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REVIEWER'S REPORT

Manuscript No.: IJAR-55540

Title: Anthocyanin content, lipid peroxidation inhibition and anti-salmonellosis activity of Vitelaria paradoxa Gaertn and Parkia biglobosa (Jacq.) Benth bark extracts.

Recommendation:
Accept as it is

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

Reviewer Name: Dr. Manju M

Date: 03-01-2026

Detailed Reviewer's Report

1. Background and Scientific Rationale

The rapid rise of antimicrobial resistance has significantly reduced the effectiveness of conventional antibiotics, particularly against enteric pathogens such as Salmonella spp. This challenge necessitates the exploration of alternative therapeutic agents of natural origin. Medicinal plants traditionally used in ulcer management are promising reservoirs of bioactive compounds. Vitellaria paradoxa and Parkia biglobosa are widely used in African ethnomedicine, justifying their scientific evaluation. Their stem bark was selected due to its known richness in secondary metabolites. This study integrates antioxidant and antimicrobial investigations to assess their therapeutic relevance.

2. Study Objectives

The primary objective was to evaluate the therapeutic potential of V. paradoxa and P. biglobosa stem bark extracts. Specifically, the study aimed to quantify total anthocyanin content, assess lipid peroxidation inhibition capacity, and determine antibacterial activity against clinical Salmonella strains. A comparative analysis between plant species and extraction solvents was performed. The work also sought to characterize bactericidal versus bacteriostatic effects. Overall, the study links phytochemical content to biological activity.

3. Selection of Plant Species

Vitellaria paradoxa and Parkia biglobosa were selected based on their extensive traditional use in gastrointestinal disorders. Both species are native to West Africa and commonly used in herbal medicine. Their bark has been reported to possess antimicrobial and antioxidant properties. However, comparative scientific data on their efficacy against Salmonella spp. remain limited. This study fills that gap through systematic experimentation.

4. Collection and Authentication of Plant Material

Stem bark samples were collected in February 2023 from Sèmèrè village, Donga Department, northern Benin. Geographic coordinates were precisely recorded to ensure reproducibility. Samples were air-dried under controlled laboratory conditions (20 ± 2 °C) for 15 days. Drying minimized enzymatic degradation of bioactive compounds. The dried bark was ground into fine powder to increase extraction efficiency.

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5. Preparation and Selection of Extraction Solvents

Multiple solvents of varying polarity were employed to extract a broad range of phytochemicals. These included methanol, acetone, ethyl acetate, ethanol (50%, 70%, 97%), and methanol acidified with 1% HCl. Maceration was performed for 72 hours with continuous agitation to maximize compound diffusion. Solvent diversity allowed evaluation of polarity effects on biological activity. Ethanol extracts were prioritized due to biocompatibility.

6. Extract Concentration and Storage

Following maceration, filtrates were concentrated using a rotary evaporator under reduced pressure. Drying was completed at 40 °C to preserve thermolabile compounds. The dried residues were weighed to calculate extraction yield. Extracts were stored in airtight containers to prevent oxidation and moisture absorption. These standardized extracts were used consistently in subsequent assays.

7. Determination of Total Anthocyanin Content

Total anthocyanin content was determined using the pH differential method, a reliable spectrophotometric technique. Absorbance was measured at 520 and 700 nm under pH 1.0 and pH 4.5 conditions. This method exploits the structural transformation of anthocyanins under different pH environments. Calculations were expressed as mg/g of plant powder. Triplicate analysis ensured statistical reliability.

8. Anthocyanin Distribution between Species

Anthocyanins were detected exclusively in V.paradoxa bark extracts, while P. biglobosa extracts were below the limit of quantification. This highlights strong interspecific phytochemical variation. The absence of detectable anthocyanins in P. biglobosa suggests alternative bioactive compounds dominate its activity. This specificity may arise from genetic and metabolic differences. Such findings underline the importance of species-level phytochemical profiling.

9. Effect of Solvent Polarity on Anthocyanin Extraction

Among V. paradoxa extracts, 70% ethanol yielded the highest anthocyanin content, followed by 50% and 97% ethanol. Intermediate polarity solvents favored anthocyanin solubilization. Highly aqueous or highly alcoholic solvents were less efficient. This confirms that hydroalcoholic mixtures optimize phenolic extraction. Solvent polarity is therefore a critical determinant of phytochemical yield.

10. Assessment of Lipid Peroxidation Inhibition

The antioxidant activity of extracts was evaluated using the lipid peroxidation inhibition assay. Egg yolk emulsion served as a lipid-rich substrate, while Fe^{2+} ions induced oxidative stress. Thiobarbituric acid reactive substances (TBARS) were measured at 532 nm. This method reflects the capacity of extracts to prevent oxidative membrane damage. Ascorbic acid was used as a reference antioxidant.

11. Antioxidant Performance of V. paradoxa Extracts

All ethanolic extracts of V. paradoxa demonstrated strong lipid peroxidation inhibition (54–65%). Vp50 and Vp97 showed statistically superior activity compared to ascorbic acid. This suggests potent antioxidant capacity beyond standard antioxidants. The activity likely results from synergistic effects between anthocyanins and other polyphenols. These findings highlight V. paradoxa as a valuable antioxidant source.

12. Antioxidant Performance of P. biglobosa Extracts

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P. biglobosa extracts exhibited moderate lipid peroxidation inhibition (32–44%). Although lower than V. paradoxa, their activity was comparable to ascorbic acid in some cases. This suggests the presence of non-anthocyanin antioxidants such as tannins or flavonoids. The results confirm antioxidant potential despite low anthocyanin levels. Thus, multiple phytochemical classes contribute to activity.

13. Isolation and Characterization of Bacterial Strains

Twenty-two clinical Salmonella strains were isolated from blood and stool samples. Strains were obtained from patients with gastro-duodenal ulcers at CNHU. Confirmation was performed using Salmonella–Shigella agar. Clinical origin increases the relevance of the findings. The use of pathogenic strains strengthens translational significance.

14. Antibiotic Resistance Profile of Salmonella Strains

Antibiogram analysis revealed alarming resistance patterns. All strains were resistant to ampicillin, penicillin, and clindamycin. Resistance to tetracycline exceeded 95%. In contrast, no resistance was observed to ceftriaxone and norfloxacin. These results underscore the urgent need for alternative antimicrobial agents.

15. Initial Antibacterial Screening of Plant Extracts

Disc diffusion assays demonstrated strong antibacterial activity for both plant species. Most strains showed high sensitivity, with inhibition zones exceeding 9 mm. Ethanolic, methanolic, and acetonic extracts were particularly effective. Resistance to extracts was rare and sporadic. These findings confirm broad-spectrum antimicrobial potential.

16. Comparative Sensitivity Patterns

V.paradoxa extracts showed 85–95% "highly sensitive" responses among strains. P. biglobosa extracts exhibited even higher sensitivity levels (>90%). Variations were observed depending on solvent polarity. Ethyl acetate and acidified methanol extracts showed slightly reduced consistency. Overall, both species demonstrated remarkable antibacterial efficacy.

17. Determination of Minimum Inhibitory Concentrations

MIC values ranged from 0.78 to 50 mg/mL across extracts and strains. Lower MICs indicate higher antibacterial potency. Ethanolic and acetonic extracts consistently produced lower MIC values. Acidified methanol extracts showed selective activity. These quantitative results validate the qualitative diffusion assay findings.

18. Determination of Minimum Bactericidal Concentrations

MBC values ranged between 6.25 and 50 mg/mL. Many extracts showed MBC values close to MIC values, indicating strong killing capacity. The ethanol and acetone extracts were particularly effective. MBC determination confirmed true bactericidal action rather than growth inhibition alone. This enhances therapeutic relevance.

19. Bactericidal versus Bacteriostatic Classification

The MBC/MIC ratio was used to classify antimicrobial effects. Ratios ≤ 4 indicated bactericidal activity. Most V. paradoxa extracts were predominantly bactericidal (72–95%). This suggests lethal effects on Salmonella cells. Such activity is desirable in severe infections.

20. Activity Profile of P. biglobosa Extracts

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P. biglobosa extracts displayed more variable antimicrobial profiles. Ethanolic and acetonic extracts showed higher bactericidal proportions. Highly polar solvents produced more undetermined effects. This variability suggests solvent-dependent extraction of active compounds. Nonetheless, all extracts showed bactericidal action on at least one strain.

21. Role of Solvent Polarity in Antimicrobial Activity

Solvent polarity strongly influenced antibacterial outcomes. Intermediate polarity solvents extracted compounds with optimal antimicrobial efficacy. Highly polar solvents extracted heterogeneous compounds with variable effects. This emphasizes the importance of extraction optimization. Solvent choice directly affects therapeutic potential.

22. Correlation Between Antioxidant and Antibacterial Activity

Extracts with higher antioxidant activity generally exhibited stronger antibacterial effects. This suggests shared or synergistic mechanisms, such as membrane stabilization or oxidative stress modulation. Anthocyanins and polyphenols may contribute to both activities. However, the correlation was not strictly linear. Multiple bioactive pathways are likely involved.

23. Mechanistic Implications

Although mechanisms were not directly studied, the results suggest possible membrane disruption, enzyme inhibition, or interference with bacterial metabolism. Antioxidant compounds may enhance antimicrobial efficacy by reducing oxidative defense systems in bacteria. Phenolic compounds are known to affect quorum sensing. Further mechanistic studies are warranted.

24. Overall Significance and Research Implications

This study demonstrates that V. paradoxa and P. biglobosa bark extracts possess strong antioxidant and antibacterial properties. Their effectiveness against multidrug-resistant Salmonella highlights their therapeutic promise. The findings support their traditional medicinal use. Further chemical characterization, toxicity evaluation, and in vivo studies are essential for drug development.

25. Recommendation

- Advanced phytochemical profiling of bioactive compounds
- In vivo validation of antioxidant and antibacterial efficacy
- Comprehensive toxicological and safety evaluation studies
- Optimization of extraction and formulation strategies
- Investigation of molecular antimicrobial mechanisms