CRITIQUE OF PAPER PUBLISHED BY RATHOD, DHADUK, AND JETHAVA, TITLED, “A REVIEW ON QUALITATIVE AND QUANTITATIVE ANALYSIS OF CARBOHYDRATES EXTRACTED FROM BACTERIA

BY

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**INTRODUCTION**

The review article by Rathod, Dhaduk, and Jethava (2022), "A Review on Qualitative and Quantitative Analysis of Carbohydrates Extracted from Bacteria", attempts to synthesize various classical techniques used for the detection and quantification of bacterial carbohydrates. While the topic is timely—especially considering the increasing relevance of bacterial carbohydrate profiling in diagnostics, vaccine development, and biotechnology—the article ultimately falls short of being a comprehensive, analytical resource. Rather than offering a critical comparative assessment, the review primarily presents a list of traditional tests with minimal mechanistic detail, comparative evaluation, or discussion of their practical relevance. This limits its value for students and professionals looking to make informed decisions on appropriate testing strategies.

The authors include foundational qualitative tests such as Molisch’s, Iodine, Benedict’s, Fehling’s, Barfoed’s, and Seliwanoff’s tests. While these methods are indeed classical and widely used in carbohydrate analysis, the explanations provided are overly simplistic. For example, the review correctly identifies Molisch’s test as a general assay for carbohydrates, relying on the formation of a purple ring through the dehydration of sugars by concentrated sulfuric acid followed by a reaction with α-naphthol. However, it fails to mention that this test reacts with virtually all carbohydrates—including polysaccharides and non-reducing sugars—thus lacking specificity. Yadav and Yadav (2021) emphasize that while the Molisch test is valuable for initial screening, its non-selectivity renders it unsuitable for detailed compositional analysis, particularly in complex biological samples.

The Iodine test is another instance where the paper's presentation is superficial. It correctly notes that iodine reacts with starch and glycogen to yield a blue-black color; however, it fails to mention that this interaction is reversible upon heating and that the color response may vary depending on the structure of the polysaccharide. Rinaldi et al. (2023) highlighted these limitations, explaining that the test’s utility is reduced when analyzing mixed or modified polysaccharide samples.

A particularly problematic point in the article is the treatment of Benedict’s and Fehling’s tests. While both assays detect reducing sugars through the reduction of copper (II) ions to copper (I) oxide, the review treats them as functionally identical without acknowledging their practical differences. Barman et al. (2022) noted that Benedict’s reagent is more stable and safer to handle, making it preferable in many laboratory settings. The review misses this nuance, which is essential for selecting the most suitable assay based on laboratory conditions.

The description of Barfoed’s test also lacks depth. Though the authors note that it distinguishes monosaccharides from disaccharides based on reaction time, they omit the critical detail that the test’s acidic environment enables this selectivity. Guo et al. (2023) explain that without understanding the chemistry behind such reactions, users risk misinterpreting results—particularly when dealing with partially hydrolyzed sugars.

Seliwanoff’s test, intended to differentiate ketoses from aldoses, is briefly described but not critically evaluated. The review does not mention that prolonged heating can lead to aldoses undergoing similar dehydration reactions, producing misleading red complexes (Gonuguntla et al., 2022). This limitation is crucial when dealing with samples of unknown sugar composition.

Equally concerning is the article's omission of other classical but relevant carbohydrate tests such as the Bial’s Orcinol Test, the Mucic Acid Test, and the Osazone Formation Test. The latter remains a cornerstone in sugar differentiation based on crystal morphology (Mora-Flores et al., 2023). Their absence significantly reduces the comprehensiveness of the review.

Moreover, the article fails to integrate modern advancements in carbohydrate analysis. There is no mention of High-Performance Liquid Chromatography (HPLC), Mass Spectrometry (MS), or Capillary Electrophoresis (CE), which are now indispensable tools in microbial carbohydrate research (Zhao et al., 2022). These technologies offer superior sensitivity and specificity and are essential for both qualitative and quantitative carbohydrate analysis.

The paper also lacks methodological transparency, failing to describe the literature selection criteria, the databases searched, or the timeframe considered. Furthermore, it does not provide a structured comparison of tests in terms of sensitivity, specificity, practicality, or cost—elements critical for an effective review. The omission of practical guidelines or decision frameworks for test selection is a missed opportunity to add value.

In essence, while Rathod et al. (2022) aim to provide an overview of classical carbohydrate analysis techniques, the review falls short in analytical rigor, methodological completeness, and scientific relevance. This critique aims to address these deficiencies by offering a more structured evaluation of the tests discussed and suggesting ways to improve the review's educational and scientific utility.

**IDENTIFIED LAPSES**

1. **Lack of Analytical Depth**  
   The review functions more as a descriptive listing than a critical evaluation. It fails to address test sensitivity, specificity, or limitations. For instance, while Molisch’s test is described correctly, the authors do not clarify that it lacks specificity for differentiating carbohydrate types (Alam et al., 2021).
2. **Omission of Test Comparisons**  
   Benedict’s and Fehling’s tests are treated interchangeably without discussing their differences in reagent composition, stability, and safety. This omission prevents readers from understanding why one might be preferred over the other in practice (Singh & Prasad, 2020).
3. **Inadequate Explanation of Chemical Principles**  
   The chemical basis of each test is often missing or incomplete. For example, Barfoed’s test selectively detects monosaccharides due to its acidic conditions—a point that should be highlighted to avoid misinterpretation of results.
4. **Failure to Address Test Limitations**  
   Limitations like the reversibility of the Iodine test upon heating and the false positives of the Seliwanoff test with prolonged exposure are not discussed, risking an overestimation of these tests’ reliability (Pathania et al., 2023).
5. **Exclusion of Classical Tests**  
   Key carbohydrate tests such as Bial’s Orcinol, Mucic Acid, and Osazone Formation are not included. These remain valuable tools in distinguishing sugar types in bacterial samples (Tuli et al., 2022).
6. **Neglect of Quantitative Techniques**  
   Despite the title referencing both qualitative and quantitative analysis, the paper fails to mention any modern quantitative tools such as spectrophotometry, HPLC, or enzymatic assays (Yadav & Sharma, 2023).
7. **Lack of Recent Literature**  
   The article does not reference newer technologies or methodologies, such as biosensor-based detection or chromatography-mass spectrometry integrations, thus limiting its relevance (Ahmed et al., 2022).

**LITERATURE REVIEW**

The authors present a range of classical biochemical tests used to detect bacterial carbohydrates, such as Molisch’s, Fehling’s, Benedict’s, Iodine, Barfoed’s, and Seliwanoff’s tests. However, they fail to situate these tests within a broader scientific framework. For instance, they do not explore the historical development of these assays, their adaptations for modern microbiological or biochemical use, or their comparative efficacy in complex matrices like bacterial lysates.

Recent literature highlights how traditional carbohydrate tests are increasingly supplemented—or replaced—by more sensitive techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and enzyme-linked immunosorbent assays (ELISA) (Ahmed et al., 2022; Yadav & Sharma, 2023). Furthermore, studies also point to the use of spectrophotometric methods for precise sugar quantification, especially in bacterial exopolysaccharide analysis, which the article does not mention at all (Li & Zhang, 2021).

By not integrating these developments, Rathod et al. (2022) fall short of offering a comprehensive review. A more effective literature review would have traced both foundational and contemporary techniques to create a holistic picture of carbohydrate analysis.

### **RESEARCH DESIGN**

Since the article is a review paper, it does not follow a formal research design. However, as a literature-based review, it lacks methodological transparency. The authors do not specify the criteria for selecting the included tests, the databases used, the time frame of literature considered, or how comprehensiveness and relevance were ensured. Without a clear methodology, the objectivity and completeness of the review remain questionable (Zhang et al., 2021).

### **DISCUSSION OF FINDINGS**

Because Rathod et al. (2022) did not present original data or reanalysis of findings, the article lacks a substantive discussion. A well-developed discussion section in a review should synthesize findings across multiple sources and critically evaluate the strengths and weaknesses of each test in various bacterial contexts. Additionally, no attempt is made to assess which tests are most suited for particular bacterial carbohydrate types (e.g., capsular polysaccharides vs. intracellular sugars), which would have been useful to researchers.

### **CONCLUSION**

In summary, while the article provides a basic overview of classical carbohydrate tests, it is limited by its lack of analytical depth, methodological transparency, and integration of recent advancements. The failure to discuss test limitations, exclude modern quantitative techniques, and differentiate between the strengths of individual assays undermines the paper’s utility as a scientific reference.

Although the review offers introductory insight into qualitative carbohydrate analysis, it does not meet the standards of a critical scientific review. Readers hoping for guidance on selecting the most effective methods for bacterial carbohydrate characterization will likely find the article insufficient.

### **RECOMMENDATIONS**

1. **Include Recent Advances** – Future iterations should include modern analytical tools such as HPLC, spectrophotometry, and biosensor technologies.
2. **Provide Comparative Analysis** – Rather than listing tests, the authors should compare them in terms of sensitivity, specificity, and suitability for bacterial samples.
3. **Expand Literature Coverage** – More recent studies and a wider scope of sources should be included to improve scientific rigor.
4. **Add Application Context** – Descriptions should explain which bacterial carbohydrate types each test is suited for, such as capsular polysaccharides or intracellular glucose derivatives.
5. **Incorporate Instrumental Insight** – Discuss the required laboratory instruments, cost, ease of use, and limitations for each method.

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