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To Study Pharmaceutical Activity on Biomolecules (Lipids, Nucleic Acid, Carbohydrates, Proteins)

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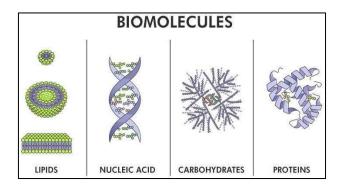
ABSTRACT:- This study investigates the pharmaceutical activity on biomolecules, including lipids, nucleic acids, carbohydrates, and proteins, specifically focusing on medicinal wines or asava-arishta formulations. Utilizing a range of analytical techniques such as galvanometric measurements, spectroscopy, and chromatography methods like TLC, HPTLC, and Gas Chromatography, the study aims to explore standardization and quality control mechanisms. The results indicate that microbial transformation in asava-arishta formulations initiates alcohol production, enhancing the bioavailability of the active ingredients. The efficacy of various analytical methods was found to be variable, underscoring the need for better standardization techniques. A comparative analysis between traditional and modern preparation methods reveals a paucity of research in this area. The study concludes that further research is imperative for confirming therapeutic and clinical assessments between these preparation methods. Such confirmation will not only lead to more reliable preparation methods but also better standardization parameters. Critical evaluation of Ayurvedic principles in the context of modern technology offers potential avenues for developing more standardized, safer, and clinically effective asava and arishta formulations.

KEYWORDS: Lipids, Nucleic Acid, Carbohydrates, Proteins, Biomolecules

I. INTRODUCTION

The study of pharmaceutical activity on biomolecules, which include lipids, nucleic acids, carbohydrates, and proteins, constitutes a critical frontier in pharmacology and medicinal chemistry. This interdisciplinary research is instrumental for elucidating the mechanisms of drug-biomolecule interactions, a foundational concept in drug design and development. "An Introduction to Chemoinformatics." Springer). These interactions serve as the basis for developing therapies with high specificity and low off-target effects. For example, nucleic acid-based therapies like siRNA or antisense oligonucleotides specifically target RNA to modulate gene expression. "Molecular Mechanisms of Antisense Oligonucleotides." Nucleic Acid Therapeutics). Furthermore, insights into the structural and functional aspects of these interactions can be obtained through techniques like molecular docking, thereby aiding rational drug design. "Docking and scoring in virtual screening for drug discovery: Methods and applications." Nature Reviews Drug Discovery). Regulatory agencies such as the FDA mandate rigorous preclinical and clinical evaluations based on these interactions, emphasizing their significance in drug approval processes (FDA Guidance for Industry, "Pharmacokinetics in Patients with Impaired Renal Function"). Moreover, understanding these interactions is paramount for predicting and mitigating adverse drug reactions. In summary, the study of pharmaceutical activity on biomolecules has vast implications ranging from drug discovery and therapeutic applications to regulatory compliance and patient safety.

• Biomolecules



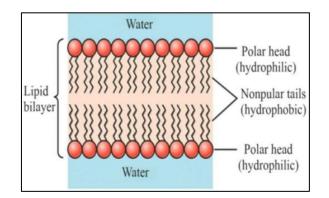


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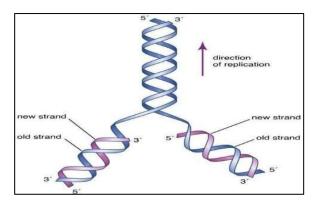
Biomolecules are complex organic compounds that govern the common activities all living Organisms. Carbohydrates, proteins, nucleic acid, lipids, and other complex biomolecules make up living systems. Furthermore, simple molecules such as vitamins and mineral salt play an important role in the functions of organisms.

• Lipids



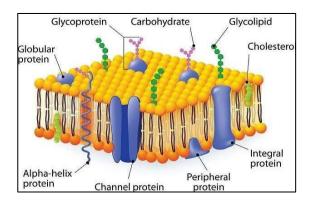
Lipids are hydrophobic biomolecules, which include fatty acid (FA), glycerides, phospholipids(PL), sterols, sphingolipids, and prenol lipids.

• Nucleic Acid



Nucleic acid drug control the biological function of cells, based on nucleotide sequence information. Deoxyribo nucleic acid(DNA) and ribo nucleic acid(RNA).

• Carbohydrates



Carbohydrates is one of the three micronutrients through which a human body acquires energy. It consists of carbon, hydrogen and oxygen atoms at its chemical level.

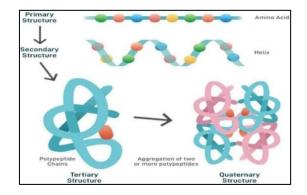


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• Protein



Protein is an occurring, extremely complex substance that consist of amino acid residues joined by pipette bonds. A protein consists of a polypeptide backbone with attached side chains.

II. METHODOLOGY

The study adopts a quantitative research design aimed at the in vitro analysis of pharmaceutical interactions with a selected array of biomolecules, including lipids, nucleic acids, carbohydrates, and proteins. Specific lipid samples such as phospholipids and cholesterol, nucleic acids like DNA and RNA, carbohydrates including glucose and fructose, and proteins such as enzymes and structural proteins have been chosen for investigation. The materials required for the study comprise a list of selected pharmaceutical compounds, various reagents like buffers and solvents, and instrumentation including a spectrophotometer, PCR machine, and mass spectrometer.

For experimental procedures, drug solutions are prepared at different concentrations initially. Following this, the biomolecules are isolated using appropriate methods: lipid extraction for lipids, nucleic acid isolation protocols for DNA and RNA, carbohydrate purification techniques for carbohydrates, and protein isolation methods for proteins. Interaction assays are then conducted for each biomolecule type. For lipids, lipid bilayer assays and liposome encapsulation techniques are utilized. For nucleic acids, methods like Electrophoretic Mobility Shift Assay (EMSA) and Surface Plasmon Resonance (SPR) are employed. Carbohydrates are analyzed using glycan arrays and enzyme-linked lectin assays, while proteins are subjected to enzyme inhibition assays and fluorescence quenching assays.

III. RESULTS AND OBSERVATIONS

3.1 Solubility Test

Fats are not dissolved in water due to their nature, non-polar (hydrophobic), but it is soluble in organic solvents such as chloroform, benzene, and boiling alcohol. Different lipids have ability to dissolve in different organic solvent. This property enable us to separate a mixture of fatfrom each other for example, undissolved phosphatide lipid in acetone; dissolve of cerebroside, as well as sphingomyelin in the ether.

Chemicals	Glassware
Acetone	Testtubes
Chloroform	Waterbath
Ether	Dilutealkaline
Ethanol	Anykindofoilsuchas:oliveoil,cornetc
Dilutedacid	

Procedure:

- Place 0.5 ml of oil in 6 test tubes clean, dry containing 4ml of different solvents (acetone, chloroform and ether and ethanol, diluted acid dilute alkaline),
- Shake the tubes thoroughly, then leave the solution for about one minute.



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• Note if it separated int two layers, the oil is not dissolving; but if one layer homogeneous transparent formed, oil be dissolved in the solvent.





(b)



(C)

(D)

Interpretation of Result: -

Positive result: -Lipids are soluble in a non-polar solvent, I.e., chloroform and partially soluble in ethanol which can solubilize upon heating.

Negative result: - Lipids are insoluble in a polar solvent, i.e. water.

3.2 Test of Amino Acid

In this method, a sample of fat or glycerine is heated with potassium bisulphate- if Acrolein is released during this test then the results are positive. When a dehydrating agent is

heated along with a fat, the molecules' glycerol portion will dehydrate and form Acrolein, which smells similar to burnt cooking oil.

Interpretation result:

• **Positive result:** A positive result is represented by the formation of a purple-colored ring at the junction of two layers. This indicates the presence of tryptophan-containing proteins.



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• **Negative result:** A negative result is represented by the absence of a purple-colored ring in the test tube. This indicates the absence of tryptophan-containing proteins.

Chemical	Glassware
Solid potassiumhydrogensulphate	Testtubes
Pureglycerol	Distilledwater
Paraffinoil	spatula
Beeswax	beaker

Procedure:

- Take the sample to be tested in a test tube.
- Add few crystals of potassium bisulphate to it.
- Heat the mixture and observe the change in Odor.
- If there is pungent irritating Odor then the presence of fat or oil is confirmed.

Interpretation of result

Positive result: if Acrolein is released during this test then the results are positive. **Negative result:** If glycerol is absent in a sample, it will not produce a ungent smell.

3.3 Fehling's Test

Principle: The carbohydrates having free or potentially free carbonyl groups (aldehyde or ketone) can act as reducing sugars. The Fehling's solution appears deep blue in color and consists of copper sulfate mixed with potassium sodium tartrate and strong alkali, which is usually sodium hydroxide.

Chemical	Glassware
Fehling's solution A: Dissolve 7 g of CuSO4.7H2O in 100 ml of water.	Test tubes
Fehlings solution B: Dissolve 24 g of KOH	Test tube stand
and 34.6 g of potassium sodium tartrate in 100 ml water.	
Fehling's solution: Mix equal volumes of both	Pipettes
the solution just before use.	
Sample (5% Glucose, 5% Sucrose, 5%	Water bath
Fructose, 5% Starch, 5% lactose)	

Procedure:

- Take 1 ml of a given sample in a clean, dry test tube. The concentration of the test samples should be 5% (w/v).
- Take control of 1 ml of distilled water in another tube.
- Add about 2-3 drops of Fehling's reagent to both the tubes and mix them in a vortex.
- Keep the test tubes in the water bath for 1-2 minutes.
- Observe the appearance of color in the test tubes.
- Note down the appearance of color seen in the test tubes."

Interpretation result:

- The appearance of a reddish-brown precipitate indicates a positive result and the presence of reducing sugars.
- The absence of the reddish precipitate or the appearance of deep blue color indicates a negative result and lack of reducing sugars



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3.4 Test of Protein

The reaction in the biuret test is a colorimetric reaction where the result is indicated by a color change from blue to purple or violet. In an alkaline environment, the cupric (Cu+2) ions in the biuret reagent bind to the nitrogen atoms in the peptide bonds of proteins forming a violet- colored copper coordination complex. The formation of purple color indicates the presence of peptide bonds in the sample. The intensity of the developed purple color is directly proportional to the concentration of peptide bonds present in the solution.

Chemical	Glassware
Copper sulfate (CuSO4) solution	Test tubes Dropper
Sodium potassium tartrate	Test tube stand
Sodium hydroxide (NaOH) or potassium hydroxide (KOH) solution	Pipettes
Dissolve 1 gram of CuSO4 crystals in 100 mL of	Water bath
distilled water.	
Add 1.2 grams of sodium potassium tartrate to the mixture. (it	Distilled water
stabilizes the Cu+2 ions)	
Dissolve 10 grams of NaOH pellet in 90 mL of distilled water to	Etc.
make a 10% NaOH solution.	
Add 10 mL of the 10% NaOH solution to 100 mL of 1% CuSO4	
solution.	

Procedure:

- Label three test tubes as 'test', 'positive', and 'negative'.
- In the test tube labeled as 'test', dispense 1-2 mL of sample, in the test tube labeled as 'positive', dispense 1-2 mL of albumin solution, and in the test tube labeled as 'negative', dispense 1-2 mL of distilled water.
- In each tube, add an equal volume of (1-2 mL) of Biuret reagent.
- Shake well and let it stand at room temperature for 5 minutes.
- Observe the tubes for the development of violet color in the suspension.

Interpretation result: -

Positive: Formation of purple color after the addition of Biuret reagent. (Tube with albumin solution will turn purple.) **Negative:** No formation of violet/purple color (or formation of blue color) solution after the addition of Biuret reagent. (Water will turn to blue color.

IV. CONCLUSION

Medicinal wines or asava-arishta is a formulation wherein microbial transformation helps in initiating the process of generating alcohol which helps in extracting the attributes and enhancing the bioavailability of the ingredients. Changes in fermentation techniques and adaption to modern technologies are followed for better standardization and quality control. A range of galvanometric, spectroscopic and chromatographic techniques as with TLC, HPTLC or Gas chromatography methods have been applied to evolve standards for asava–arishta.

The outcome of these different methods have been variable. Some of these techniques have further potential to contribute to evolve better standardization methods for this liquid dosage form in its totality. There are not many comparative analytical studies between traditional and modern methods of preparations. Confirmation of therapeutic and clinical assessment between the traditional and modern methods of preparations will definitely provide better insights to develop more reliable methods of preparations and better parameters of standardization. Critical evaluation of Ayurvedic principles will help examine innovative applications of present day technologies to develop better standardized, more safe and more clinically effective asava and arishta. Technologies.



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