, terpenoids, alkaloids, flavonoids, cardiac glycosides, saponins, coumarins, amino acids, etc., in the *Eragrostis plana* Nee. leaves extracts [3].

The presence of alkaloids, tannins, and phenolics in the methanolic extracts of *Leptochloa uniflora* Hochst fresh leaves was confirmed [4]. The phytochemical study revealed flavonoids, alkaloids, and steroids in *Cymbopogon coloratus*, *Cynodon dactylon*, *Panicum repens*, and *Sporobolus wallichii* [5]. *Cymbopogon citratus* is well exploited in the pharmaceutical industry [6]. Favaretto *et al*. (2015) revealed the occurrence of alkaloids, anthraquinones, flavonoids, tannins, and saponins in the tough love grass extracts [7]. Flavonoids, lactones, and certain phenolic acids are the main chemical constituents of bamboo leaves [8].

Eragrostis tenella (Poaceae), phenolic acids, glycosides, flavonoids, alkaloids, leuco anthocyanidins, saponins, fatty acids, steroids, and emodins presence were reported [9]. But there is no report on the biochemical composition of *Eragrostis amabilis* and *Eragrostis pilosa*. With this knowledge, the present study was carried out to find the quantitative and qualitative phytoprofile of *Eragrostis amabilis* (L.) Wight. Arn. and *Eragrostis pilosa* (L.) Beauv. aerial and underground parts extracts.

The brine shrimp lethality bioassay (BSLB) has been employed to measure the toxicity of the plant crude extracts or isolated compounds. BSLB assay has been exploited to predict the pesticide residues, mycotoxins, stream pollutants, anesthetics, morphine-like compounds, the carcinogenicity of phorbol esters, and toxicants in the marine environment. A number of novel antitumor and pesticide natural products have been isolated using this bioassay [10,11]. The *in vitro* anticancer potential of aqueous and ethanolic extracts of *Cyperus rotundus* (L.) showed great effects [12]. But there is no report on the toxicity and cytotoxicity of aerial and underground parts of *Eragrostis amabilis* (L.) Wight. Arn. and *Eragrostis pilosa* (L.) Beauv. different extracts.

The objective of the present study is to assess the toxicity and cytotoxic activity of aerial and underground parts of *Eragrostis amabilis* (L.) Wight. Arn. and *Eragrostis pilosa* (L.) Beauv. extracts using Brine shrimp lethality bioassay and Dalton's lymphoma ascites cell (DLA).

2. Materials and Methods

2.1. Collection of plant samples.

The whole plants of *Eragrostis amabilis* (L.) Wight. Arn. and *Eragrostis pilosa* (L.) Beauv. were collected from A. Thirumalapuram, Tirunelveli district, Tamil Nadu, South India. The plants were washed with tap water to remove the soil and other debris. The aerial and underground parts of *E. amabilis* and *E. pilosa* were separated properly and dried under shade conditions at room temperature for fifteen days. The dried samples (aerial and underground parts) were grounded to a fine powder using a mechanical grinder. The powdered sample was then stored in a refrigerator at 7°C for further analysis.

2.2. Preparation of extract.

The dried and powdered aerial and underground plant parts (30 g) of *Eragrostis amabilis* (L.) and *Eragrostis pilosa* (L.) were extracted with 400 ml of petroleum ether, acetone, chloroform, ethanol, and ethyl acetate by using a soxhlet extractor for 12 hrs at a room temperature not exceeding the boiling point of the solvents. After 12 hrs, the extracts were collected in Petri dishes and evaporated the excess solvents. The obtained residues of the plant extracts were stored in sterile bottles for further analysis.

2.3. Quantitative determination of primary metabolites.

According to the following literature report, the total glucose, the amino acid, protein concentration, and the indole acetic acid were determined, respectively [13-16].

2.4. Quantitative determination of secondary metabolites.

2.4.1. Estimation of flavonoids.

The total flavonoid content of aerial and underground parts of *E. amabilis* and *E. pilosa* was estimated by Zhishen *et al.* (1999) [17]. The results are expressed as mg of flavonoids as quercetin equivalent/gm of dried sample.

2.4.2. Determination of total phenolics and Tannins.

The total flavonoid content of aerial and underground parts was estimated by the method described by Siddhuraju and Becker (2003) [18]. The tannin content of the sample was calculated as follows:

Tannins (%) = Total phenolics (%) – Non-tannins phenolics (%)

2.4.3. Estimation of total terpenoids and total sterols.

The total concentration of terpenoids and sterols was determined according to the methodology described by Johnson *et al*. (2020) [19].

2.5. Toxicity analysis.

2.5.1. Preparation of samples.

The toxicity analysis was carried out with five test tubes for each concentration of *E. amabilis* and *E. pilosa* various extracts (5 x 10 nauplii = 50 nauplii / concentration) and 5 tubes for control and performed according to the method described by (McLaughlin and Rogers 1988) [20]. Tubes without the extract were added to prepare the control solution. After 24 hours, the number of survived nauplii in each tube was counted, and $LC_{50,}$ LC_{90}, LCL, and UCL values were calculated.

2.5.2. *In vitro* cytotoxicity (anticancer activity).

The test compounds were studied for short-term *in vitro* cytotoxicity using Dalton's lymphoma ascites cell [21]. These assay mixtures were incubated for 3 h at 37^{o}C. The further cell suspension was mixed with 0.1ml of 1% trypan blue, kept for 2-3 minutes, and loaded on

a hemocytometer. Dead cells take up the blue color of trypan blue, and white live cells do not take up the dye. The numbers of stained or unstained cells were counted separately, and the cytotoxicity was determined by:

% of Cytotoxicity = No of dead cells / No of live cells + number of dead cells x100

3. Results and Discussion

3.1. Quantification of primary metabolites.

The estimated total protein, amino acid, glucose, and Indole acetic acid of *E. amabilis* and *E. pilosa* aerial and underground parts were illustrated in Fig. 1. The maximum amount of protein 31.2 mg/g, amino acid 50.5 mg/g, glucose 62.8 mg/g, and indol acetic acid 29.5 mg/g were found in *E. amabilis* aerial parts.

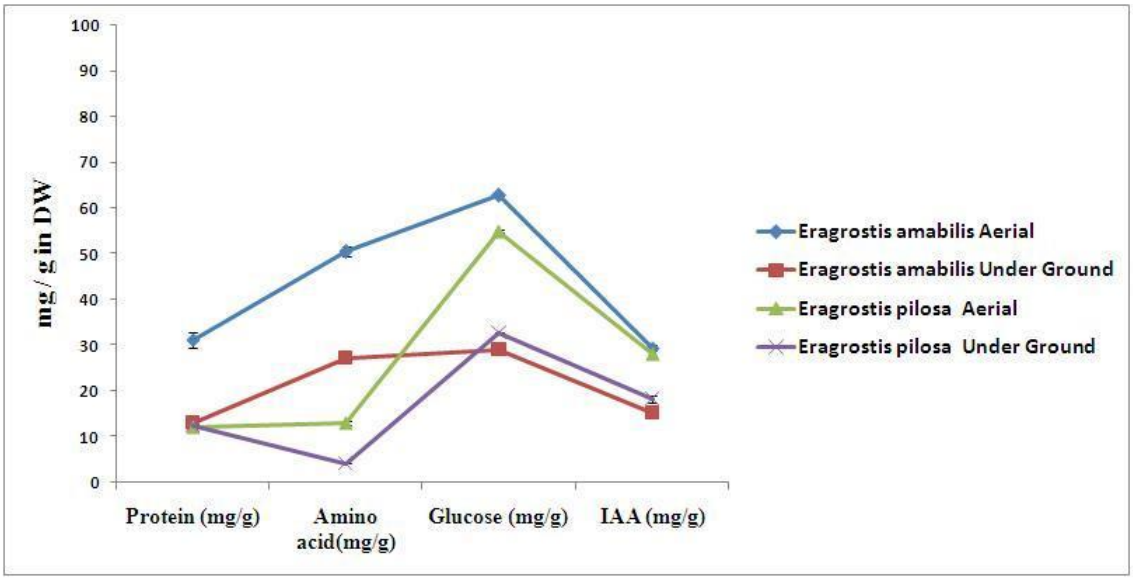

Figure 1. Biochemical profile of *E. amabilis* and *E. pilosa.*

3.2. Total phenolics contents of E. amabilis aerial and underground parts.

The total extractable total phenolics, tannins, flavonoids, and triterpenoids of *E. amabilis* aerial and underground parts were demonstrated in Fig. 2. The maximum amount of total phenolics (218.21 mg/g) was observed in ethanolic extracts of *E. amabilis* aerial parts, and the least value (151 mg/g) was noticed in *E. amabilis* underground parts acetone extracts. The highest amount of tannins (88.5 mg/g) was observed in ethanolic extracts of *E. amabilis* aerial parts. The chloroform extracts of *E. amabilis* underground parts showed a minimum amount of tannins (17 mg /g). The highest value of flavonoids was stated in chloroform extracts of *E. amabilis* underground parts (544 mg/g). Ethyl acetate extracts of *E. amabilis* underground parts revealed the minimum amount of flavonoids (65.7 mg /g). Among the observed values, ethyl acetate extracts of *E. amabilis* aerial parts showed the highest terpenoids (285 mg/g), and petroleum ether extract of *E. amabilis* aerial parts revealed the least amount of terpenoids (94.8 mg/g). The maximum amount of sterols (7.36 mg/g) was found in ethanolic extracts of *E. amabilis* aerial parts, and a minimum amount (1.73 mg /g) was observed in acetone extracts of *E. amabilis* underground parts.

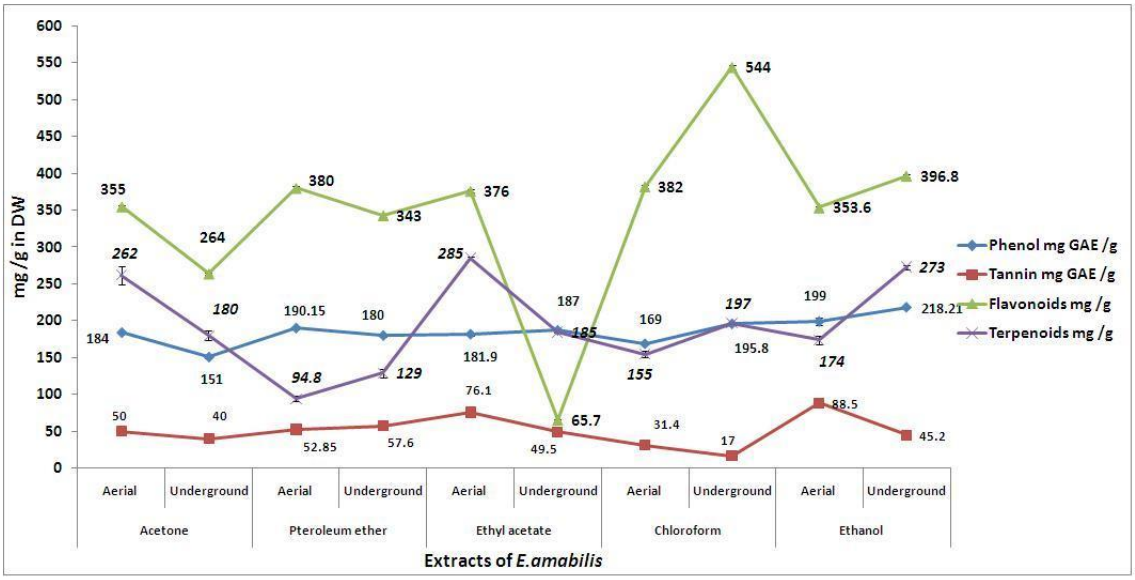

Figure 2. Secondary metabolites of *E. amabilis* (mg/g in DW).

3.3. Total phenolics contents of E. pilosa aerial and underground parts.

The total extractable total phenolics, tannins, flavonoids, and triterpenoids of *E. pilosa* aerial and underground parts were illustrated in Fig. 3. The maximum amount of total phenolics was observed in ethyl acetate extracts of *E. pilosa* aerial parts (197 mg/g). Whereas acetone, chloroform, ethyl acetate, and petroleum ether extracts of *E. pilosa* aerial and underground parts demonstrated moderate levels of phenolics. Among the tested extracts of *E. pilosa*, total tannin was the highest in ethanolic extracts of *E. pilosa* aerial parts (64.5 mg/g) and least in ethyl acetate extracts of *E. pilosa* underground parts (18.95 mg /g). The highest amount of flavonoids was found in ethyl acetate extracts of *E. pilosa* underground parts (289.5 mg/g). The lowest (127.8mg /g) was observed in the ethanolic extracts *E. pilosa* underground parts. Among the observed values, ethyl acetate extracts of *E. pilosa* aerial parts showed maximum terpenoids (265 mg/g), and petroleum ether extracts of *E. pilosa* underground parts showed the least amount of terpenoids (119.3 mg/g). The highest amount of sterols was revealed in ethyl acetate extracts of *E. pilosa* aerial parts (33.8 mg/g), and the least amount (14.6 mg /g) was obtained in the acetone extracts of *E. pilosa* underground parts.

Brine shrimp lethality bioassay method was used to determine the general toxic properties of *E. amabilis* and *E. pilosa* aerial and underground parts. The toxicity (LC_{50} values) of E. amabilis aerial parts were as follows: ethyl acetate extracts (0.48 mg/ mL) > acetone (0.85 mg/ mL) > chloroform (1.18 mg/ mL) > petroleum ether (1.42 mg/ mL) > ethanol (1.52 mg/ mL). The toxicity (LC_{50} values) of *E. amabilis* underground parts extracts were as follows: ethyl acetate (0.012 mg/ mL) > petroleum ether (0.70 mg/ml) > ethanol (1.112 mg/ml) > acetone (1.133 mg/ml) > chloroform (1.154 mg/ mL). The LC_{50} value of E. pilosa aerial parts extract were as follows: ethyl acetate (1.089 mg/ mL) > chloroform (1.302 mg/ mL) > petroleum ether (1.423 mg/ mL) > acetone 91.464 mg/ml) > ethanol (1.904 mg/ mL). The LC_{50} value of *E. pilosa* under ground parts extract were as follows: ethyl acetate (0.038 mg/ mL) > petroleum ether (0.071 mg/ mL) > acetone (1.048 mg/ mL) > chloroform (1.397mg/ mL) > ethanol (1.726 mg/ mL). Based on the results of toxicity analysis, the ethyl acetate extracts of *E. amabilis* and *E. pilosa* aerial and underground parts were screened for the cytotoxicity against Dalton's lymphoma ascites cell (DLA). Among the tested extracts, the aerial parts of *E. amabilis* and *E. pilosa* possess more activity than underground parts. The cytotoxicity

(ICT_{50}) of ethyl acetate extracts of *E. amabilis* and *E. pilosa* aerial and underground parts were as follows: E. amabilis aerial parts (50.81 mg/mL) > *E. pilosa* aerial parts (51.5 mg/mL) > E. amabilis underground parts (60.31 mg/mL) > *E. pilosa* underground parts (76.6 mg/mL).

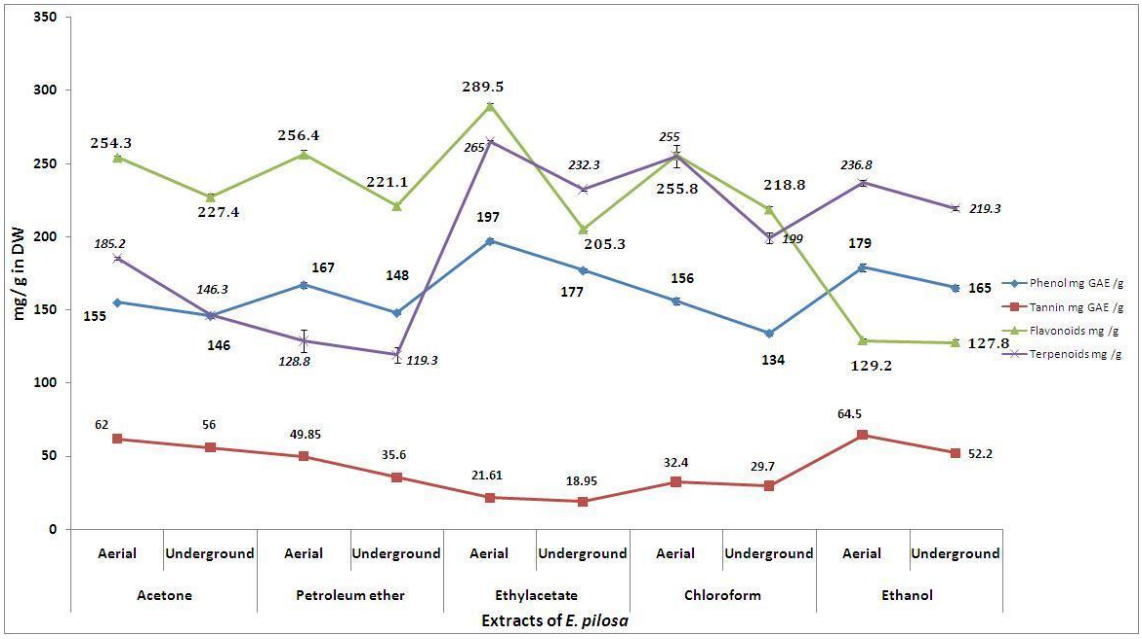

Figure 3. Phytoprofile of *E. pilosa* (mg /g in DW).

3.4. Discussion.

The primary metabolites (protein, amino acids, and glucose) existence was high in the aerial parts of *E. amabilis* and *E. pilosa*. Similarly, the amount of Indole acetic acid (IAA) existence also varied concerning parts. IAA plays a vital role in regulating plant growth and development processes, cell elongation, cell division, and the formation of meristems. Due to the presence of more meristematic regions, the aerial parts of *E. amabilis* and *E. pilosa* showed more amount of IAA than underground parts of *E. amabilis* and *E. pilosa.* The medicinal properties of the plant extracts are predominantly governed by the presence of bioactive constituents like phenolics, flavonoids, and others [20]. For ecological adaptation, survival, and protection, the plants are endowed with various phytoconstituents such as vitamins, terpenoids, phenolics, lignins, tannins, flavonoids, quinines, alkaloids, and other metabolites [21].

The presence of secondary metabolites with varying amounts is due to the solvent polarity (petroleum ether, acetone, chloroform, ethyl acetate, and ethanol) and inbound chemical constituents of the aerial and underground parts of *E. amabilis* and *E. pilosa* showed (Fig. 1-3). Phenolic compounds are widely distributed in plants, possessing various biological activities, such as anti-inflammatory, antidiabetic, anticancer, and antimicrobial [22, 23]. Flavonoids also possess anti-inflammatory, antidiabetic, and antimicrobial activities [24]. Boonya-udtayan *et al*. (2019) summarized the serratene-type triterpenoids and biological activities viz., cytotoxicity, and chemopreventive activity [25].

The present study results revealed the presence of secondary metabolites such as phenols, tannins, flavonoids, terpenoids, and sterols in the *E. amabilis* and *E. pilosa* aerial and underground parts extracts. Previous observations on the biological activities of secondary metabolites suggest that the studied *E. amabilis* and *E. pilosa* aerial and underground parts may possess antioxidant, anti-inflammatory, anticancer, antidiabetic, and antimicrobial activities. To confirm the biological properties of *E. amabilis* and *E. pilosa* aerial and underground parts,

the toxicity analysis using the brine shrimp lethality bioassay (BSLB) was carried out. McLaughlin *et al*. (1982) predicted the antitumor and pesticide properties of the plant extracts using BSLB assay.

The toxicity study results suggest that *E. amabilis* and *E. pilosa* aerial and underground parts may possess antitumor and pesticide properties. To confirm the antitumor properties of the ethyl acetate extracts of *E. amabilis* and *E. pilosa*, aerial and underground parts were subjected to cytotoxicity analysis against Dalton's lymphoma ascites cell. Similarly, Vijayalakshmi *et al*. (2012) studied the cytotoxic effects of *Trticum aestivum* ethanolic extract against the A549 cell line *in vitro* [26]. Garima *et al*. (2014) screened the cytotoxic properties of *Wheat grass* methanolic extracts [28]. They observed significant cytotoxicity and confirmed that phenols and flavonoids' presence in the methanolic extracts is responsible for the cytotoxic property. Hema *et al*. (2016) observations also identified the role of phenolics and flavonoids cytotoxicity and anticancer potential of *Cyperus rotundus* [12]. In the present investigation, the high frequency of anticancer potential was observed in the phenol, flavonoids, and tannins rich *E. amabilis* and *E. pilosa* aerial parts ethyl acetate extracts. Similar to the present investigation, *Cynodon dactylon* ethyl acetate extracts showed greater anticancer activity [28].

4. Conclusions

The present study results confirmed the cytotoxic potential of *E. amabilis* and *E. pilosa;* further studies on these crude extracts may bring out an alternative drug for cancer treatment.

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Conflicts of Interest

The authors declare no conflict of interest.

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