Study on the Phytochemical Screening and Antimicrobial Activity of the Methanolic Extract and Butanoic Fraction of Jackfruit leaves (Arthocarpus heterophyllus)

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DOI: https://doi.org/10.51584/IJRIAS.2025.10040027

Received: 25 March 2025; Accepted: 01 April 2025; Published: 01 May 2025

ABSTRACT

The leaf extract and metabolites of Jack fruit possess several bioactive compounds of relevant medicinal significance. This study investigated the phytochemical and antimicrobial activity of the methanol extract and butanol fraction. The extract and fraction were screened for their microbial activity on typed strains of bacteria and fungi. In the preliminary antimicrobial screening, the methanol extract displayed better anti-microbial and anti-fungal activities when compared with the butanol fraction, E.coli and B.cereus at IZD of 16mm and 13.8mm. The minimum inhibition concentration (MIC) of test samples against the test organisms ranged from (0.156-5mg/mL) where the lowest of the methanol extract as recorded against E.coli (0.156mg/mL) and butanol fraction against B.cereus (0.313mg/L). The physiochemical screening revealed the leaf as having heavy presence of flavonoids, saponins, tannins, alkaloids although more in the methanol extract at above 2 while that of butanol fraction less than 1. Based on the available data, it was concluded that Artocarpus heterophyllus leaf exhibits medicinal activity and opens a broader avenue for further therapeutic investigations for its usefulness.

Keywords: Jack Fruit, Medicinal, Inhibition, Flavonoids, Extract.

INTRODUCTION

The world we live in is carefully ornamented with wild and farmed plant types, each of which serves a specific purpose. Medicinal plants are severally used in local context for the treatment of different organic ailments and infections has been a long-standing worldwide practice (Bello, et al., 2008).

Plant-based foods are considered to be the most significant dietary components for optimal health. Fruits and vegetables are rich sources of antioxidants (Shirajum, et al., 2015). Thousands of plant species are known to have medical benefits in Africa and other continents, and the usage of various portions of multiple medicinal plants to heal certain aliments has been popular since ancient times (Bello et al., 2008 and Khan et al., 2003). A vast variety of analgesics, anti-inflammatory drugs, and central nervous system (CNS) depressants have been recommended for use in the treatment of many human ailments, both natural and synthetic. It has been proposed that synthetic chemicals have harmful consequences such as liver damage, mutagenesis, and so on.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue IV April 2025

To deal with this issue, there has been a significant surge in the attempt to identify novel plant-based medicines in recent years, as can be observed all around the globe is paramount. (Shirajumet al., 2015; Chawdharyet al., 1997; Tulyathan et al., 2002 and Charles, 2006).

This study was designed to evaluate the phytochemical constituents and anti-microbial potency of the methanol extract as the crude and the butanol fraction.

MATERIALS AND METHOD

Fresh leaves were sourced from Abba town in Njikoka LGA in Anambra State, Nigeria, and were brought to the Department of Pharmaceutical and Medicinal Chemistry, NnamdiAzikiwe University, Awka, Anambra State, Nigeria. The leaf was assigned voucher number of PCG/474/009 in the Department of Pharmacognosy and Traditional Medicin, Faculty of Pharmaceutical Sciences,. Nnamdi Azikiwe University, Anambra State.

Methods

The dried leaves were pulverized and 500 g was extracted by cold maceration for 48 hours using 2 L methanol respectively with intermittent agitation. The macerate was filtered and the filtrate was evaporated to dryness using rotary evaporator.

Liquid-liquid chromatographic fractionation

Ten gram of the crude methanol extract was dissolved in 250 mls distilled water and fractionated using n-hexane, ethyl acetate and n-butanol in increasing order of their polarities. The filtrate obtained from various fractions were then concentrated using the rotary evaporator at 50 ^{o}C. The fractions obtained were stored at 4 ^{o}C.

Antimicrobial Evaluation

The antimicrobial study was carried out on the methanolic extract and the n-butanol fraction using Muller Hinton agar for bacteria and Potato dextrose agar for the fungi. The organisms used are Staphylococcus aureus, Bacillus cereus and Escherichia coli as bacteria isolates while Aspergillus niger, Aspergillus flavus and Candida albicans as fungi isolates

Determination of Inhibitory Zone Diameter, (IZD)

This was carried out using agar well diffusion method as described by (Felix et al., 2014).

Determination of Minimum Ihibitory Concentration (MIC).

This was carried out using the MIC. Method as described by Uhama et al., (2022).

Phytochemical analysis

This test was carried out using standard method as described by Harbone, (1988) and screened for bioactive constituents or secondary metabolites

Data analysis

The results were presented as mean SEM and analyzed using SPSS Version 16.00. Significant between control and the extract/fraction treated groups using oneway ANOVA (Turkey LSD Alpha Post Hoc Test).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue IV April 2025

RESULTS

Table 1.0 Phytochemical screening

Using only Methanol and Butanol fractions

Test	**Qualitative**		**Quantitative/%**	
Type	Me	Bu	Me	Bu
Alkaloids	+	+	2.30 ± 0.14	0.78 ± 0.01*
Saponins	++	+	12.12 ± 0.03	0.93 ± 0.01*
Flavonoids	++	+	6.20 ± 0.14	0.62 ± 0.01*
Tannins	++	++	7.10 ± 0.00	0.96 ± 0.01*
Terpenoids	-	-	-	-
Glycosides	++	+	-	-
Steroids	+	+	-	-
Phenolic	++	++	5.10 ± 0.14	0.89 ± 0.01*

Presence of constituent- ++ - Massively detected, +- Sparsely detected. - Not detected. *P<0.05: Statistically significant difference from quantitative phytoconstituents of methanol sample.

*Me- Methanol Extract

*Bu- Butanol Fraction

Table 2.0 Results of antimicrobial properties of the methanol extract showing the mean Inhibition Zone Diameters (IZDs) mm

Test Organism	**Mean Inhibition Zone Diameter (IZDs)/(mm)**		
	Methanol Extract (200 mg/mL)	**Positive Control**	**Negative Control**
		Chloramphenicol (100 µg/mL)	**DMSO**
E.coli	21.50 ± **0.71*[a]**	24.00 ± **0.00***	0.00 ± **0.00**
B.cereus	18.00 ± **0.00*[a]**	23.00 ± **0.00***	0.00 ± **0.00**
S.aureus	15.50 ± **0.71*[a]**	26.00 ± **1.41***	0.00 ± **0.00**
		Miconazole (100 µg/mL)	**DMSO**
C.albicans	16.00 ± **0.00*[a]**	21.00 ± **0.00***	0.00 ± **0.00**
A.flavus	12.80 ± **0.00*[a]**	22.00 ± **1.41***	0.00 ± **0.00**
A.niger	10.85 ± **0.71***	19.00 ± **7.07***	0.00 ± **0.00**

*P<0.05: Statistically significantly different from IZDs of negative control. [a]P<0.05: Statistically significantly different from IZDs of positive control.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue IV April 2025

Table 3.0 Results of antimicrobial properties of the butanol fraction showing the mean Inhibition Zone Diameters (IZDs) mm

Test Organism	**Mean Inhibition Zone Diameter(IZDs)(mm)**		
	N-butanol Extract (200 mg/mL)	**Positive Control**	**Negative Control**
		Chloramphenicol (100 µg/mL)	**DMSO**
E.coli	18.50 ± **0.71*[a]**	24.00 ± **0.00***	0.00 ± **0.00**
B.cereus	20.00 ± **0.00***	23.00 ± **1.41***	0.00 ± **0.00**
S.aureus	11.25 ± **1.06*[a]**	26.00 ± **0.00***	0.00 ± **0.00**
		Miconazole (100 µg/mL)	**DMSO**
C.albicans	13.00 ± **0.00**	0.00 ± **0.00**	0.00 ± **0.00**
A.flavus	13.80 ± **0.00**	0.00 ± **0.00**	0.00 ± **0.00**
A.niger	12.00 ± **0.00**	0.00 ± **0.00**	0.00 ± **0.00**

*P<0.05: Statistically significantly different from IZDs of negative control. [a]P<0.05: Statistically significantly different from IZDs of positive control.

Table 4.0 Results of Minimum Inhibitory Concentration (MICs) of the plant extract/fraction on test organisms

Test Organism	**MIC (mg/mL)**	
	Methanol Extract	**Butanol Fraction**
E. coli	0.156	0.625
B. cereus	0.625	0.313
S. aureus	2.5	5
C. albicans	1.25	5
A. flavus	2.5	2.5
A. n iger	5	5

DISCUSSION

The results obtained in this study provides scientific evidence to back the statements made by the locals in many regions of Nigeria (especially in the Southeast region of Nigeria from where this study was carried out) insinuating that this plant can be used to cure stomach upsets and inflammations thus serving as an anti-inflammatory and anti-microbial treatment dosage.

The phytochemical results showed the presence of alkaloids, saponins, tannins, phenolic compounds, flavonoids. However, there was a significant reduction in alkaloid, saponins, flavonoids, tannins and phenolic contents of butanol fraction when compared to the contents of the methanol extract. The presence of the above secondary metabolites in both the extract and butanol fraction in quantitative and Qualitative results as reflected in table 1 above suggests that the leaves exhibit medicinal activity as well as physiological activity and quantification within range to activate response, in accordance with Omale et al., 2010; Edeoga et al., 2005; Mills and Bone, 2000 and Singh, et al., 1991). The variability in the phytochemical could be attributed to geographic and phonological factors such as collection time, plant part, and notably processing prior to solvent extraction (Yang et al., 2018; Kategunya, 2011 and Fasset (1996). Herbs that have heavy presence of tannins as seen from the result here are astringent in nature and these explains their usage in treatment of intestinal disorder which affirms the its use by locales for treatment of different ailments. Studies have reported the presence of saponins as anti-inflammatory, anti-oxidants for various ailments (Yebpella, et al., 2011).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue IV April 2025

The preliminary antimicrobial screening result revealed that the minimum inhibitory concentrations (MIC) showed a narrower bacterial spectrum for methanol extract as compared to n-butanol fraction. There was also a significant increase in IZDs of methanol extract, positive control against E.coli, B.cereus, S.aureus, C.albicans, A.flavus, and A.niger when compared to negative control. More so, there was a significant reduction in IZDs of methanol extract against E.coli, B.cereus, S.aureus, C.albicans and A.flavus, except A.niger when compared to positive control. The crude methanol extract of (Artocarpus heterophyllus) demonstrated efficacy against all test organisms at 200 mg/ml, with inhibition zone diameters (IZDs) ranges between 10.8-22.0 mm, according to the preliminary antimicrobial screening results. The best antibacterial and antifungal activity were found against E.coli (with an IZD of 22 mm) and Candida albicans (with an IZD of 16 mm).

There was a significant increase in IZDs of methanol extract, positive control against E.coli, B.cereusand S.aureus. Also, there was a significant reduction in IZDs of methanol extract against E.coli, and S.aureus, when compared to positive control. The methanol extract produced varying IZDs against C.albicans, A. flavus and A. niger while the positive control did not produce such response.The n-butanol component of the plant also showed antibacterial activity against all test isolates with IZDs ranges between 11 mm to 20 mm at 200 mg/L. The antibacterial and antifungal activity of the n-butanol fraction were the greatest with B.cereus (with an IZD of 20mm) and A.flavus (with an IZD of 13.8 mm), respectively. According to Khan et al., 2003 and Loizzo et al., 2010, Jack Fruit leaves extract is effective against test organisms at a minimum inhibitory concentration within a range of less than seven, which is consistent with the IZDs determination against chloramphenicol, which is also consistent with the reference minimum values in millimeters as determined by (CLSL, 2011). More so, the narrower the bacterial spectrum as shown by methanol over n-butanol in this research, the smaller the bacterial spectrum better efficiency. It is worth noting that the solvent can influence the level of bioactive compound in plants (Do et al., 2014),

The extract/fraction Minimum Inhibitory Concentrations (MICs) were measured against the test organisms and the MICs of the extract/fraction on the test organisms varied from 0.156-5mg/ml at the doses studied (0.078-10 mg/mL). The methanol crude extract had the lowest MIC against E.coli, with a MIC of 0.156 mg/mL. The lowest MIC values for the n-butanol fraction were found against B.cereus (0.313 mg/mL). The Jack fruit leaf extract showed stronger antibacterial and antifungal activity than the n-butanol fraction, according to the findings of the antimicrobial test. Sousa, (2021) believes that the leaves should be considered as a homemade tea for the oral treatment of bacterial caused disorders because of this impact. An extended study has also been reported on the methanolic extract and other fractions of the compounds elucidated from the HPLC studies Catechin, Septicine, Naamine , Vitexin which displayed anti-inflammatory and anti microbial activity as revealed by the IZD for the methanolic extract showing action on both gram negative E.coli and S.aureus gram positive, indicating a broad spectrum microbial activity (Ajawobu et al.,).

CONCLUSION

This study showed that Arthocarpus heterophyllus leaf can exhibit medicinal properties that are indicative of anti-microbial and anti-inflammatory potentials. Thereby providing an alternative and simple scientific proof to authenticate the practices by locals in the treatment of mostly stomach inflammation obstructions as claimed. Therefore the isolation from this active fraction with a view of zeroing on the anti-microbial and anti-inflammatory properties is recommended.

ACKNOWLEDGEMENT

We are thankful to the Department of Pharmaceutical and Medicinal Chemistry for the use of their laboratory where most of the work was carried out.

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ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue IV April 2025

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