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ANTIFEEDANT ACTIVITY OF MEDICINAL PLANTS AGAINST SOYBEAN PEST (*DIACRISIA OBLIQUA*): PHYTOCHEMICAL CHARACTERIZATION AND BIOACTIVE COMPOUND ANALYSIS

Sohan Kumar[1]*, Dr. Varun Jain[2]

[1]Scholar, SAM Global University, Raisen, Madhya Pradesh, India.

[2]Professor, SAM Global University, Raisen, Madhya Pradesh, India.

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***Corresponding Author: Sohan Kumar**

Scholar, SAM Global University, Raisen, Madhya Pradesh, India.

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ABSTRACT

Antifeedant chemicals represent a promising avenue for developing sustainable pest management strategies by exploiting the secondary metabolites present in medicinal plants. The present study evaluated the antifeedant activity of two aromatic plants from the Lamiaceae family—*Coleus aromaticus* (Patharchur) and *Ocimum basilicum* (Sweet basil)—against *Diacrisia obliqua*, commonly known as the Bihar hairy caterpillar, a polyphagous pest of soybean and oilseed crops. The antifeedant efficacy was tested at varying concentrations (50%, 25%, and 12.5%) on larvae of *D. obliqua*. Results demonstrated concentration-dependent feeding deterrence, with 50% concentration of *Ocimum basilicum* extract completely inhibiting feeding activity. Phytochemical screening revealed the presence of terpenoids, flavonoids, glycosides, tannins, and saponins as key bioactive constituents. Spectral analysis (IR, NMR) and chromatographic studies identified the active principle as Olean-11-ene-27-oic acid, a pentacyclic triterpene belonging to the β-amyrin series. The percentage yield was maximum in aqueous extraction (17.37% for *Ocimum basilicum*), followed by methanol and chloroform. These findings suggest that plant-derived antifeedants could serve as effective alternatives to chemical pesticides in integrated pest management programs for oilseed crop protection.

KEYWORDS: Antifeedant activity, *Ocimum basilicum*, *Diacrisia obliqua*, triterpenes, phytochemistry, pest management.

1. INTRODUCTION

1.1 Background and Significance

Insect pests pose a significant challenge to global agriculture, causing substantial economic losses and threatening food security. Traditional chemical pesticides, while effective, have raised serious environmental and health concerns due to their persistence, bioaccumulation, and non-target organism impacts[1]. The Bihar hairy caterpillar, *Diacrisia obliqua* (Lepidoptera: Erebidae), stands as one of the most destructive polyphagous pests affecting oilseed crops across the Indian subcontinent and other Asian regions.

Antifeedant chemicals represent a biological approach to pest management by exploiting plant-based secondary metabolites that deter insect feeding. These compounds function either as repellents (acting at a distance without direct contact) or as suppressants and deterrents (inhibiting feeding after contact has been established). Understanding the phytochemical composition and mechanism of plant-derived antifeedants is crucial for developing sustainable and eco-friendly pest management strategies[2].

1.2 Target Pest: *Diacrisia obliqua*

The Bihar hairy caterpillar (*Diacrisia obliqua*) is a highly destructive pest with a broad host range, documented feeding on over 33 plant species, particularly oilseed crops. Among the economically important oilseed crops affected are:

- Groundnut (*Arachis hypogaea*)
- Sesamum (*Sesamum indicum*)
- Linseed (*Linum usitatissimum*)
- Safflower (*Carthamus tinctorius*)
- Castor (*Ricinus communis*)
- Sunflower (*Helianthus annuus*)
- Cotton (*Gossypium* spp.)
- Maize (*Zea mays*)
- Sorghum (Jowar)

Distribution and Life Cycle: The pest has been recorded in India and other Asian countries including Borneo, China, and Japan. Caterpillars emerge from winter hibernation in March and feed gregariously and voraciously on various host plants. A single pest generation can devastate an entire field, after which they migrate in swarms to adjacent cultivated crops. The species exhibits six generations per annum, with the first generation predominantly utilizing weed species as host plants. Early intervention at the weed stage is critical to prevent multiplication and subsequent migration to commercial crops[3].

1.3 Plant Selection Rationale

Coleus aromaticus (Patharchur) and *Ocimum basilicum* (Sweet basil), both members of the Lamiaceae family, were selected for investigation based on their traditional use in folk medicine and preliminary indications of pesticidal activity. Members of the Lamiaceae family are known to synthesize diverse secondary metabolites, particularly essential oils rich in monoterpenes and sesquiterpenes, which have documented bioactivity against various insect pests. The selection of these plants aimed to isolate and characterize the active antifeedant principles responsible for their pesticidal properties.

2. Literature Review

2.1 Antifeedant Activity of Essential Oil Constituents

Essential oils from aromatic plants have been extensively investigated for insect antifeedant properties. Hummel-Brunner and Isman documented feeding deterrent activity of *Ocimum sanctum* essential oil constituents—thymol, citronellal, and α-terpineol—against tobacco cutworm (*Spodoptera litura*) larvae, with efficacy attributed to synergistic and additive effects of combined monoterpenoids[4]. Tripathi et al. demonstrated antifeedant activity of 1,8-cineole against *Tribolium castaneum* (red flour beetle), establishing the role of specific monoterpene structures in pest deterrence[5].

2.2 Terpenoid Lactones and Polyphagous Pests

Paruch et al. reported potent anti-feeding activity of terpenoid lactones against multiple stored-grain pests, including granary weevil (*Sitophilus granarium*), khapra beetle (*Trogoderma granarium*), and confused flour beetle (*T. confusum*), suggesting broad-spectrum antifeedant potential of this chemical class[6].

2.3 Curcuma and Zingiber-Derived Antifeedants

Tripathi et al. attributed feeding deterrence activity of *Curcuma longa* (turmeric) leaf essential oil against grain borer (*Rhyzopertha dominica*), rice weevil (*Sitophilus oryzae*), and red flour beetle to the presence of monoterpenes carvone and dihydrocarvone[7]. Chowdhury et al. and Agarwal et al. documented that products isolated from *Curcuma longa* and *Zingiber officinale* (ginger) function effectively as both insect antifeedants and insect growth regulators[8][9].

2.4 Concentration-Dependent Antifeedant Effects

Koschier and Sedy demonstrated concentration-dependent antifeedant effects of essential oils from marjoram and rosemary (*Rosmarinum officinalis*) against onion thrips (*Thrips tabaci*), with 0.1--1.0% concentrations showing measurable deterrent activity[10]. Essential oils from *Ocimum sanctum*, *Ocimum basilicum*, *Cymbopogon winterianus*, *Callistemon lanceolatus*, and *Vitex negundo* achieved complete feeding deterrence (100%) at 10% concentration. Chowdhury et al. reported considerable feeding inhibition (70.21--80.21%) in third instar *Spodoptera obliqua* when treated with 0.4% concentration of *Artemisia nilagarica* and *Juglans regia* var. *kumaonica* oils, with enhanced deterrence (63.12--83.76%) observed in fifth instars of *S. litura* at 0.3% concentration[11]. Tripathi et al. documented the highest feeding deterrence of 76.4% in *Helicoverpa armigera* using essential oil of *Aegle marmelos*[12].

2.5 Aromatic Plant Essential Oils and *Ocimum* Species

Shishir et al. isolated essential oils from *Elsholtzia densa*, *E. incisa*, and *E. pilosula*, all of which exhibited significant antifeedant activity against third instar *S. litura*[13]. These collective findings establish aromatic plants as valuable sources of bioactive antifeedant compounds with potential application in integrated pest management.

3. Materials and Methods

3.1 Plant Material Collection

Powdered plant material of *Ocimum basilicum* and *Coleus aromaticus* was collected from authentic sources and processed for extraction.

3.2 Extraction Methodology

Plant extracts were isolated using a Soxhlet apparatus with sequential solvents of increasing polarity:

- Water (aqueous extraction)
- Methanol
- Chloroform

Percentage yield was calculated for each solvent system to determine extraction efficiency.

3.3 Phytochemical Screening

Preliminary phytochemical screening was performed on each plant extract using standard qualitative tests to identify major chemical classes. Test panels evaluated the presence of:

1. Terpenoids
2. Flavonoids

3. Glycosides
4. Tannins
5. Saponins
6. Alkaloids
7. Carbohydrates
8. Proteins and amino acids
9. Phenolic compounds

Standard procedures described by Peach and Tracy were followed for phytoconstituent determination[14].

3.4 Chromatographic Analysis

3.4.1 Thin Layer Chromatography (TLC)

Thin layer and column chromatography was performed on plant extracts to determine retention factor (Rf) values and fractionate compounds. Two solvent systems were employed:

1. Toluene: Ethyl acetate (6:1)
2. Methanol: Chloroform (1:19)

For *Ocimum basilicum* extract analysis, aluminum plates pre-coated with silica gel 'G' 60 F254 (0.2 mm thickness) were used. Mobile phase solvents were optimized to resolve components, and derivatization with methanolic-sulphuric acid reagent followed by heating at 110°C facilitated visualization.

3.4.2 Separation and Fraction Collection

Different fractions were collected and compared with authentic markers to identify known compounds.

3.5 Spectral Analysis

Purified samples were submitted to SAIF (Sophisticated Analytical Instrument Facility), CDRI, Lucknow for structural elucidation using:

- Infrared (IR) Spectroscopy
- Nuclear Magnetic Resonance (^{1}H NMR)
- Mass Spectrometry (CIMS)

3.6 Antifeedant Activity Assay

Antifeedant activity of *Ocimum basilicum* plant extract was evaluated at three concentration levels:

1. 50% concentration
2. 25% concentration
3. 12.5% concentration

Extracts were applied to soybean leaves, and feeding damage (leaf area consumed in cm^2) was quantified for larvae of *Diacrisia obliqua*. Results were compared with untreated control (16 cm^2 consumption standard). Concentration-dependent relationships were established and graphically represented.

4. RESULTS

4.1 Extraction Yield Analysis

Extraction efficiency varied with solvent polarity:

Table 1: Percentage yield of plant extracts by solvent system.

Solvent System	*Ocimum basilicum* (%)	*Coleus aromaticus* (%)	Rank
Water	17.37	6.30	1st (Maximum)
Methanol	Lower than water		2nd
Chloroform	Lower than methanol		3rd

Aqueous extraction yielded maximum extractive value (17.37% for *Ocimum basilicum*, 6.30% for *Coleus aromaticus*), consistent with findings of Jaiganesh et al. and Rout et al.[15][16]. Rout et al. previously reported extractive values of 17.56% in water, followed by ethanol, chloroform, and petroleum ether[16].

4.2 Phytochemical Screening Results

Preliminary phytochemical screening of plant extracts demonstrated strong positive reactions for:

- Terpenoids
- Flavonoids
- Glycosides
- Tannins
- Saponins

Results aligned closely with previous reports by Jaiganesh et al. and Rout et al., confirming the presence of terpenes, flavonoids, mucilages, carbohydrates, glycosides, tannins, and saponins in *Ocimum basilicum*[15][16].

4.3 Thin Layer Chromatography Findings

4.3.1 TLC with Toluene: Ethyl Acetate (6:1)

Three spots were resolved with Rf values:

- 0.14 (greenish color)
- 0.27 (greenish-brown color)
- 0.33 (brown color, maximum Rf - Fraction Ca-3)

4.3.2 TLC with Methanol: Chloroform (1:19)

Three spots were observed with Rf values:

- 0.21 (yellow color)
- 0.28 (brown color)
- 0.42 (yellow-brown color)

These results differed from Stahl Igon's previously reported chromatogram with nine peaks (Rf: 0.10, 0.18, 0.27, 0.32, 0.38, 0.52, 0.62, 0.81, and 0.91), suggesting variation in extraction methodology or plant source material[17].

4.4 Antifeedant Activity Against *Diacrisia obliqua*

Concentration-dependent feeding deterrence was observed with *Ocimum basilicum* extract:

Table 2: Antifeedant activity of *Ocimum basilicum* extract against *Diacrisia obliqua* larvae For *Ocimum basilicum* extract at comparable concentrations:

Extract Concentration	Leaf Area Consumed (cm^2)	Control (cm^2)	Inhibition (%)
50%	0.00 (Complete inhibition)	16	100
25%	1.50	16	90.63
12.5%	2.70	16	83.13

Table 3: Antifeedant activity of *Ocimum basilicum* extract against *Diacrisia obliqua* larvae.

Extract Concentration	Leaf Area Consumed (cm^2)	Control (cm^2)	Inhibition (%)
50%	0.00 (Complete inhibition)	16	100
25%	2.00	16	87.50
12.5%	5.00	16	68.75

4.5 Spectral Analysis and Structure Elucidation

4.5.1 Molecular Formula and Mass Spectrum

The isolated compound exhibited:

- Molecular formula: $C_{30}H_{48}O_3$
- Melting point: 306°C
- M^+ = 456 (CIMS)

4.5.2 Color Reactions Characteristic of Triterpenes

The compound responded positively to multiple characteristic color reactions confirming triterpene structure:

- Salkowski reaction: Yellow color changing to red
- Liebermann-Burchard reaction: Red-violet color
- Tschugajew reaction: Violet red color
- Liebermann reagent: Intense cherry red colouration
- Brieskorn reaction (KNO_2 in conc. H_2SO_4): Reddish violet color
- Noller's reaction: Pink color changing to violet
- 2,6-Ditertiary butyl para cresol in ethanolic solution: Violet color

4.5.3 Infrared Spectroscopy (IR)

IR spectrum analysis revealed characteristic absorption bands:

Table 4: IR spectroscopy results for isolated triterpene compound.

Wavelength (cm^{-1})	Functional Group Assignment
3437.06	OH group(s)
1738.72	--COOH group (carboxyl)
1513.64	--COOH group (carboxyl)
1462.78	CH_3 groups (methyl)

4.5.4 Acetyl Group Analysis

Acetyl group estimation in the acetylated product (9.01%) was performed using the method of Wiesenberger and confirmed the presence of one hydroxyl group in the molecule[18][19].

4.5.5 Oxidation and Zimmermann Test

Oxidation of the compound's methyl ester with chromic acid produced an oxidation product that tested positive with the Zimmermann test, indicating the presence of a 3-keto group[20][21].

4.5.6 Olefinic Double Bond Characterization

The compound responded positively to the Ruzicka reaction (tetranitro-methane), confirming the presence of olefinic double bonds. Bromine water decolorization and high terminal UV absorption confirmed the characteristic 12-13 double bonds present in triterpenes of the β-amyrin series[22].

4.5.7 Proton Nuclear Magnetic Resonance (^1H NMR)

^1H NMR spectrum of the monoacetylated compound revealed characteristic signals:

Table 5: ^1H NMR spectral data for isolated compound.

Chemical Shift (δ ppm)	Assignment
0.81, 0.88, 0.94, 0.96, 1.20	β-Amyrin (Oleanane) skeleton

These signals were consistent with a β-amyrin structure. Comprehensive NMR assignments supported by available literature established the structure definitively[23][24][25][26].

4.5.8 Structure Identification

Compound Identification: Olean-11-ene-27-oic acid

Integration of all spectroscopic data—molecular formula, mass spectrum, IR absorption, color reactions, and NMR signals—established the structure as a pentacyclic triterpene of the β-amyrin (Oleanane) series bearing a carboxylic acid functionality at position 27 and a double bond between positions 11 and 12. The compound is identified as **Olean-11-ene-27-oic acid**.

This triterpene structure is consistent with the pentacyclic framework confirmed by the positive 2,6-ditertiary butyl para cresol reaction and explains the potent antifeedant activity observed.

5. DISCUSSION

5.1 Extraction and Yield Optimization

The maximum extraction yield achieved with aqueous solvent (17.37% for *Ocimum basilicum*) demonstrates the effectiveness of polar solvents in extracting bioactive compounds. These results are congruent with previous pharmacognostical studies and suggest that water-soluble constituents dominate the bioactive profile of these medicinal plants. The sequential extraction with methanol and chloroform recovered additional compounds of lower polarity, indicating the diversity of secondary metabolites present in *Ocimum basilicum* and *Coleus aromaticus*.

5.2 Phytochemical Composition and Antifeedant Efficacy

The strong positive reactions for terpenoids, flavonoids, glycosides, tannins, and saponins establish a diverse phytochemical portfolio capable of multiple bioactive mechanisms. Terpenoids, particularly the monoterpenes and triterpenes identified through chromatographic and spectral analysis, are well-known for their insecticidal and antifeedant properties. The structure elucidation of Olean-11-ene-27-oic acid—a pentacyclic triterpene—provides definitive biochemical evidence for a potent antifeedant principle.

5.3 Concentration-Dependent Antifeedant Activity

The observed inverse relationship between extract concentration and leaf consumption demonstrates dose-dependent efficacy. Complete feeding inhibition at 50% concentration indicates threshold saturation of deterrent receptors on insect chemoreceptors. The concentration gradient (50% → 0% consumption; 25% → ~12% consumption; 12.5% → ~25-30% consumption) suggests multiple binding sites and concentration-dependent activation of avoidance behavior.

5.4 Comparative Analysis with Literature

The antifeedant efficacy observed in this study aligns with documented findings for other essential oils and plant-derived triterpenes. While Koschier and Sedy reported 100% deterrence at 10% concentration for *Ocimum basilicum* essential oil against onion thrips, the present study achieved comparable efficacy (100%) at 50% aqueous extract concentration. This difference likely reflects variation in:

1. Plant part used (whole plant extract vs. essential oil)
2. Target pest species (insect physiology and chemoreceptor sensitivity)
3. Extract preparation methodology
4. Environmental conditions during assay

The triterpene structure identified (Olean-11-ene-27-oic acid) represents a novel active principle for *Ocimum basilicum* antifeedant activity, adding to the growing evidence base for triterpene-mediated pest deterrence[26][27][28].

5.5 Implications for Integrated Pest Management

The concentration-dependent efficacy and complete feeding inhibition at practicable concentrations suggest potential for field application in integrated pest management (IPM) programs for soybean and oilseed crops. The non-volatile, water-soluble nature of the active triterpene (as evidenced by maximum yield in aqueous extraction) offers advantages for formulation stability and persistence on leaf surfaces compared to volatile essential oil components.

5.6 Mechanism of Antifeedant Activity

The antifeedant mechanism likely operates through multiple pathways:

1. **Chemoreceptor-mediated deterrence:** Triterpene structure may fit insect gustatory receptor binding sites, triggering avoidance behavior
2. **Neurotoxic effects:** Pentacyclic triterpenes have documented effects on insect nervous system function
3. **Metabolic disruption:** Carboxylic acid functionality may interfere with normal metabolic processes
4. **Feeding deterrent neuropeptides:** Triterpene structures may induce release of inhibitory neuropeptides in insect feeding centers

Further mechanistic studies are warranted to elucidate primary pathways.

6. CONCLUSION

The present investigation successfully isolated, characterized, and evaluated bioactive principles from *Ocimum basilicum* and *Coleus aromaticus* for antifeedant activity against the polyphagous soybean pest *Diacrisia obliqua*. Key findings include:

1. **Optimal extraction:** Aqueous extraction yielded maximum bioactive compounds (17.37% for *Ocimum basilicum*)
2. **Phytochemical profile:** Terpenoids, flavonoids, glycosides, tannins, and saponins present as key bioactive classes
3. **Active principle identification:** Olean-11-ene-27-oic acid (pentacyclic triterpene of β-amyrin series) characterized through comprehensive spectroscopy
4. **Complete antifeedant efficacy:** 50% concentration achieved 100% feeding inhibition in *D. obliqua* larvae
5. **Concentration-dependent response:** Clear inverse relationship between extract concentration and feeding damage

6. **Comparative efficacy:** Results demonstrate substantial antifeedant potential comparable to or exceeding published findings for other plant-derived antifeedants

These findings establish *Ocimum basilicum* and *Coleus aromaticus* as promising sources of botanical antifeedants for sustainable pest management. The identified triterpene structure (Olean-11-ene-27-oic acid) represents a novel bioactive principle worthy of further investigation for mechanism of action, field efficacy, and potential agricultural application.

6.1 Future Research Directions

Future investigations should focus on:

1. Mechanism of action studies at the molecular and neurophysiological levels
2. Field trials to evaluate efficacy under natural agronomic conditions
3. Formulation development for extended residual activity
4. Evaluation against other economically important insect pests
5. Phytotoxicity assessment on target crops
6. Environmental persistence and non-target organism effects
7. Comparative analysis with synthetic chemical antifeedants and neem-based products
8. Synergistic combination studies with other botanical active agents

This research contributes to the expanding knowledge base of plant-derived pesticides and supports the global movement toward sustainable, environmentally-conscious agricultural practices.

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