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Bioethanol Content Estimation in *Lantana camara* at different Altitudinal Gradients in Mussoorie Forest Division, Uttarakhand

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***Abstract*:** The study investigated ethanol yield from different parts of *Lantana camara* at two distinct altitudes: 560 m and 1550 m. The plant parts analyzed include leaves, stems, and a combination of leaves and stems. Ethanol content revealed significant variations based on altitude and plant part. Leaves at 560 m produced a significantly higher ethanol yield (7.5 ml/l) compared to leaves at 1550 m (5.1 ml/l), due to more favorable photosynthesis conditions and higher fermentable sugar accumulation at lower altitudes indicating higher carbohydrate content in leaves at lower altitudes. Conversely, stems showed no significant difference in ethanol yield between the two altitudes (2.7 ml/l and 2.6 ml/l at 560 m and 1550 m, respectively), suggesting that stem metabolic processes and carbohydrate content are less affected by altitude. The combined leaves and stems demonstrated a significant difference, with higher ethanol yields at 560 m (3.2 ml/l) compared to 1550 m (2.9 ml/l), underscoring the altitude sensitivity of leaves in contributing to overall ethanol yield.

***Keywords*:** Ethanol contents, Altitude, *Lantana camara*, Bioethanol

The growing worldwide energy demand, fueled by increasing urbanization, industrialization, and population expansion, has driven home the importance of sustainable and environmentally acceptable energy solutions. Traditional fossil fuels, which have been the main source of energy for many years, are limited and have a major impact on climate change and environmental damage. Finding less polluting and renewable energy sources is imperative in light of this urgent problem (Hahn-Hagerdal et al., 2006). Because it is renewable and has a lower carbon footprint than fossil fuels, bioethanol, a form of biofuel, has become viable option among others (Nigam and Singh 2011). Microorganisms ferment carbohydrates to generate bioethanol, which may be made from various biomass feedstocks such as forestry and agricultural waste, as well as energy crops. A vast range of plant materials, from non-food sources like lignocellulosic biomass to food crops like maize and sugarcane, have been investigated in the hunt for appropriate biomass feedstocks. Lignocellulosic biomass, which includes woody plants, grasses, and leftover materials from agriculture and forestry, is particularly attractive because it is readily available and has little impact on food security (Mohan et al., 2006).

Lantana camara, a floral plant species that is frequently referred to as wild sage or lantana, is commonly found in the family of verbenas (Verbenaceae). It is well known for its capacity to thrive in various environments, from arid and degraded soils to tropical rainforests, and for its adaptation to different climatic circumstances and land uses (Sharma et al., 2005, Thakur et al., 2005, 2011). The plant is distinguished by its clusters of tiny, vibrant blooms, ovate leaves, and sturdy, woody branches. *Lantana camara* is frequently found in dense thickets and can reach a height of two meters (Thakur et al., 2017). However, because it frequently displaces native ecosystems and lowers biodiversity, its invasive nature presents ecological concerns (Sundram et al., 2012). *Lantana camara* is regarded as one of the worst invasive plants in the world due to its relentless expansion. It is a powerful invader in many areas because it can flourish in various climates, from the wet tropics to dry settings.

Lantana camara, has caught attention as a potential source for bioethanol because it produces a lot of biomass and can spread quickly (Sharma et al., 2005, Thakur et al., 2007). Although *L. camara* is known for being an invasive plant, it has a significant amount of potential for producing bioethanol. Due to its high lignocellulosic content, researchers can use it as a feedstock for fermentation and enzymatic hydrolysis to produce bioethanol (Kumar et al., 2009, Kumar et al., 2016). Furthermore, using this invasive plant to produce biofuel might both provide a sustainable energy source and lessen its detrimental effects on the ecosystem.

The three primary constituents of lignocellulosic biomass are cellulose, hemicellulose, and lignin. Microorganisms may hydrolyze polysaccharides like cellulose and hemicellulose to produce fermentable sugars, which are then transformed into bioethanol (Hamelinck et al., 2005, Kim and Lee 2007). Conversely, lignin is a complex aromatic polymer that gives plant cell walls structural support, but because of its resistance, it presents a problem for biomass conversion (Sun and Cheng 2002).

The physiology, biomass composition, and growth of plants are all significantly impacted by altitude. Temperature, humidity, solar radiation, and soil composition all change with altitude, and these variations impact the biochemical characteristics of plants (Körner 2007). These environmental gradients may impact plants' suitability for producing bioethanol, leading to notable changes in their lignocellulosic composition. Research has shown that altitude influences the amount of structural carbohydrates needed to make bioethanol, such as cellulose and hemicellulose. To maximize bioethanol production, one must understand how altitude affects *L. camara* biomass composition. To clarify that how altitude affects the biomass composition and bioethanol output of *L. camara*, this study examines the bioethanol potential of the plant taken from two distinct elevations. Considering the information presented above, the current study is being carried out to evaluate the amount of bioethanol that may be obtained by the treatment of lignocellulosic biomass with acid hydrolysis and fermentation.

MATERIAL AND METHODS

Plant biomass collection: The current study was carried out at the research facility of Department of Forestry, Dolphin (PG) Institute of Biomedical and Natural Sciences, Manduwala. The fresh plants were collected from two distinct altitudinal locations that were separated by a vertical distance of 1000 m. The first sample was taken at an elevation of 560 m from Manduwala, Uttarakhand, while the second sample was taken at an elevation of 1550 m from Kolukhet area, Mussoorie Road, Uttarakhand.

Study area: This study covered the two altitudinal transects namely Manduwala (30.3880^0 N, 77.94110^0 E) and Kolukhet (30.4152^0 N, 78.0809^0 E) forest area situated in Mussoorie Forest division, Dehradun Valley of Uttarakhand.

Sample processing: The stems, foliage, and flowers were separated subsequent to their collection. After being cut into 2–3 cm pieces, the stems were left to dry in the sun for three to four days until sufficient dryness for grinding was attained. The biomass from the stems and leaves was crushed separately with a leaf grinder, and a fine powder was produced and sieved. The powdery leaf and stem biomass was quantified individually at 20 g, and composite samples of

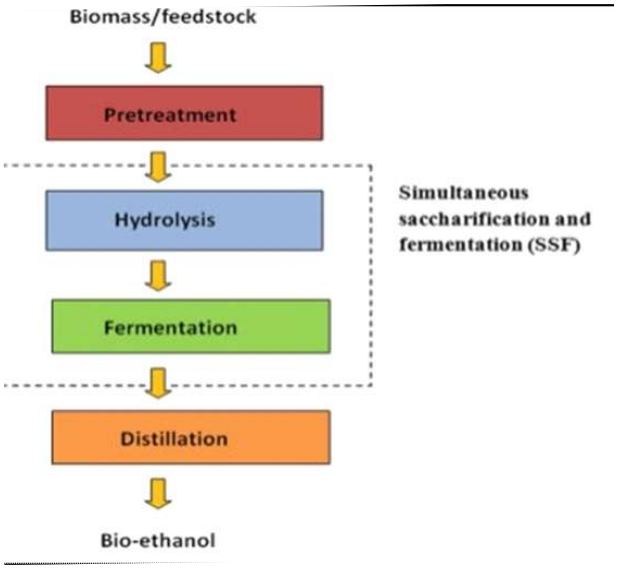

Fig. 2. Flowchart of Ethanol processing

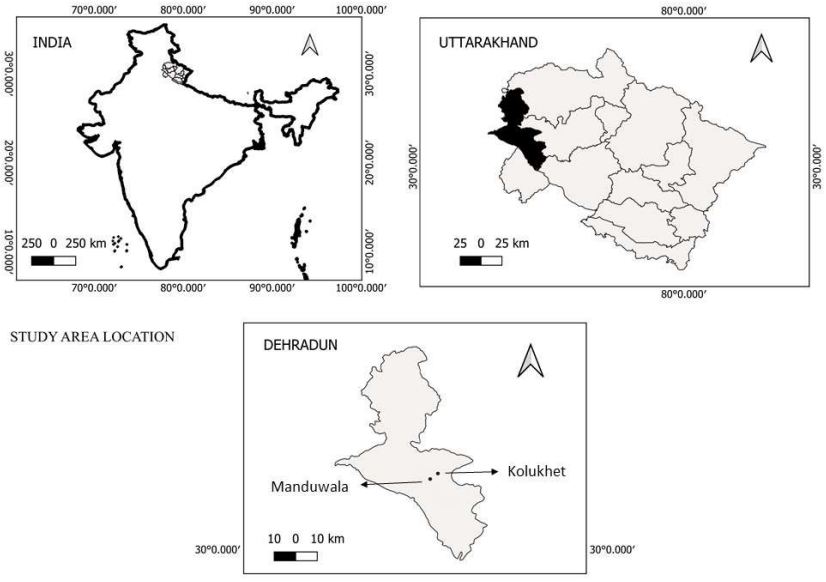

Fig. 1. Location map of study area

10 g each were prepared. These powdered samples were placed in 500 ml conical flasks. A 3% solution of 98% sulfuric acid was then added to each flask. After incubation for 30 minutes, the samples were autoclaved for 20 minutes at 121 °C and 15 PSI. Following autoclaving, the pH of the samples was neutralized with a sodium hydroxide solution (Braide et al., 2016).

Preparation of culture medium for saccharomyces cerevisiae: A total of 20 ml of distilled water was used for dissolving 10 g of nutritional broth in 60 ml of water. The mixture was then autoclaved for 20 minutes at 15 PSI and 121°C. To avoid contamination, saccharomyces cerevisiae was injected under laminar air flow. The injected samples were incubated for 24 hours to facilitate fungal growth. After this period, 20 ml of the fungal culture were added to each biomass sample under laminar air flow.

Ethanol determination: The amount of ethanol present was measured using the potassium dichromate technique. To separate the liquid from the solid, the samples were first centrifuged for five minutes at 5000 rpm. A 250 ml volumetric flask was then filled with 10 ml of the sample. The volume was increased to 100 ml by the addition of water. From this diluted material, a 20 ml aliquot was taken and placed in a conical flask. To prepare 10 ml of acid dichromate solution, a 500 ml conical flask was filled with 125 ml of distilled water. Pure sulfuric acid (70 cc) was carefully added with continuous swirling. After cooling under running water, 0.75 grams of potassium dichromate were added. Using a measuring cylinder, 250 ml of distilled water was poured into each flask for dilution. Each flask was loosely stoppered and heated in a water bath at 40 to 50 degrees Celsius for 10 minutes. After the flasks had been removed from the water bath for twenty minutes, 1 ml of potassium iodide solution (1.2 mol/l) was added to each. The standard thiosulphate solution was placed in a burette, and the contents of the flask were titrated with it. When the brown hue of the solution turned green, 1–2 ml of starch was added. The equivalence point of titration was reached when the blue color of the starch–iodine complex disappeared and was replaced by a clear solution (Table 1a, 1b, 1c, 1d, & 1e) (Shilpa et al., 2013).

Statistical analysis: Statistical tool used to compare ethanol contents among two altitudes and three plant parts (replications) were Heat Map and Scatter plot using “R – software” and the simple statistical analysis of mean (Sharma 1998).

RESULTS AND DISCUSSION

Ethanol yield: Leaf samples from both elevations had the highest ethanol concentrations (7.5 ml/l and 5.1 ml/l), whereas samples from 560 m and 1550 m altitudes had the lowest ethanol contents (2.6 ml/l and 2.7 ml/l, respectively) (Table 2). Darker colors represent higher ethanol concentrations. It also reveals that leaf samples from 560 m showed the highest values, while stems at both altitudes contained the lowest ethanol concentrations (Fig. 3). Further, correlation scatter plot reveals that points aligned closely to a straight line, confirm the strong positive correlation ($r \approx 1.00$). The leaf point is farthest from the origin, reflecting higher ethanol contents (Fig. 4). Altitude greatly influences *L. camara* leaf and leaf-stem ethanol output, but not stem alone.

The ethanol production of leaves at 560 m was substantially greater. A greater buildup of fermentable sugars is probably the result of more favorable photosynthetic conditions at lower elevations. increased carbohydrate content in leaves at lower elevations has also been noted in earlier research (Han et al., 2020). With averages of 2.7 ml/l and 2.6 ml/l at 560 m and, respectively, the ethanol production from stems did not significantly differ between the two elevations. This implies that altitude has less of an impact on the metabolic activities and glucose content of stems. Rao et al. (2015) found steady ethanol production from stem biomass at different elevations, which is consistent with this constancy.

The production of ethanol from the combined leaves and stems varied significantly across the two elevations, with larger yields at 560 m (3.2 ml/l) than at 1550 m (2.9 ml/l). The leaves, which are more susceptible to variations in altitude, account for the majority of the total ethanol output in the combined biomass. In lower altitude regions, *L. camara* leaves produced higher bioethanol yields (Sudha et al., 2014) and attributed this to higher temperatures and sunlight exposure, which increased photosynthetic activity and carbohydrate accumulation in the leaves, resulting in higher ethanol production. Current findings are in line with these conclusions, supporting the notion that lower elevations' climates are better suited for higher leaf ethanol outputs. Furthermore, height affects biomass output and the subsequent generation of ethanol (Gupta and Sharma 2017). Their results demonstrated that improved growth conditions at lower elevations led to larger yields of biomass and ethanol. Current study also indicate greater ethanol yields for leaves at 560 m compared to 1550 m. Rao et al. (2015) concluded that the ethanol output varied little with height, suggesting that altitude had less impact on stems. This study supports our finding that the amount of ethanol produced by *L. camara* stems is constant at both 560 m and 1550 m.

These findings have significant ramifications for the synthesis of bioethanol from *L. camara*. Lower elevations are ideal for optimizing ethanol output, particularly from leaves. It

may be more appropriate to use raw material for the production of bioethanol at elevations of about 560 m. Focusing on leaf harvesting might be more effective given the large yield from leaves at lower elevations. However, stems remain a viable option due to their consistent production regardless of height.

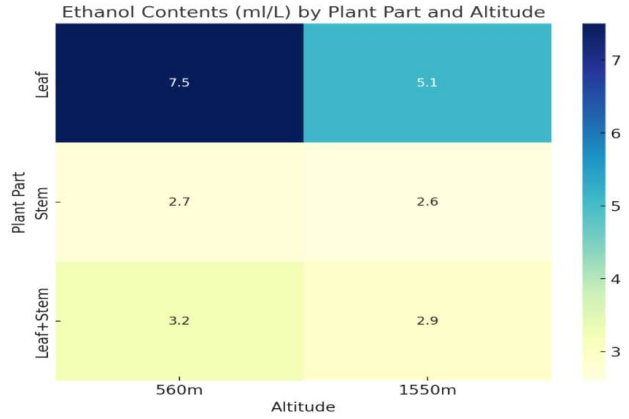

Fig. 3. Ethanol contents by plant part and altitude (Heat map)

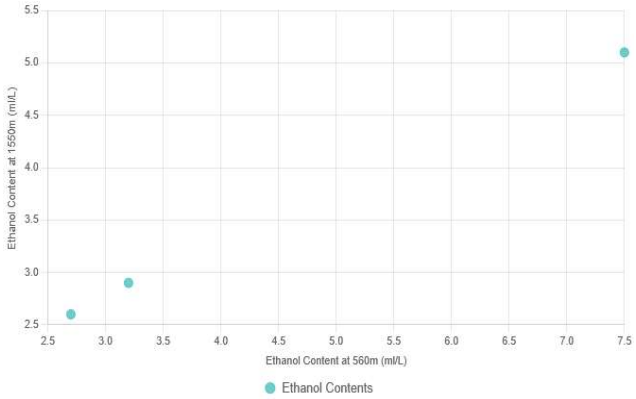

Fig. 5. Scatter plot reflecting ethanol contents in two altitudes

Table 1a. Average volume of Sodium thiosulphate used for titrated samples

Average volume of Sodium thiosulphate used for blank titration	Average volume of Sodium thiosulphate used for samples Altitude					
	560 m			1550 m		
	Leaf	Stem	Leaf + Stem	Leaf	Stem	Leaf + Stem
21.5	17.0	9.6	11.0	15.2	9.6	10.4
22.1	17.6	9.0	11.4	14.8	7.8	9.8
21.8	16.8	8.8	10.8	15.2	9.8	10.0
Average= 21.8	17.2	9.1	11.1	15.1	9.1	10.1

Table 1b. Final volume used to determine alcohol concentration

560 m			1550 m		
Leaf	Stem	Leaf + Stem	Leaf	Stem	Leaf + Stem
(21.8-17.2) = 4.6	(21.8-9.1) = 12.7	(21.8-11.1) = 10.7	(21.8-15.1) = 6.7	(21.8-9.1) = 12.7	(21.8-10.1) = 11.7

Table 1c. Number. of moles of sodium thiosulphate

560m			1550m		
Leaf	Stem	Leaf + Stem	Leaf	Stem	Leaf + Stem
M1V1 = M2V2 M1 (4.6) = 0.03 (1000) Or M1 = 6.52	M1V1 = M2V2 M1 (12.7)= 0.03(1000) Or M1 = 2.36	M1V1 = M2V2 M1 (10.7) = 0.03 (1000) Or M1 = 2.80	M 1 V 1 = M 2 V 2M 1 (6.7) = 0.03 (1000) Or M 1 = 4.48	M 1 V 1 = M 2 V 2 M 1 (12.7) = 0.03(1000) Or M 1 = 2.36	M 1 V 1 = M 2 V 2 M 1 (11.7) = 0.03 (1000) Or M 1 = 2.56

Table 1d. Number of moles of ethanol in sample dilution

560m			1550m		
Leaf	Stem	Leaf + Stem	Leaf	Stem	Leaf + Stem
6.52× 0.25 =1.63 mol	2.36× 0.25 =0.59 mol	2.80× 0.25 = 0.70 mol	4.48× 0.25 = 1.12 mol	2.36× 0.25 = 0.59 mol	2.56× 0.25 = 0.64 mol

Table 1e. Percentage of ethanol produced per 100 ml

560m			1550m		
Leaf	Stem	Leaf + Stem	Leaf	Stem	Leaf + Stem
(1.63 × 46.07)/100 = 0.75 ml	(0.59 × 46.07)/100 = 0.27 ml	(0.7× 46.07)/100 = 0.32 ml	(1.12× 46.07)/100 = 0.52 ml	(0.59× 46.07)/100 = 0.27 ml	(0.64× 46.07)/100 = 0.29 ml

Table 2. Ethanol yield from different plant parts of *Lantana camara* at two altitudes

Plant part	Ethanol contents (ml/l)	
	560 m	1550 m
Leaf	7.5[a]	5.1[a]
Stem	2.7[a]	2.6[a]
Leaf+Stem	3.2[a]	2.9[a]

Mean values followed by same letter within a column are not significantly different at 0.05%

CONCLUSION

The lower elevation of 560 m as opposed to 1550 m, the ethanol output from leaves was higher. This implies that lower elevations may offer more conducive circumstances for *L. camara* leaves to produce ethanol. This oscillation may be caused by variables including temperature, exposure to sunshine, and nutrition availability. Altitude has no discernible effect on the amount of ethanol produced by *L. camara* stems, as seen by the stems' same ethanol output at both elevations. This consistency implies that the metabolic mechanisms in stems that produce ethanol are less susceptible to variations in altitude. Higher altitude results in a statistically significant decrease in ethanol yield for the combined leaves and stems indicating that altitude affects the plant's overall ethanol production, especially when taking the biomass of the entire plant into account. By choosing appropriate growth conditions and concentrating on the most productive plant portions, our findings support the possibility of enhancing the production of bioethanol from *L. camara*.

AUTHORS' CONTRIBUTIONS

Conceptualization, methodology, writing, reviewing and editing by A.K. Uniyal, Vikaspal Singh, Sandhya Goswami, Manish Kumar and Prabhakar Manori, Data collection and analysis by Soumya Pathak. All the authors have read and approved the final manuscript.

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