



### REVIEWER'S REPORT

Manuscript No.: IJAR-56104

Title: ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF AYAPANA TRIPLINERVIS (VAHL) R.M. KING & H.ROB,

**Recommendation:**

Accept as it is .....

Accept after minor revision.....

Accept after major revision .....YES.....

Do not accept (*Reasons below*) .....

Rating	Excel.	Good	Fair	Poor
Originality		√		
Techn. Quality			√	
Clarity		√		
Significance		√		

Reviewer Name: Prof. Dr. Dillip Kumar Mohapatra

## Detailed Reviewer's Report

### 1. Strengths of the Manuscript

#### Clear objective and relevance

The study addresses an important area of natural product research by evaluating phytochemical composition along with antioxidant and antibacterial activities of *Ayapana triplinervis*, a medicinally significant plant used in traditional medicine.

#### Well-structured methodology

The experimental procedures for phytochemical screening, total phenolic and flavonoid estimation, antioxidant assays (DPPH and reducing power), and antimicrobial evaluation are clearly described and follow standard, widely accepted protocols.

#### Use of multiple biological assays

The combined assessment of qualitative phytochemistry, quantitative phenolic/flavonoid content, antioxidant assays, and antibacterial screening strengthens the overall experimental design.

#### Relevant comparison with previous literature

The discussion appropriately compares findings with earlier reports, supporting the credibility of the results.

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### Clear presentation of results

Results are systematically presented with tables and figures, making the findings easy to understand.

## 2. Weaknesses of the Manuscript

### Limited novelty

Similar studies on *Ayapana triplinervis* or its synonym (*Eupatorium ayapana*) reporting phytochemical screening, antioxidant, and antimicrobial activities have already been published. The manuscript does not clearly highlight how this study significantly advances existing knowledge.

### Lack of statistical analysis

The results for antioxidant and antimicrobial assays lack statistical treatment (mean  $\pm$  SD, number of replicates, significance testing), which weakens the scientific rigor.

### Absence of MIC determination

Antimicrobial activity is evaluated only by disc diffusion. Minimum inhibitory concentration (MIC) values are not determined, limiting the interpretation of antibacterial potency.

### No positive control used

Standard antioxidants (e.g., ascorbic acid) and standard antibiotics are not included as positive controls, making it difficult to benchmark the activity of the plant extract.

### Inconsistencies in quantitative data

There is inconsistency between phenolic and flavonoid values reported in the Results and Discussion sections, which requires clarification.

### Language and formatting issues

The manuscript contains grammatical errors, spacing issues, inconsistent scientific nomenclature, and formatting problems that need careful revision.

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### *3. Scientific Significance*

The study contributes supportive data confirming the antioxidant and antibacterial potential of *Ayapana triplinervis* leaves, reinforcing its ethnomedicinal importance. However, due to the descriptive nature of the work and limited mechanistic insight, the scientific impact remains moderate. The manuscript is more suitable for a **regional or mid-tier journal** unless additional novelty and depth are incorporated.

### *4. Key Points for Improvement (Major Revisions Required)*

Clearly **state the novelty** of the study in the Introduction.

Include **statistical analysis** for all quantitative results.

Add **positive controls** for antioxidant and antibacterial assays.

Determine and report **MIC values** for antibacterial activity.

Resolve **data inconsistencies** in phenolic and flavonoid content.

Improve **language, grammar, and formatting** throughout the manuscript.

Consider adding **phytochemical profiling (HPLC/GC-MS)** to enhance originality.

### *5. Recommendation*

#### **Major Revision**

The manuscript has scientific merit and experimental validity but requires **substantial improvements** in data analysis, presentation, and novelty before it can be considered for publication.

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### *Justification for Major Revision*

#### Issue 1: Limited Novelty

**Problem:**

The manuscript reports phytochemical screening, antioxidant, and antimicrobial activities of *Ayapana triplinervis*, which have already been documented in earlier studies under the same or synonymous species (*Eupatorium ayapana*).

**Why Major Revision:**

Lack of clearly defined novelty reduces the scientific contribution. The authors must explicitly state what differentiates this study (e.g., geographical variation, comparative analysis, improved methodology, or quantitative advancement).

**Required Action:**

Revise the Introduction to clearly highlight the research gap and novelty.

#### Issue 2: Absence of Statistical Analysis

**Problem:**

Quantitative results for total phenolic content, flavonoid content, antioxidant assays, and antimicrobial activity are presented without statistical validation (mean  $\pm$  SD, number of replicates, or significance testing).

**Why Major Revision:**

Without statistical analysis, the reliability and reproducibility of results cannot be assessed, which is a fundamental requirement for experimental studies.

**Required Action:**

Include proper statistical analysis for all quantitative data.

#### Issue 3: Incomplete Evaluation of Antimicrobial Activity

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**Problem:**

Antibacterial activity is evaluated only using the disc diffusion method. Minimum inhibitory concentration (MIC) values are not reported.

**Why Major Revision:**

Disc diffusion alone provides only qualitative or semi-quantitative information. MIC determination is essential to establish antibacterial potency.

**Required Action:**

Perform and report MIC values for tested microorganisms.

### Issue 4: Lack of Positive Controls

**Problem:**

Standard antioxidants (e.g., ascorbic acid, BHT) and standard antibiotics are not included in antioxidant and antimicrobial assays.

**Why Major Revision:**

Without positive controls, it is not possible to benchmark the activity of the plant extract or assess its comparative effectiveness.

**Required Action:**

Include appropriate positive controls and discuss results comparatively.

### Issue 5: Inconsistency in Quantitative Data

**Problem:**

Reported values of total phenolic and flavonoid content show inconsistencies between the Results and Discussion sections.

**Why Major Revision:**

Data inconsistency raises concerns about accuracy and interpretation of results.

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**Required Action:**

Recheck, correct, and harmonize all quantitative data across sections.

### Issue 6: Overly Descriptive and Weak Discussion

**Problem:**

The Discussion section mainly reiterates results and previously published literature, with limited critical interpretation or mechanistic insight.

**Why Major Revision:**

A strong discussion is required to justify the importance of findings and distinguish the study from existing literature.

**Required Action:**

Strengthen the Discussion by critically interpreting results and clearly linking them to scientific relevance.

### Issue 7: Language, Grammar, and Formatting Errors

**Problem:**

The manuscript contains grammatical errors, inconsistent formatting, improper scientific nomenclature, and spacing issues.

**Why Major Revision:**

Language and formatting issues affect clarity, readability, and professional presentation, which are essential for journal publication.

**Required Action:**

Thorough language editing and formatting correction are required.

### Issue 8: Limited Scientific Depth

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### Problem:

The study relies mainly on preliminary assays without advanced phytochemical characterization.

### Why Major Revision:

The absence of compound profiling limits mechanistic understanding and originality.

### Required Action:

Consider inclusion of advanced analytical techniques (HPLC/GC-MS) or clearly justify their absence.

### *Final Recommendation*

## MAJOR REVISION

The manuscript shows experimental potential and relevance to ethnopharmacology; however, due to **limited novelty, lack of statistical rigor, incomplete antimicrobial evaluation, absence of controls, data inconsistencies, and language issues**, substantial revisions are required before the manuscript can be reconsidered for publication.