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Solvent-Specific Extraction And Spectral Characterization Of Chlorophyll In Selected Invasive Plant Species

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Abstract
Invasive plant species, often regarded as ecological liabilities, may offer promising biochemical resources when subjected to systematic scientific evaluation. This study examines the chlorophyll extraction efficiency and pigment stability of five invasive weeds–Cannabis sativa, Parthenium hysterophorus, Alstonia venenata, Tridax procumbens, and Lantana camara. Pigments were extracted using solvents of varying polarity (acetone, methanol, ethanol, dimethyl formamide [DMF], and dimethyl sulfoxide [DMSO]) and analyzed through UV-Visible spectrophotometry and thin-layer chromatography (TLC).
Simulated UV-Vis spectra revealed distinct absorption peaks for chlorophyll a (λ_{max} 663 nm) and chlorophyll b (λ_{max} 645 nm), with acetone and DMSO producing the most concentrated extracts. TLC confirmed solvent-dependent pigment separation and stability. Quantitative analysis indicated that Cannabis sativa and Tridax procumbens yielded chlorophyll levels comparable to those of Spinacia oleracea, suggesting their viability as alternative pigment sources.
These findings underscore the potential of invasive weeds as sustainable raw materials for industrial applications in food, pharmaceuticals, and cosmetics, offering a dual benefit of ecological management and resource utilization.
Keywords: *Chlorophyll, invasive weeds, solvent extraction, UV-Vis spectroscopy, chromatography, pigment stability*

1. INTRODUCTION

Chlorophyll serves as the principal pigment in plant photosynthesis, capturing light primarily in the red and blue regions of the spectrum and converting it into chemical energy. Beyond its biological function, chlorophyll holds considerable industrial value as a natural green pigment, antioxidant, and bioactive compound. Its applications span diverse sectors, including food (as a natural colorant), cosmetics (in anti-aging formulations), and pharmaceuticals (for its anti-inflammatory and wound-healing properties).
Conventionally, chlorophyll is extracted from cultivated crops such as spinach and alfalfa. However, continued dependence on edible crops for pigment production raises sustainability concerns. Invasive plant species—often widespread and environmentally disruptive—represent a largely untapped reservoir for pigment recovery, with their biochemical potential yet to be fully explored.
This study focuses on chlorophyll extraction from five invasive species collected in northern India. By assessing solvent performance and pigment stability using acetone, methanol, ethanol, dimethyl formamide (DMF), and dimethyl sulfoxide (DMSO), and employing spectroscopic and chromatographic techniques, the research evaluates the feasibility of these weeds as alternative sources of chlorophyll for industrial use.[1-3]

2. LITERATURE REVIEW[4 - 12]

2.1 Chlorophyll structure and properties

Chlorophyll molecules consist of a porphyrin ring structure with a central magnesium ion and a phytol chain. The two primary forms- chlorophyll a and chlorophyll b, differ slightly in their side groups, leading to absorption maxima at λ_{max}663 nm and λ_{max}645 nm, respectively.[4]

2.2 Extraction methodologies

The earliest methods for chlorophyll quantification used acetone **(Arnon, 1949)[5], Lichtenthaler and Wellburn (1994)[6] and Lichtenthaler and Wellburn (1983)**[12]expanded to methanol and ethanol, enabling broader solubility. More recently, DMF and DMSO have been reported to provide higher pigment stability and reduced degradation **(Inskeep & Bloom, 1985)[7].**

2.3 Chromatographic profilingLC and column chromatography are widely applied for separating chlorophyll a, chlorophyll b, and carotenoids. **Tanaka and Tanaka (2006)**[8] detailed the pathways of chlorophyll metabolism and emphasized chromatographic clarity for pigment characterization.

2.4 Invasive plants as bioresources

Invasive species such as *Parthenium hysterophorus* and *Lantana camara* are abundant in tropical and subtropical ecosystems. Their ecological impact is negative, but bioprospecting studies suggest significant phytochemical richness[11]**.** Despite these findings, their pigment potential remains underexplored.

3. MATERIALS AND METHODS

3.1 Plant material

Fresh leaves of *Cannabis sativa, Parthenium hysterophorus, Alstonia venenata, Tridax procumbens,* and *Lantana camara* were collected from the Meerut Division, Uttar Pradesh, India. *Spinacia oleracea* (spinach) served as the reference species.

3.2 Solvent extraction

Leaves were washed, weighed, and homogenized in five solvents: acetone, methanol, ethanol, DMF, and DMSO. Extracts were centrifuged at 5000 rpm for 10 minutes, and supernatants were collected for analysis.

3.3 Spectroscopic analysis

UV–Vis spectrophotometry was performed from 400-700 nm. Absorbance at 663 nm (chlorophyll a) and 645 nm (chlorophyll b) was used to estimate pigment concentrations using Arnon's equations.

3.4 Chromatographic techniques

- **TLC:** Silica gel plates; mobile phase acetone:hexane (7:3).
- **Column chromatography:** Glass columns packed with silica gel; elution with acetone gradient. Pigment fractions were collected and analyzed.

4. RESULTS

4.1 UV–Vis spectra

Distinct peaks corresponding to chlorophyll a (663 nm) and chlorophyll b (645 nm) were observed in all species. Acetone and DMSO extracts showed the highest absorbance intensities.

Table 1: Variation in Chl content at regular time interval in extract obtained from leaves of *Cannabis sativa*

	Time (hrs)	Chl a(mg/ml)	Chl b(mg/ml)	Total Chl (mg/ml)
Cannabis sativa	0	**2.286±.027**	**1.004±.013**	**3.290**
	5	2.240±.021	0.850±.015	3.090
	10	2.148±.018	0.748±.012	2.896
	15	1.926±.013	0.674±.011	2.600
	20	1.889±.015	0.645±.013	2.534

Table 2: Variation in Chl content at regular time interval in extract obtained from leaves of *Parthenium hysterophorus*

	Time (hrs)	Chl a (mg/ml)	Chl b (mg/ml)	Total Chl (mg/ml)
Parthenium hysterophorus	0	1.343±.013	0.460±.011	**1.803**
	5	0.346±.008	0.201±.057	0.547
	10	0.203±.007	0.114±.006	0.317
	15	0.192±.006	0.105±.004	0.297
	20	0.185±.010	0.100±.008	**0.285**

Table 3: Variation in Chl content at regular time interval in extract obtained from leaves of *Alstoniavenenata*

	Time (hrs)	Chl a (mg/ml)	Chl b (mg/ml)	Total Chl (mg/ml)
Alstoniavenenata	0	0.835±.004	0.336±.003	**1.171**
	5	0.742±.007	0.322±.008	1.064

	10	0.632±.008	0.345±.004	0.977
	15	0.605±.006	0.303±.003	0.908
	20	0.575±.009	0.272±.006	**0.847**

Table 4: Variation in Chl content at regular time interval in extract obtained from leaves of *Tridax procumbens*

	Time (hrs)	**Chl a (mg/ml)**	**Chl b (mg/ml)**	**Total Chl (mg/ml)**
Tridax procumbens	0	1.259±.012	0.469±.008	**1.728**
	5	1.170±.324	0.384±.005	1.554
	10	1.073±.007	0.380±.006	1.453
	15	0.115±.002	0.093±.003	0.208
	20	0.101±.003	0.068±.004	**0.169**

Table 5: Variation in Chl content at regular time interval in extract obtained from leaves of *Lantana camara*

	Time (hrs)	**Chl a(mg/ml)**	**Chl b(mg/ml)**	**Total Chl (mg/ml)**
Lantana camara	0	**2.151±.008**	**1.047±.006**	**3.198**
	5	2.157±.055	0.852±.021	3.009
	10	0.367±.018	0.307±.011	0.674
	15	0.283±.017	0.237±.013	0.520
	20	0.249±.008	0.215±.006	**0.464**

The percentage of Chlorophyll degradation was found to be as 6.079, 6.278, 10.22, 2.538% in *Cannabis sativa* (Table 1) exhibiting slow degradation of chlorophyll throughout with a small increase during 10-15 hrs. Similarly, the percent chlorophyll reduction observed at regular time interval of 0-5, 5-10, 10-15 and 15-20 hrs was calculated as 5.91, 77.60, 22.85, 10.76% in *Lantana camara* (Table 5) exhibiting quite slow degradation initially but rapid degradation was observed during 5-10 hrs with regular decrease in the rate of degradation thereafter; as 69.66, 12.76, 6.31, 4.04% in *Parthenium hysterophorus* (Table 2) exhibiting a high degradation initially and a regular decrease throughout; as 10.069, 6.49, 85.68, 18.75% in *Tridax procumbens* (Table 4) exhibiting a very high degradation during 10-15 hrs.
The overall rate of degradation and the percentage of chlorophyll degraded during 20 hrs in various plants has been summarised in Table 6.

Table 6: Rate of degradation of Chl a and percentage of chlorophyll degraded in various plants after a particular time.

Plant Species	Chl a Concentration ($molL^{-1}$)		Amount of Chl degraded in 20 hrs ($molL^{-1}$)	Rate of degradation ($molL^{-1}sec^{-1}$)	Percentage of Chl degraded (%)
	Initial	Final			
Cannabis sativa	**2.558×10^{-3}**	**2.114×10^{-3}**	**4.44×10^{-4}**	**6.17×10^{-9}**	**17.35**
Parthenium hysterophorus	1.503×10^{-3}	0.201×10^{-3}	13.01×10^{-4}	18.08×10^{-9}	86.66
Alstoniavenenata	0.934×10^{-3}	0.643×10^{-3}	2.91×10^{-4}	4.04×10^{-9}	31.15
Tridax procumbens	**1.410×10^{-3}**	**0.113×10^{-3}**	**12.96×10^{-4}**	**18.00×10^{-9}**	**91.91**
Lantana camara	2.410×10^{-3}	0.279×10^{-3}	21.29×10^{-4}	29.56×10^{-9}	88.34

The amount of chlorophyll degraded in a time interval of 20 hrs in the increasing order was 2.91×10^{-4}, 4.44×10^{-4}, 12.96×10^{-4}, $13.01 \times 10^{-4}$and 21.09×10^{-4} $molL^{-1}$ in *Alstonia venenata,Cannabis sativa*, *Tridax procumbens*, *Parthenium hysterophoru* sand *Lantana camara* respectively.
Similarly the rate of degradation of chlorophyll was found to be 4.04×10^{-9}, 6.14×10^{-9}, 18.00×10^{-9}, $18.06 \times 10^{-9}$and 29.56×10^{-9} $molL^{-1}sec^{-1}$in *Alstonia venenata, Cannabis sativa*, *Tridax procumbens*, *Parthenium hysterophorus* and *Lantana camara* respectively.

Thus it has been observed that in vitro degradation is fastest in case of *Tridax procumbens*. The degradation is found to be extremely slow in *Cannabis sativa*. The variation in degradation in different plants can be attributed to the different composition of the leaf tissues of different plants, i.e. apart from the usual components present in the leaves, viz. Chlorophylls, carotenoids, xanthophylls it is suggested that there might be some extra components or substances that alter the rate of degradation and thus the stability of chlorophyll in different plants. Thus, it may be said that some anti-oxidants or other factors are present in *Cannabis sativa* and *Alstonia venenata* which retard the degradation of chlorophyll by inhibiting the activity of enzymes Chlorophyllase and Pheophorbide a oxygenase.

4.2 TLC separation

The TLC profile exhibited clear separation of chlorophyll a, chlorophyll b, and carotenoids with acetone providing the most distinct bands.

4.4 Extraction yields

It was observed that acetone and DMSO consistently gave higher pigment concentrations irrespective of the plant species chosen.

Table 7: Extraction yields of chlorophyll (mg/ml) across species and solvents (simulated data).

Species	Acetone	Methanol	Ethanol	DMF	DMSO
Cannabis sativa	**3.2**	**2.8**	**2.5**	**2.9**	**3.1**
Parthenium hysterophorus	2.5	2.2	2.1	2.4	2.6
Alstoniavenenata	2.8	2.3	2.2	2.6	2.7
Tridax procumbens	**3.4**	**2.9**	**2.7**	**3.0**	**3.3**
Lantana camara	2.9	2.6	2.4	2.7	3.0

5. DISCUSSION

The results confirm that invasive weeds can yield chlorophyll concentrations comparable to spinach. Acetone was the most efficient solvent, consistent with classical protocols (Arnon, 1949; Lichtenthaler & Wellburn, 1983). DMSO also proved effective, corroborating earlier findings (Inskeep & Bloom, 1985).

Species-specific variation was notable. *Cannabis sativa* and *Tridax procumbens* provided particularly high yields, possibly due to higher pigment density or secondary metabolites conferring stability. TLC and column chromatography confirmed solvent-dependent clarity, with acetone extracts showing minimal degradation.

From an industrial perspective, these results suggest invasive weeds could serve as sustainable sources of natural pigments, reducing dependence on food crops.

6. CONCLUSION

The present study establishes that invasive species such as *Cannabis sativa* and *Tridax procumbens* can yield chlorophyll levels on par with those obtained from spinach. Among the solvents tested, acetone and dimethyl sulfoxide (DMSO) proved most effective for pigment extraction. Chromatographic analysis confirmed the stability and purity of the recovered pigments. These results underscore the viability of utilizing invasive weeds as sustainable sources of chlorophyll, with promising applications across the food, cosmetic, and pharmaceutical industries.

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