RESEARCH

Composition and Antimicrobial Activity of Different Plant Parts of Parthenium hysterophorus L.

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ABSTRACT

This study was aimed to determine the lignocellulosic biomass in different parts of Parthenium hysterophorus and evaluate its antimicrobial activity against selected microbial plant pathogens. Compositional analyses were conducted on live whole plants and their leaves, stems, flowers, and roots. The lignocellulosic biomass components were estimated gravimetrically as a percentage of dry weight, using the standard equation, while reducing sugar was quantified using a glucose standard curve. Significant differences (p < 0.05) in cellulose content were observed among different plant parts, with the highest dry weight percentage in the whole mature plant (48±0.33), followed by the stem (45±0.21), whole young plant (41±0.10), root (21±0.00), leaf (28±0.01), and inflorescence (21±0.14). Additionally, reducing sugar content in mg/mL was significantly higher in the stem (1.94±0.01) and root (1.17±0.00), followed by the mature whole plant (0.95±0.20), leaf-stem mixture (0.93±0.11), inflorescence (0.67±0.02), young whole plant (0.23±0.19), and leaf (0.17±0.01). The stem and root extracts from mature plants inhibited soil-borne plant pathogenic bacteria Pseudomonas sp. and Ralstonia sp. respectively. The leaf and inflorescence extracts of P. hysterophorus showed inhibitory effects only against Pseudomonas sp., not Ralstonia sp. Furthermore, the mature Parthenium plant extract inhibited all tested soil-borne fungi, with significantly higher inhibition percentages observed for Scelerotium sp. (81.93%) and Colletotrichum sp. (45.45%) compared to Fusarium sp. and Pythium sp. Significantly higher cellulose and lignin contents in the whole mature P. hysterophorus plant, along with its antimicrobial activity against major soil-borne plant pathogenic microbes was prominent than the individual plant parts and the young immature plant.

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INTRODUCTION

*Parthenium hysterophorus* is an invasive and noxious weed that belongs to the family Asteraceae, causing a loss of biodiversity in agricultural crops and severe health problems in humans and livestock. It possesses several notable biological characteristics, such as continuous and profuse flowering until senescence, high seed productivity, and a large viable seed bank both below and above the soil. Additionally, its strong regenerative capability makes it a highly fecund weed (Dagar et al., 1976; Sukhada et al., 1979). Global efforts are underway to minimize the abundance of *Parthenium* sp. to a manageable level. One effective control method on a small scale involves pulling out the weed and burning it, preferably before flowering. This process must be performed continuously for 3-4 years to achieve complete weed removal from cultivation lands. However, in the Jaffna peninsula of Sri Lanka, no measures have been taken thus far to control the spread of *Parthenium*. Controlling this prolific weed to a manageable level remains challenging due to ignorance and socio-economic limitations.

Despite the effectiveness of diverse chemical formulations in minimizing the spread of this weed at pre- and post-emergence stages, these methods are economically expensive and pose hazards to the environment. Moreover, their action spectrum may lead to the destruction of certain useful plant species, resulting in their unwanted removal. As an alternative, the use of botanicals and allelochemicals in the control of *Parthenium* sp. shows promise and could potentially replace environmentally harmful synthetic herbicides (Rice, 1995).

Utilizing *Parthenium* plants as a low-cost raw material for other products opens a new avenue for the effective management of this invasive weed. This noxious weed and its parts, are gaining consideration as a countermeasure to global warming and as an alternative to traditional fuel as biofuel resources, because they can exhibit high productivity (Singh et al., 2011, Joshi et al., 2011, Nguyen et al., 2010).

Biofuels, fuels produced from biomass, offer promising alternatives to secure energy (Gunatilake, 2014). Weed biomass, especially from *Parthenium hysterophorus*, presents a highly promising, low-cost, non-conventional feedstock for second-generation biofuel production, specifically cellulosic ethanol. Lignocellulosic biomass primarily consists of cellulose (30-50%), hemicellulose (15-35%), and lignin (10-20%) (Han et al., 2017). Cellulose, a key component of the cell wall, represents a linear homopolymer of fermentable sugar D-glucose. Consequently, *P. hysterophorus* plant parts with high cellulose content can serve as ideal feedstock for cellulosic bioethanol production (Saini et al., 2015). Cellulose is embedded in the cell wall matrix, which also contains hemicelluloses (Marriott et al., 2016).

Figure 1. Different growth stages of *Parthenium hysterophorus* growing in the paddy fields around the Farm School at Thurnelvelly, Jaffna. a) Leaves forming rosette (seedling) b) Inflorescence bearing small white flowers with five distinct corners c) Lobed leaves on the woody octangular stem with longitudinal groves (mature plant)
Beyond its potential as a biofuel resource, *P. hysterophorus* has been utilized in traditional medicine to treat various ailments across different regions of the world. This therapeutic potential is attributed to the secondary metabolites produced by *Parthenium*, which exhibit antimicrobial activity (Kumar & Pandey, 2014). Several studies have reported the antimicrobial properties of *P. hysterophorus* against both plant and animal pathogenic microbes (Harsha et al., 2011).

The present study focuses on identifying plant parts containing high amounts of cellulose and exhibiting bio-pesticidal activity, to utilize this plant as feedstock for bioethanol production after appropriate pretreatment, as well as a primary substrate for compost. The study objectives encompass analyzing the composition of different parts of *P. hysterophorus* and screening the antimicrobial activity of aqueous extracts from these various parts against selected plant pathogenic microbes.

**METHODOLOGY**

**Sample Preparation**

Live specimens of *P. hysterophorus* L. were collected from local sites in Thirunelvely, Jaffna, by uprooting the entire plant. The collected plants underwent a thorough washing process with tap water. Subsequently, distinct plant parts, including leaves, stems, flowers, and roots, were separated and cut into pieces measuring 1-2 cm. These pieces were then sun-dried for two days and further dried in a hot air oven at 50°C until a constant weight was achieved. Following this, the material was finely ground into a powder and sieved through a 500 µm mesh sieve plate. Finally, the separated components were stored in air-tight containers.

**Compositional Analysis of the Components of Lignocellulosic Biomass**

**Estimation of Lignin:**

The amount of lignin was determined using a modified method as described by Yao et al. (2010). The dried biomass (w0) was subjected to hydrolysis using 72% H2SO4 at 20°C for two hours, with a bath ratio of 1:20. This hydrolysis process resulted in the breakdown of cellulose and hemicellulose present in the biomass. The hydrolysate was then filtered through a Gooch-type glass crucible with a sintered disc, having a pore size of 40-90 µm (Grade 2). Before filtration, the crucibles were dried and pre-weighed (w1). The solid residue obtained in the crucible was subsequently washed with hot water and further dried in an oven at 105°C until a constant weight was attained (w2).

\[
\text{Cellulose content} \% = \frac{W2 - W1}{W0} \times 100
\]

**Estimation of Cellulose:**

One gram of oven-dried biomass was combined with 10 mL of 80% acetic acid and 1.5 mL of HNO3. This mixture was fluxed for 20 minutes to facilitate the dissolution of lignin and hemicellulose components within the biomass, following the method described by Updegraff in 1969. The resultant solution was filtered through a crucible that had been dried and pre-weighed (w1), utilizing a vacuum pump. The solid residue left in the crucible was then subjected to oven drying at 105 °C until a constant weight was achieved (w2).

\[
\text{Cellulose content} \% = \frac{W2 - W1}{W0} \times 100
\]

**Estimation of Hemicellulose:**

The hemicellulose content was estimated using the method described by Lin et al. (2010). To 1 g of the dried biomass (w0), 10 mL of a 0.5 mol NaOH solvent was added. The resulting mixture was filtered through a glass crucible that had been dried and pre-weighed (w1), and then washed with distilled water until the pH of the wash solution reached a neutral value. Subsequently, the solid residue in the crucible was dried in an oven at 105 °C until a constant weight was attained (w2). The difference between the initial weight of the sample and its weight after the treatment provided the % w/w of hemicellulose content,
as per the method outlined by Blasi et al. (1999).

\[ Hemicellulose \text{ content (\%)} = \frac{W_0 - (W_2 - W_1)}{W_0} \times 100 \]

Estimations of the three components mentioned above were conducted both for the entire Parthenium plant and for each part separately. The approximate percentages of these different components in the various plant parts were carefully recorded and documented.

**Estimation of Reducing Sugar**

The reducing sugar content of the hydrolysates obtained during lignin estimation was determined using the Di Nitro Salicylic acid (DNS) method, as described by Miller (1959). To perform this analysis, one milliliter of the sample was extracted from the hydrolysis flask and mixed with 3 mL of DNS reagent. The resulting mixture was thoroughly blended and then boiled at 100°C for 10-15 minutes until a yellowish-brown color of the solution developed. The absorbance of the solution was measured at 540 nm using a UV-visible spectrophotometer (Shimadzu: UV mini-1240). The quantification of the reducing sugar released from different plant parts was accomplished using a glucose standard curve.

**Effect of Extracts of Different Parts of Parthenium on Plant Pathogenic Microorganisms**

One gram of dried biomass from various plant parts was individually soaked in 20 mL of water (bath ratio 1:20) for one week. After the soaking period, the extracts were obtained by separating the solid particles through centrifugation at 1000 rpm. The resultant solution was then subjected to testing for its inhibitory effect on selected plant pathogens. To evaluate the effect on plant pathogenic bacteria (Pseudomonas sp. and Ralstonia sp.), the agar well diffusion method described by Cleidson et al. (2007) was employed. For this method, spread plates of pathogenic bacteria were prepared, and wells were created where 100 µL of the extracts from different plant parts were added. The plates were then incubated at 37°C for 2 days, and the diameters of the zones of inhibition were measured. Both young and mature plant extracts were also tested on the same bacteria. Similarly, to investigate the effect on plant pathogenic fungi (Sclerotium sp., Colletotrichum sp., Pythium sp., Fusarium sp., etc.), the "poison food technique" as outlined by Nene and Thapliyal (1979) was employed. This involved mixing 1 mL of each UV-sterilized (30 min.) extract of different plant parts with sterilized molten PDA, along with the addition of streptomycin. The mixture was poured into Petri dishes and allowed to solidify. Discs of various pathogenic fungi were then placed on both control plates (without any plant extract) and treated plates with the extracts. The Petri dishes were incubated at room temperature for 3-4 days, and the radial growth of the fungi’s mycelia on each test plate was compared with that on the control plate.

\[ \text{Mycelial inhibition (\%)} = \frac{\text{Mycelial growth (con.}) - \text{Mycelial growth (trt.)}}{\text{Mycelial growth (con.)}} \times 100 \]

**Statistical analysis**

Statistical analyses were conducted using Minitab v 17.0. All estimations were performed in triplicate for each sample. The data were then subjected to one-way ANOVA. Tukey's multiple comparison test was applied to determine significant differences at a significance level of \( p < 0.05 \). All experiments
were carried out in triplicate and within a completely closed environment to ensure precision and accuracy. Measures were taken to prevent the spread of pollen or any plant parts to the external surroundings, thereby maintaining a controlled experimental setting.

**RESULTS AND DISCUSSION**

In *P. hysterophorus*, both roots and leaves exhibited significantly higher hemicellulose content and lower lignin content compared to stems. Furthermore, mature plants (those with flowers) of *P. hysterophorus* had significantly higher quantities of cellulose, hemicellulose, and lignin in comparison to younger plants at the pre-flowering stage. Notably, even the inflorescence of *P. hysterophorus* showed the presence of cellulose (Table 1).

Further, when comparing the different plant parts, the leaves and roots of *P. hysterophorus* had a significantly higher amount of hemicellulose than the other plant components (Fig. 2).

**Table 1. Estimates of components of lignocellulosic biomass of different plant parts**

<table>
<thead>
<tr>
<th>Mature plant part used</th>
<th>Components in the lignocellulosic biomass of <em>P. hysterophorus</em> (% dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cellulose</td>
</tr>
<tr>
<td>Leaf</td>
<td>28±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stem</td>
<td>45±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Root</td>
<td>21±0.00&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leaf+Stem</td>
<td>31±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inflorescence</td>
<td>21±0.14&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Whole plant (young)</td>
<td>41±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Whole plant (mature)</td>
<td>48±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Lignocellulosic biomass composition of *P. hysterophorus* (% dry weight). Significantly higher cellulose content was observed in the whole mature plant. Values with different superscripts within the same column are statistically significant (p < 0.05). Values are represented as mean (n = 3) ± SD.

**Figure 2. Components in the lignocellulosic biomass of *P. hysterophorus* (% dry weight).**

**Table 2. Amount of reducing sugar in the hydrolysates from lignin estimation for different parts of *Parthenium hysterophorus* glucose standard curve (R² = 0.9974)**
The hydrolysate of the stem contained the highest amount of reducing sugars, followed by the root, whole mature plant, leaf-stem mixture, inflorescence, whole young plant, and leaf (Table 2). The cellulose content varied significantly (p < 0.05) among different plant parts, with the whole mature plant showing the highest value as a percentage of dry weight (48±0.33), followed by the stem (45±0.21), whole young plant (41±0.10), root (21±0.00), leaf (28±0.01), and the inflorescence (21±0.14). Moreover, the stem (1.94±0.01) and root (1.17±0.00) exhibited significantly higher reducing sugar content in mg/mL, followed by the mature whole plant (0.95±0.20), leaf-stem mixture (0.93±0.11), inflorescence (0.67±0.02), young whole plant (0.23±0.19), and leaf (0.17±0.01). These findings indicate that all parts of the Parthenium plant can serve as valuable sources of cellulose, which in turn can be utilized for bioethanol production.

Soil-borne plant pathogenic bacteria, *Pseudomonas* sp., was significantly more inhibited by root and stem extracts compared to extracts from leaves and inflorescence, as supported by previous findings from the study on in vitro inhibition of plant pathogenic bacteria using different solvent extracts from various parts of *P. hysterophorus* (Harsha et al., 2011). Conversely, *Ralstonia* sp. showed no inhibition by leaf and inflorescence extracts of *P. hysterophorus* (Fig. 3 & Table 3). Both aqueous extracts from the entire young and mature Parthenium plants significantly inhibited bacterial growth. However, the entire mature *P. hysterophorus* plant demonstrated significantly larger zones of inhibition compared to the young plant (Fig. 4 & Table 4).

The aqueous extract from the entire mature Parthenium plant exhibited a significantly higher percentage of inhibition on the radial mycelial growth of the majority of tested fungi (Table 5). Specifically, *Scelerotium rolfsi* and *Colletotrichum* sp. were significantly inhibited by the aqueous extract from the entire mature Parthenium plant (Fig. 5 & Table 5).
Figure 3. Effect of Parthenium extracts from different parts (L-leaf, S-stem, R-root, inf.-inflorescence) of a mature Parthenium plant on plant pathogenic bacteria. a) Control plate b) Pseudomonas treated plate c) Ralstonia treated plate

Table 3. Effect of extracts of different plant parts of mature Parthenium sp. on bacterial pathogens

<table>
<thead>
<tr>
<th>Part of mature plant</th>
<th>Zone of Inhibition (ZOI) in cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pseudomonas sp.</td>
</tr>
<tr>
<td>Leaf</td>
<td>0.8±0.00c</td>
</tr>
<tr>
<td>Stem</td>
<td>1.8±0.13b</td>
</tr>
<tr>
<td>Root</td>
<td>2.6±0.21a</td>
</tr>
<tr>
<td>Inflorescence</td>
<td>0.6±0.01c</td>
</tr>
</tbody>
</table>

Figure 4. Control and test plates showing the effect of young and mature Parthenium extracts on plant pathogenic bacteria Pseudomonas sp. and Ralstonia sp. a) Control plate-Pseudomonas b) Treated plate- Pseudomonas c) Control plate- Ralstonia d) Treated plate- Ralstonia

Table 4. Effect of young and mature whole plant extracts of Parthenium sp. on different bacterial plant pathogens

<table>
<thead>
<tr>
<th>Plant used</th>
<th>Zone of inhibition in cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pseudomonas sp.</td>
</tr>
<tr>
<td>Young</td>
<td>1.70</td>
</tr>
<tr>
<td>Mature</td>
<td>2.40</td>
</tr>
</tbody>
</table>
Niranjan et al. (2024) Tropical Agricultural Research, 35(1): 94-93

Figure 5. Control plates and test plates showing the effect of mature Parthenium plant extract on different plant pathogenic fungi a) Fungi on control b) Fungi on Parthenium treated

Table 5. Effect of Parthenium extract on the radial growth of different fungal mycelium

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Mean diameter of radial mycelial growth (cm)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control plate</td>
<td>Treatment</td>
</tr>
<tr>
<td>Sc- Scelerotium sp.</td>
<td>8.3±0.10</td>
<td>1.5±0.01</td>
</tr>
<tr>
<td>Coll.- Colletotrichum sp.</td>
<td>2.2±0.02</td>
<td>1.2±0.00</td>
</tr>
<tr>
<td>F- Fusarium sp.</td>
<td>1.0±0.01</td>
<td>0.8±0.01</td>
</tr>
<tr>
<td>P- Pythium sp.</td>
<td>0.8±0.11</td>
<td>0.8±0.11</td>
</tr>
</tbody>
</table>

Different parts of the *P. hysterophorus* plant displayed variable responses to the inhibition of plant pathogens, possibly attributed to inherent differences in the physiological and morphological characteristics of the microorganisms involved (Shaukat et al., 1983).

It was observed that the aqueous extract of *P. hysterophorus* demonstrated inhibition against most of the pathogens. This finding is consistent with the observations made by other researchers, where aqueous extracts have shown significant inhibition of fungi even at low concentrations (Bajwa et al., 2003; Zunera Zaheer, 2012). Consequently, utilizing aqueous extracts from various parts of *P. hysterophorus* proves to be an economical and effective method for controlling essential plant pathogens.

Despite being a rich source of lignocellulosic biomass, *P. hysterophorus* exhibits diversity in the quantity and distribution of its components among different plant parts. Specifically, the stem of *P. hysterophorus* contains significantly higher cellulose than the leaf and root. Conversely, the roots and leaves of this plant contain significantly higher amounts of hemicellulose than the stems. Moreover, all parts of the *P. hysterophorus* plant demonstrate a significantly lower amount of lignin compared to the other components. These observations align with the findings of a study on other lignocellulosic biomass conducted by Mansor Adila Maisyarah et al. in 2019.

While the amount of cellulose in the whole mature plant and the whole young plant is more or less the same, the reduced sugar yield from the young plant is slightly lower than that of the mature plant. This discrepancy may be attributed to the active and abundant usage of cellulose for rapid growth in young plants compared to mature ones.
Roots and inflorescence contain approximately 21% cellulose on a dry weight basis. However, due to its rubbery texture, challenging handling, and vague odour, the root is not a preferable choice for bioethanol production. Additionally, the root contains about 11% suberin, which is present in the root epidermis, root endodermis, and periderm (cork) – the outer layer of the bark. This characteristic contributes to its leathery nature. For the production of bioethanol, the leaf and stem can be employed as feedstock, but it is advisable to exclude roots from the feedstock used for ethanol production.

Apart from its well-known benefits in composting, the leachates derived from different parts of *P. hysterophorus* also exhibit inhibitory effects on plant pathogenic microbes, making it an advantageous choice as the main substrate in compost production. However, caution should be exercised when using *P. hysterophorus* plants with inflorescence for composting, as not all the seeds will be destroyed during the composting process, potentially leading to weed emergence issues. Therefore, it is recommended to utilize young and mature plant parts at their pre-flowering stage for composting purposes.

Despite being an invasive and dangerous weed, *P. hysterophorus* possesses various utilities. Utilizing appropriate methods, such as large-scale production of bioethanol and organic compost from *P. hysterophorus*, represents one of the most promising approaches for controlling this weed effectively.

**CONCLUSIONS**

While the stem, leaf, and root of *P. hysterophorus* L. are indeed rich sources of lignocellulosic biomass, significantly higher quantities of cellulose and lignin are present in the mature whole plant. Remarkably, the extract obtained from the mature, well-grown *P. hysterophorus* L. whole plant exhibited notably higher antimicrobial activity against major soil-borne plant pathogenic microbes, such as *Colletotrichum* sp., *Sclerotium* sp., *Pseudomonas* sp., and *Ralstonia* sp., in comparison to the root, stem, leaf, and the young immature whole plant. Hence, the mature *P. hysterophorus* L. whole plant holds promising potential as a substrate for bioethanol production and organic compost preparation.

**REFERENCES**


current state and prospects. Applied Microbiological Biotechnology, 6, 627-642.