Effects of Seasonal change on the Phytochemical composition of the Flora-Calyx of Bombax ceiba obtained from Guinea Savanna Nigeria

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Hemen Terseer Joseph
[1]Department of Biological Sciences, Faculty of Sciences, Nigeria Police Academy, Kano, Nigeria.
Corresponding author: hemenjoseph@yahoo.com

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Abstract: The impact of seasonal variation on the phytochemistry of the flora-calyx of Bombax ceiba sourced from the Guinea Savanna region of Nigeria was investigated. The study focused on assessing the physical characteristics, qualitative presence, and quantitative concentrations of bioactive compounds in the calyx extract during both dry and wet seasons. Physical characterization revealed consistent properties with light brown coloration, woody odor, and a soft, sticky texture, yielding 20% during both seasons. Qualitative assessment indicated the presence of tannins, phenols, alkaloids, and flavonoids in varying degrees during both seasons, while saponins, and glycosides were absent during the dry and wet seasons respectively. Quantitative analysis techniques using chromatography and spectrophotometry showed fluctuation in the concentrations of bioactive compounds between seasons. Specifically, during the dry season, phenols were found at 1030 mg/g, alkaloids at 3.14%, flavonoids at 7.6%, and glycosides at 810 mg/g, whereas during the wet season, saponins were at 2.3%, tannins at 1120 mg/g, phenols at 1.90 mg/g, alkaloids at 8.23%, and flavonoids at 15.02%, with glycosides not detected. These findings underscore the significant influence of seasonal variations on the phytochemical composition of Bombax ceiba, potentially impacting its medicinal properties and applications. Understanding the influence of seasonal variations is also crucial for ensuring the consistency, quality, and efficacy of medicinal products derived from Bombax ceiba across different seasons. Further research and methodological advancements are needed to develop robust analytical approaches capable of accounting for seasonal variations in bioactive compound concentrations and also to optimize harvesting practices for maximizing their therapeutic potential.

Keywords: Bioactive compounds, Bombax ceiba, flora-calyx, phytochemistry, seasonal variation

Introduction

Bombax ceiba belonging to family Bombacaceae also known as silk cotton tree and commonly called semal. Bombax ceiba is found in Northern Australia, India, Sri Lanka, Pakistan, Bangladesh, Myanmar, Malaysia, Java and Sumatra. Bombax ceiba belongs to the family Bombacaceae, also known as the silk cotton tree and commonly called semal (Raunmoon et al., 2024). Bombax ceiba has many significant. medicinal values. The tree is a powerful, fast-growing light demander. It thrives, especially in valleys, on sandy loams that are deep, and in regions with an annual rainfall of 50 to 460cm (Raunmoon *et al*.,2024). *Bombax ceiba,* found in India, is a tall deciduous tree with characteristic woody thorns on the bark of the tree (Bhadkariya,, & Patley, 2024). This tree produces huge, crimson, ornithophilous flowers. The flowers have a firm perianth with rigid filaments and well-protected ovaries (Saklani *et al.*, 2013). Nearly all parts of Bombax ceiba are known to have different medicinal properties, which ethno botanists prove Bark has hard-sharp conicles and grey-brown or silver-grey colored. The leaves are broad, spreading, glabrous, and lanceolate with 3-7 leaflets. The seeds of the plant are shiny, black or brown, embodied in wool viz long and white, irregularly shaped obovoid, oily and shiny with thick, silky hair. The gum of the tree is light brown to translucent, locally known as "KATIRA"(Karole *et al.*, 2017) and (Huang *et al.*, 2023). In *Bombax ceiba* presence of xanthones, flavonoids, quinines, triterpenes, sterols, hydrocarbons, fatty acids, and their esters is distinguished phytochemically and for its various pharmacological activities such as cytotoxicity, anti-oxidant, hypotensive, antiangiogenic, anti-inflammatory, anti-diabetic, and anti-microbial (Safdar *et al*.,2023).

All over the world, plants have served as the richest source of raw materials for traditional as well as modern medicine. The medicinal value of plants is mainly due to the presence of some chemical substances known as phytochemicals. These are basically plant metabolites synthesized in all parts of the plant body by themselves and have some definite physiological action on animals (Huang *et al.*, 2023). Plants are a major source of secondary metabolites, which are formed as products of primary metabolism and produced for defense against predators (Safdar *et al*., 2023). The information available on B. ceiba depicted the fact that it is a popular delicacy soup and remedy among the various ethnic groups in Benue State, Nigeria. It is interesting to note that crude extract and aqueous extracts of stem bark, root and the leaf of B. ceiba have been screened for some pharmacological activities and hepatoprotective, antiangiogenic, analgesic and antioxidant, hypoglycemic, antimicrobial activity and cholinesterase activity. Still, other parts of plants, most especially the flora calyx, which are documented to possess important medicinal virtues, are not explored scientifically for their biological potential. Therefore, this study is designed to evaluate the changes in the phytochemistry of the flora-calyx extract due to seasonal variation.

Materials and Methods

Sample collection and Authentication

Flora calyx of the *B. ceiba* plant were obtained in February from Benue State, Nigeria, and the practical was carried out in March and August of 2023, which correspond to the rainy

and dry seasons of the year. It was authenticated by a Botanist in Department of Biological Sciences, Nigeria Police Academy, Wudil, Kano State.

Preparation of plant extract

The plant material after collection was washed with distilled water to remove all fibrous and soil debris and then sun dried for 15 days. An electric blender was used to powder the dried sample (electric grinder). After the fine powder (200 gm sample extracted with 800 ml of each solvent) was extracted, it was soxhed using a series of solvents in increasing polarity order, including petroleum ether, ethanol, chloroform, distilled water, and ethyl acetate (Shukla et al., 2020) and (Raunmoon *et al.*,2024). After practically all of the moisture and solvents were eliminated from the extract by drying it, the finished products were placed in airtight containers and refrigerated at 4°C to facilitate further research.

Determination of extraction yield

The extraction yield (%) was calculated as follows: Extraction yield (%) = weight of the extract after evaporating solvent and freeze drying/ dry weight of the sample ×100.

Qualitative phytochemical analysis

The extracts were tested for the presence of bioactive components by using following standard methods (Huang *et al.*, 2023)

Preliminary phytochemical investigation

To identify different phytochemicals like alkaloids, flavonoids, steroids, phenols, tannins, saponins, terpenoid, glycoside, a preliminary phytochemical screening was performed on all the extracts using standard procedures.

Detection of alkaloids

After dissolved in diluted HCL, filter the extracts. To find out if an alkaloid is present in the filtrate, the following color tests can be used: Wager's reagent (reddish brown), Mayer's reagent (cream ppt), and Hager's reagent (yellow ppt).

Detection of flavonoid

Alkaline reagent test

Apply a few drops of sodium hydroxide solution to the extracts. When diluted acid is added, the formation of a bright yellow tint that turns colorless indicates the presence of flavonoids.

Test for Steroids

Liebermann-burchard reaction

Add 1-2 ml of acetic anhydride, 2 drops of conc, and 2 ml of plant extract to the chloroform mixture. The green color developed by sulfuric acid on the test tube's sidewalls indicates a positive steroid moiety test.

Salkowiski reaction

Pour in two milliliters of the extract. Chloroform (2 ml) and concentrated sulfuric acid (2 ml). The presence of greenish yellow fluorescence in the acid layer and red coloration in the chloroform layer after shaking indicates a positive test result for the steroids moiety.

Test for phenolic components and tannins

A small amount of test solution was dissolved in water and put through the next test to find out if tannins and phenolic compounds were present.

Test for saponins

In a graduated cylinder, add 1 milliliter of the test solution, diluted with distilled water, to 20 milliliters, and shake for 15 minutes. The presence of saponins is indicated by a 1 cm layer of foam.

Test for terpenoids

Salkowski test

Add 3 milliliters of concentrated sulfuric acid to 5 milliliters of each extract combined with chloroform. Terpenoids are present at the contact as shown by a reddish-brown precipitate appearance.

Detection of glycosides

After using diluted HCl to hydrolyze the extracts, filter them. The following tests for glycosides are run on the obtained filtrate.

Modified borntrager's test.

After treating extracts with a 5 percent ferric chloride solution, boil them for approximately five minutes. After cooling the mixture, extract using the same volume of benzene. After separating the top layer, apply an ammonia solution to it. Glycosides are present when the ammonical layer begins to form a rose-pink color (anthranol, glycosides).

Results

Physical Characterization of Calyx Extract of Bombax Ceiba with Seasonal Variations

The physical parameters such as color, odor, texture, and percentage yield of the Calyx extract of *Bombax ceiba* during both dry and wet seasons are similar as presented in Table 3.1. This physical characterization of the Calyx extract of *Bombax ceiba* during both dry and wet seasons provides valuable insights into the consistency of its appearance and texture across different environmental conditions. More so, both extracts exhibit a light brown color, woody odor, and soft and sticky texture, with a percentage yield of 20%. This suggests that the physical characteristics of the extract remain consistent regardless of the season.

Table 3.1: Physical characterization of Calyx extract of *Bombax ceiba* during Dry and Wet seasons

	Physical parameters of the extract			
Season	**Colour**	**Odour**	**Texture**	**Percentage (%) yield**
Dry	Light brown	Woody	Soft and sticky	20
Wet	Light brown	Woody	Soft and sticky	20

Qualitative Assessment of Bioactive compounds in the Calyx extract of Bombax Ceiba during Dry and Wet seasons

The results of qualitative assessment of bioactive compounds in the Calyx extract of Bombax Ceiba during the dry and wet seasons revealed notable differences in the presence of certain compounds as indicated in Table 3.2. During the dry season, the extract was devoid of saponins but contained tannins, phenols, alkaloids, flavonoids, and glycosides. In contrast, during the wet season, all bioactive compounds except glycosides were present. This suggests a

shift in the composition of bioactive compounds between seasons, with the wet season extract exhibiting a more diverse range of compounds. And also, this indicates some variability in the presence of bioactive compounds between the dry and wet seasons, with the wet season extract exhibiting a slightly higher diversity of compounds.

Table2 Qualitative Assessment of Bioactive compounds in the Calyx extract o Extract of *Bombax Ceiba* during Dry and Wet seasons

	Bioactive compounds					
Season	**Saponins**	**Tannins**	**Phenols**	**Alkaloids**	**Flavonoids**	**Glycosides**
Dry	-	+	+	+	+	+
Wet	+	+	+	+	+	-

Key: + = Present - =Absent

Quantitative Assessment of Bioactive compounds present in the Calyx extract of Bombax Ceiba during Dry and Wet seasons.

The quantitative analysis of bioactive compounds in the Calyx extract of Bombax Ceiba during both dry and wet seasons provided insights into the concentrations of specific compounds. According to the results presented in Table3.3, significant ($P<0.05$) differences were observed in the concentrations of saponins, tannins, phenols, alkaloids, flavonoids, and glycosides between the two seasons. For instance, phenol concentration was higher during the dry season, while alkaloid and flavonoid concentrations were higher during the wet season, meanwhile, Saponins and glycosides were not found during the dry and wet seasons respectively, such variations in compound concentrations highlight the influence of seasonal factors on the chemical profile of the extract.

Table3.3; Quantitative Assessment of Bioactive compounds present in the Calyx extract of *Bombax Ceiba* during dry and wet seasons

	Bioactive compounds					
Season	**Sap**	**Tan**	**Phe (mg/g)**	**Alk (%)**	**Flav (%)**	**Gly (mg/g)**
Dry	**NF**	1030	3.14	7.6	5.4	810
Wet	2.3	1120	1.90	8.23	15.02	**NF**

Key: Spa= Saponins, Tan= Tannin, Phe=Phenol, Alk=Alkaloid, Fla= Flavonoid, Gly= Glycosides, **NF=** Not found

Discussion

Effects of Bioactive Compounds Present in the Calyx Extract of Bombax Ceiba during Dry and Wet Seasons

It had been reported that, various parts of the Bombax ceiba tree, including the bark, leaves, flowers, and seeds, have been utilized in traditional medicine for centuries due to their potent medicinal properties. And among the different parts of the Bombax ceiba tree, the calyx extract, derived from the flower's protective covering, has garnered significant attention for its rich content of bioactive compounds. These compounds include flavonoids, alkaloids, terpenoids, phenolics, and polysaccharides, among others, which exhibit diverse pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and hepatoprotective properties (Rastogi *et al.*, 2016) and (Raunmoon *et al.*,2024)

However, it is essential to consider that the composition and concentration of bioactive compounds within the calyx extract of Bombax ceiba may vary under different environmental conditions, particularly between dry and wet seasons. Seasonal variations in temperature, humidity, rainfall, and sunlight can significantly influence the growth, development, and metabolic activities of plants, ultimately affecting the synthesis and accumulation of secondary metabolites, including bioactive compounds (Nawaz et al., 2020).

During the dry season, characterized by low humidity and limited rainfall, plants often experience water stress, which can trigger various physiological responses, including the activation of stress-related pathways and alterations in secondary metabolism. Studies have shown that under water-deficit conditions, plants may produce higher concentrations of certain bioactive compounds as part of their defense mechanisms against environmental stressors (Hossain et al., 2018). Therefore, it is plausible that the calyx extract of Bombax ceiba harvested during the dry season may exhibit increased levels of specific bioactive compounds, potentially enhancing its therapeutic efficacy in managing conditions such as oxidative stress, inflammation, and microbial infections (Huang *et al.*, 2023).

Conversely, during the wet season, characterized by higher humidity and abundant rainfall, plants generally experience optimal growth conditions with ample water availability. Under such favorable conditions, plants allocate resources towards growth and development rather than stress response mechanisms. Consequently, the synthesis and accumulation of certain bioactive compounds in the calyx extract of Bombax ceiba may be relatively lower during the wet season compared to the dry season (Hossain et al., 2018).

Effects of Seasonal variations on the quantitative Analysis of Bioactive Compounds in the Calyx Extract of Bombax Ceiba.

The calyx extract derived from Bombax ceiba contains various bioactive compounds, including flavonoids, alkaloids, terpenoids, phenolics, and polysaccharides, which contribute to its pharmacological activities (Rastogi et al., 2016) and (Huang *et al*., 2023). Therefore, the quantitative analysis of these bioactive compounds is essential for assessing the potency and efficacy of the extract for therapeutic applications.

However, the quantitative analysis of bioactive compounds in the calyx extract of Bombax ceiba can be significantly influenced by seasonal variations, particularly between dry and wet seasons. Environmental factors associated with these seasons, such as temperature, humidity, rainfall, and sunlight, play critical roles in modulating plant metabolism and secondary metabolite production (Nawaz et al., 2020). Consequently, the concentration and composition of bioactive compounds in the calyx extract may vary, posing challenges for consistent quality control and standardization in medicinal product development.

During the dry season, characterized by low humidity and limited rainfall, plants often experience water stress, which can trigger physiological responses aimed at survival. Under such conditions, plants may allocate resources towards the synthesis of certain bioactive compounds involved in stress adaptation, such as antioxidants and osmoprotectants (Hossain et al., 2018). As a result, the calyx extract of Bombax ceiba harvested during the dry season may exhibit higher concentrations of these stress-responsive compounds, impacting its overall pharmacological profile.

Conversely, the wet season, characterized by higher humidity and abundant rainfall, provides favorable conditions for plant growth and metabolism. During this period, plants may prioritize growth and development over stress response mechanisms, leading to altered patterns of secondary metabolite production (Nawaz et al., 2020). Consequently, the concentration of certain bioactive compounds in the calyx extract of Bombax ceiba may be lower during the wet season compared to the dry season, affecting its potency and therapeutic efficacy.

Quantitative analysis techniques such as chromatography (e.g., high-performance liquid chromatography, HPLC) and spectrophotometry are commonly employed to quantify specific bioactive compounds in plant extracts. However, variations in environmental conditions between dry and wet seasons can introduce additional challenges in standardizing analytical methods and interpreting results (Bako et al., 2024). Factors such as sample preparation, extraction efficiency, and calibration standards may need to be adjusted to account for seasonal variations in bioactive compound concentrations.

Conclusion and Recommendation

Conclusion

In conclusion, the effects of bioactive compounds present in the calyx extract of Bombax ceiba are subject to seasonal variations, with environmental factors playing a crucial role in determining the composition and concentration of these compounds. More so seasonal variations associated with dry and wet seasons can significantly influence the quantitative analysis of bioactive compounds in the calyx extract of Bombax ceiba. Understanding these effects is also crucial for ensuring the consistency, quality, and efficacy of medicinal products derived from Bombax ceiba across different seasons.

Recommendation

Further research and methodological advancements are needed to develop robust analytical approaches capable of accounting for seasonal variations in bioactive compound concentrations and also to optimize harvesting practices for maximizing their therapeutic potential.

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