https://chemclassjournal.com/
ChemClass Journal Vol. 8 Issue 1 (2024); 232-244
e-ISSN:0198-2734 p-ISSN:0198-5680
doi.org/10.33003/chemclas_2024_0801/020

Phytochemical Screening and Antimicrobial Activity of the Seed Extracts of *Acacia ataxacantha*

Gambo, Nanbol Ndam [1]; Duguryil, Zipporah Pewat [1]; Chukwu, Anthonia Chilagorom[1]; Ogori, Boniface Otokpa [1] and Denji, Kitka Bulus [1]

[1] *Chemistry Department, Federal College of Education, P.M.B. 27, Pankshin, Plateau State, Nigeria.*

(*) Corresponding author: gambonanbol@gmail.com

Abstract

Acacia ataxacantha is a plant that is traditionally used in various forms of herbal medicine due to its diverse therapeutic properties, including antimicrobial, anti-inflammatory, and antioxidant activities. The seed extracts of *A. ataxacantha*, like other Acacia species, are known to contain an array of bioactive phytochemicals that contribute to its pharmacological potential. The study aims to investigate the phytochemical composition and antimicrobial properties of *A. ataxacantha* seed extracts as well as their potential applications in drug development. The dried ground seeds of the plant were used for the analyses. The coarse-powdered sample was separately subjected to cold extraction (maceration) using ethanol and hexane with intermittent stirring for 2 days. The extracts were obtained through filtration and the filtrate was concentrated using a rotary evaporator. The various extracts were subjected to phytochemical screening using standard techniques. The methods described by the Clinical and Laboratory Standards Institute (CLSI, 2004 and 2012) were used for the *in vitro* antimicrobial analysis. Phytochemical analysis revealed the presence of various bioactive compounds, including alkaloids, flavonoids, tannins, and saponins. The ethanolic extract exhibited significant antibacterial activity, particularly against *Bacillus subtilis*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, with the highest inhibition observed against *Bacillus subtilis* (15 mm at 100 mg/mL concentration). However, it was ineffective against fungal strains such as *Candida albicans* and *Aspergillus sporogenes*. The combined ethanolic and hexane extracts also showed antibacterial activity, particularly against Gram-positive bacteria but limited effectiveness against Gram-negative bacteria like *Escherichia coli.* The study concludes that ethanol is a more effective solvent for extracting antimicrobial compounds from *A. ataxacantha* seeds and recommends further research into phytochemical components, extraction methods, and broader antimicrobial testing, along with safety and toxicity studies to assess potential medical applications.

Keywords: Antibacterial activity, bacteria, bioactive compounds, fungi, maceration, seeds

Introduction

The increasing reliance on medicinal plants for healthcare, particularly in developing countries, draws attention to their critical role in traditional and modern medicine. Among such plants, *Acacia ataxacantha* has drawn attention for its wide range of potential therapeutic applications. Commonly known as the Flame Thorn or Guinea Senna, *A. ataxacantha* belongs to the Fabaceae family and is native to tropical and subtropical regions of Africa. It is a hardy, fast-growing shrub or climbing plant often found in savannas, woodlands, and riparian habitats [1].

Traditional knowledge attributes several medicinal uses to *A. ataxacantha*. In ethnomedicine, various parts of the plant, including its leaves, bark, roots, and pods, are employed to treat ailments such as diarrhea, malaria, skin infections, and inflammatory conditions [2]. These uses suggest that the plant may possess bioactive compounds with pharmacological properties. Phytochemical studies on related species of the *Acacia* genus have identified the presence of flavonoids, tannins, saponins, and alkaloids, many of which have demonstrated antimicrobial, antioxidant, and anti-inflammatory effects [3]. Such findings underscore the importance of further exploring the phytochemistry and bioactivity of *A. ataxacantha* to substantiate its traditional uses. Despite its documented ethnomedicinal applications, scientific research on *A. ataxacantha* remains limited, particularly with respect to its efficacy, safety, and underlying mechanisms of action. This study aims to investigate the medicinal potential of *A. ataxacantha* by evaluating its phytochemical composition and assessing its effects in experimental models of disease.

The seed of *Acacia ataxacantha* holds considerable promise in traditional medicine. In Nigeria, the plant has been utilized in ethnomedicine to address a variety of health challenges, ranging from gastrointestinal disorders to infections and inflammatory conditions [4]. While the leaves, bark, and roots of *A. ataxacantha* have been widely studied for their medicinal properties [5], its seeds are an underexplored resource with unique therapeutic potential. Preliminary investigations suggest that *A. ataxacantha* seeds are rich in bioactive compounds, including flavonoids, tannins, saponins, and essential oils, known for their antimicrobial, antioxidant, and anti-inflammatory properties [3]. Ethnobotanical records indicate their use in managing ailments such as diarrhea, intestinal parasites, and respiratory infections, particularly in rural Nigerian communities where access to conventional medicine may be limited [5]. Recent studies have highlighted the pharmacological activities of various parts of *A. ataxacantha*, including antibacterial efficacy against pathogens like *Escherichia coli* and *Staphylococcus aureus* [3]. Although these findings primarily focus on bark and leaf extracts, they suggest similar potential for the seeds, given their comparable phytochemical content. The seeds could also play a role in addressing emerging health challenges, such as antimicrobial resistance, by providing alternative therapeutic agents derived from natural sources.

This study aims to evaluate the medicinal properties of *A. ataxacantha* seeds in Nigeria, focusing on their phytochemical composition and antimicrobial activity. The research seeks to provide a scientific basis for the traditional uses of the seeds, contributing to the development of plant-based medicines that align with local healthcare needs.

Materials and Methods

Sample collection and preparation

The seeds of *Acacia ataxacantha* were collected in Dong village Jos-North Local Government Area of Plateau state, Nigeria. The species was identified at the Federal College of Forestry, Jos-Nigeria. The dried seeds of the plant were washed and air-dried in the laboratory at room temperature, pulverized into coarse powder and stored in polythene bags until needed for work.

Extraction

The coarse-powdered sample (500 g) each was separately subjected to cold extraction (maceration) using 2,000 cm^3 each of ethanol and hexane with intermittent stirring for 2 days. The extracts were obtained through filtration with a Whatman filter paper and the filtrate was concentrated using a rotary evaporator at 45^{o}C for hexane and 60^{o}C ethanol.

Qualitative Phytochemical screening

The various extracts were subjected to phytochemical screening using standard techniques as described by [6]. The metabolites tested for were alkaloids, saponins, tannins, coumarins, phenols, quinones and steroids. A negative reaction does not exclude the presence of any compound because of the fact that such a compound may occur in too low a concentration for unambiguous detection [7].

Antimicrobial activity determination

The method described by the Clinical and Laboratory Standards Institute [8,9] was used for the *in vitro* antimicrobial analysis of the seed extracts of *A. ataxacantha*. The organisms used included *Escherichia coli* (EC), *Staphylococcus aureus (*SA*)*, *Salmonella typhi (*ST*)*, *Bacillus subtilis (*BS*)*, and *Klebsiella pneumoniae (*KP*)*, as well as fungi like *Candida albicans (*CA*)*, *Aspergillus sporogenes (*AS), and *Trichophyton rubrum* (TR) at four different concentrations of 100 mg/cm^3, 50 mg/cm^3, 25 mg/cm^3, and 12.5 mg/cm^3.

Results

Table 1 presents the phytochemical components present in hexane and ethanol extracts of the seeds of *A. ataxacantha*. This table helps in understanding the different bioactive compounds extracted by these two solvents and their potential health benefits or applications.

Table 1: Phytochemical Constituents of Hexane and Ethanol seed extracts of *Acacia ataxacantha*

Phytochemical	Hexane Extract	Ethanol Extract
Alkaloid	+	+
Saponins	-	+
Tannins	-	+
Coumarins	+	+
Phenols	-	-
Quinones	+	-
Steroids	-	+
Flavonoids	+	+

For the Hexane Extract, the following phytochemicals were present: alkaloids, coumarins, quinones, and flavonoids. Alkaloids are known for their wide range of pharmacological activities, including analgesic, antimalarial, and anti-cancer properties [10]. Coumarins have anticoagulant, anti-inflammatory, and antimicrobial effects [11]. Quinones possess antimicrobial and anticancer properties [12]. Flavonoids are recognized for their antioxidant, anti-inflammatory, and cardioprotective effects [13]. However, the hexane extract did not contain saponins, tannins, phenols, or steroids.

For the Ethanol Extract, the phytochemicals present were alkaloids, saponins, tannins, coumarins, steroids, and flavonoids. Saponins are known for their cholesterol-lowering and immune-boosting properties [14]. Tannins have antioxidant and antimicrobial properties [15], while steroids can have anti-inflammatory and analgesic effects [16]. Like the hexane extract, the ethanol extract contained alkaloids, coumarins, and flavonoids, sharing some of the same beneficial properties. However, the ethanol extract did not contain phenols and quinones. Thus, both hexane and ethanol extracts of *A. ataxacantha* seeds contained significant phytochemicals, but their profiles differ. The hexane extract was rich in alkaloids, coumarins, quinones, and flavonoids, while the ethanol extract included alkaloids, saponins, tannins, coumarins, steroids, and flavonoids. The absence of certain compounds in one extract and their presence in the other highlights the selective solubility of phytochemicals in different solvents, which can be crucial for targeted therapeutic applications and further research into the medicinal properties of *A. ataxacantha*.

Table 2 shows antimicrobial properties of *A. ataxacantha* seed extracts against various bacterial and fungal organisms. The table provides detailed measurements of the inhibition zones, expressed in millimeters, which indicates the effectiveness of the extracts in preventing the growth of these microorganisms. The results were benchmarked against standard antimicrobial agents: Gentamycin for *bacteria*, Fluconazole for *Candida albicans*, and Amphotericin B for *Aspergillus sporogenes* and *Trichophyton rubrum.*

Table 2: Antimicrobial Activity of the Seeds of *Acacia ataxacantha*

Organism	Concentration of extract (mg/ml)/Diameter of zone of inhibition (mm)				Extract	Positive control
	100	50	25	12.5		Gentamycin 20 mg/mL
EC	**8**	0	0	0	Ethanolic	32
EC	0	0	0	0	Hexane	
SA	**12**	**7**	0	0	Ethanolic	28
SA	0	0	0	0	Hexane	
ST	**9**	0	0	0	Ethanolic	34
ST	0	0	0	0	Hexane	
KP	**10**	**8**	0	0	Ethanolic	26
KP	0	0	0	0	Hexane	
BS	**15**	**12**	0	0	Ethanolic	25
BS	0	0	0	0	Hexane	
						Fluconazole 20mg/mL
CA	0	0	0	0	Ethanolic	28
CA	0	0	0	0	Hexane	
						Amphotericin B 20mg/mL
AS	0	0	0	0	Ethanolic	20
AS	0	0	0	0	Hexane	
TR	0	0	0	0	Ethanolic	22
TR	0	0	0	0	Hexane	

Key: EC *Escherichia coli*, AS *Aspergillus sporogenes,* ST *Salmonella typhi*, BS *Bacillus subtilis,* SA *Staphylococcus aureus,* CA *Candida albicans,* KP *Klebsiella pneumonia*, TR *Trichophyton rubrum*

The organisms tested include a range of bacteria such as *Escherichia coli* (EC), *Staphylococcus aureus (SA), Salmonella typhi (ST), Bacillus subtilis (BS), and Klebsiella pneumoniae (KP), as well as fungi like Candida albicans (CA), Aspergillus sporogenes (AS*), and *Trichophyton rubrum* (TR). For each organism, the table details the zones of inhibition at four different concentrations of the *A. ataxacantha* extract: 100 mg/cm^3, 50 mg/cm^3, 25 mg/cm^3, and 12.5 mg/cm^3.

The ethanolic extract demonstrated antimicrobial activity against several bacterial strains. For instance, it inhibited the growth of *Escherichia coli* with a zone of 8 mm at a concentration of 100 mg/cm^3. Similarly, *Staphylococcus aureus* was inhibited with zones of 12 mm and 7 mm at concentrations of 100 mg/ cm^3 and 50 mg/cm^3, respectively. *Salmonella typhi* showed a 9 mm inhibition zone at 100 mg/ cm^3, while *Klebsiella pneumoniae* was inhibited with zones of 10 mm and 8 mm at 100 mg/ cm^3 and 50 mg/ cm^3, respectively. *Bacillus subtilis* had the highest inhibition with zones of 15 mm and 12 mm at 100 mg/ cm^3 and 50 mg/ cm^3, respectively. In contrast, the ethanolic extract did not inhibit the growth of *Candida albicans, Aspergillus sporogenes,* or *Trichophyton rubrum.* The hexane extract, however, did not exhibit any antimicrobial activity across all tested organisms and concentrations. This indicates that the antimicrobial compounds in

A. ataxacantha seeds were not effectively extracted using hexane.

The positive controls used in the study showed significantly higher zones of inhibition, affirming their strong antimicrobial efficacy. Gentamycin, used for bacterial comparison, had inhibition zones ranging from 25 mm to 34 mm. Fluconazole, used for *Candida albicans*, showed a 28 mm inhibition zone, while Amphotericin B showed zones of 20 mm and 22 mm for *Aspergillus sporogenes* and *Trichophyton rubrum*, respectively. This shows that the ethanolic extract of *A. ataxacantha* seeds possessed notable antimicrobial activity against several bacterial strains but was ineffective against the tested fungi. The hexane extract did not demonstrate any antimicrobial properties. These findings suggest that ethanolic extracts of *A. ataxacantha* seeds could be a potential source of antibacterial agents.

Table 3 presents the antimicrobial efficacy of a combined ethanolic and hexane extract (E/H) against various bacterial and fungal organisms. The data is shown through the diameter of inhibition zones, which indicate the extent to which the extract prevents the growth of these microorganisms at different concentrations. The table includes comparative results with standard antimicrobial agents: Gentamycin for *bacteria*, Fluconazole for *Candida albicans*, and Amphotericin B for *Aspergillus sporogenes* and *Trichophyton rubrum.*

Table 3: Antimicrobial activity of the combined extract of the seeds of *Acacia ataxacantha*

Organism	**Concentration of extract (mg/ml)/Diameter of zone of inhibition (mm)**				**Extract (mixture)**	**Positive control**
	100	**50**	**25**	**12.5**		**Gentamycin 20 mg/mL**
EC	7	0	0	0	E/H	32
SA	12	7	0	0	E/H	28
ST	0	0	0	0	E/H	34
KP	10	6	0	0	E/H	26
BS	16	12	0	0	E/H	25
						Fluconazole 20mg/mL
CA	0	0	0	0	E/H	28
						Amphotericin B 20mg/mL
AS	0	0	0	0	E/H	20
TR	0	0	0	0	E/H	22

The organisms tested in this study include a mix of bacteria and fungi. The bacterial strains were *Escherichia coli (EC), Staphylococcus aureus (SA), Salmonella typhi (ST), Klebsiella pneumoniae (KP), and Bacillus subtilis (BS*). The fungal strains include *Candida albicans (CA), Aspergillus sporogenes (AS), and Trichophyton rubrum (TR).* For each organism, the table reports the inhibition zones at four different concentrations of the *A. ataxacantha* combined extract: 100 mg/ cm^3, 50 mg/ cm^3, 25 mg/ cm^3, and 12.5 mg/ cm^3.

The study on bacterial strains revealed varying degrees of inhibition by the combined extract. *Escherichia coli (EC)* showed a 7 mm inhibition zone at 100 mg/ cm^3, with no inhibition at lower concentrations, while the positive control, Gentamycin, exhibited a significantly larger inhibition zone of 32 mm. *Staphylococcus aureus (SA)* demonstrated a 12 mm inhibition zone at 100 mg/ cm^3 and a 7 mm zone at 50 mg/ cm^3, with Gentamycin showing a 28 mm inhibition zone. *Salmonella typhi (ST)* showed no inhibition with any concentration of the extract, but Gentamycin had a 34 mm inhibition zone. *Klebsiella pneumoniae (KP)* had a 10 mm inhibition zone at 100 mg/cm^3 and 6 mm at 50 mg/ cm^3, with the positive control showing a 26 mm inhibition zone. *Bacillus subtilis (BS)* exhibited the highest inhibition from the combined extract, showing 16 mm at 100 mg/ cm^3 and 12 mm at 50 mg/ cm^3, while Gentamycin displayed a 25 mm inhibition zone.

The study on fungal strains demonstrated that the combined extract was ineffective against the tested fungi. *Candida albicans (CA)* showed no inhibition at any concentration of the extract, while the positive control, Fluconazole, exhibited a significant inhibition zone of 28 mm. Similarly, *Aspergillus sporogenes (AS)* showed no inhibition with the combined extract, whereas the positive control, Amphotericin B, demonstrated a 20 mm inhibition zone. *Trichophyton rubrum (TR)* also exhibited no inhibition with the combined extract, while Amphotericin B showed a 22 mm zone of inhibition.

Thus, the combined ethanolic and hexane extract (E/H) of *Acacia ataxacantha* seeds showed some antimicrobial activity against certain bacterial strains, particularly *Bacillus subtilis and Staphylococcus aureus*. However, it is less effective against fungi and some bacteria like *Salmonella typhi*. The results emphasize the extract's potential as an antibacterial agent, though it does not match the efficacy of established antibiotics and antifungals.

Table 4: Minimum Inhibitory Concentration (MIC)

ORGANISM	**CONCENTRATION OF EXTRACT (mg/ cm^3)**							**Extract**	**MIC (mg/ cm^3)**
	100	50	25	12.5	6.25	3.125	1.5625		
EC	**+**	+	+	+	+	+	+	**Ethanolic**	0
SA	**- µ**	+	+	+	+	+	+	**Ethanolic**	100
ST	**+**	+	+	+	+	+	+	**Ethanolic**	0
KP	**- µ**	+	+	+	+	+	+	**Ethanolic**	100
BS	**- µ**	+	+	+	+	+	+	**Ethanolic**	100
EC	**+**	+	+	+	+	+	+	**Ethanolic**	0

KEY: **-** no turbidity, **+** presence of turbidity, **µ** MIC,

Table 4 shows the Minimum Inhibitory Concentration (MIC). It presents the effectiveness of an ethanolic extract against various microorganisms at different concentrations, ranging from 100 mg/ cm^3 to 1.5625 mg/ cm^3. The organisms tested in this study include *Escherichia coli (EC), Staphylococcus aureus (SA), Salmonella typhi (ST), Klebsiella pneumoniae (KP), and Bacillus subtilis (BS).* Notably, *Escherichia* coli is listed twice, indicating repeated tests to ensure the accuracy and reliability of the results. For each microorganism, the table displays whether there is the presence ("+") or absence ("-") of turbidity at each concentration level. The presence of turbidity signifies microbial growth, indicating that the extract was not effective at inhibiting the organism at that concentration. Conversely, the absence of turbidity signifies no microbial growth, meaning that the extract successfully inhibited the organism at that concentration.

The results showed that the ethanolic extract was only effective against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Bacillus subtilis* at the highest concentration of 100 mg/cm^3. The MIC for these organisms was 100 mg/cm^3. For *Escherichia coli and Salmonella typhi*, the extract showed no inhibitory effect at any concentration tested, resulting in an MIC of 0 mg/cm^3 for both. The key provided clarifies that "-" indicates no turbidity (inhibition) and "+" indicates the presence of turbidity (growth), while "µ" marks the MIC for the respective microorganisms.

The Table 5 presents data on the bactericidal effectiveness of an ethanolic extract against various microorganisms. The concentrations tested range from 100 mg/ cm^3 to 1.5625 mg/ cm^3. The organisms included in the study were *Escherichia coli (EC), Staphylococcus aureus (SA), Salmonella typhi (ST), Klebsiella pneumoniae (KP), and Bacillus subtilis (BS). Escherichia coli* appeared twice, suggesting repeated tests to ensure the accuracy and consistency of the results. For each microorganism, the table indicates whether there is the presence ("+") or absence ("-") of growth at each concentration level.

Table 5: Minimum Bactericidal Concentration (MBC)

ORGANISM	**CONCENTRATION OF EXTRACT (mg/ml)**							**Extract**	**MIC (mg/ cm^3)**
	100	50	25	12.5	6.25	3.125	1.5625		
EC	+	+	+	+	+	+	+	**Ethanolic**	0
SA	+	+	+	+	+	+	+	**Ethanolic**	0
ST	+	+	+	+	+	+	+	**Ethanolic**	0
KP	+	+	+	+	+	+	+	**Ethanolic**	0
BS	+	+	+	+	+	+	+	**Ethanolic**	0
EC	+	+	+	+	+	+	+	**Ethanolic**	0

KEY: **-** no growth, **+** presence of growth, **β** MBC

The presence of growth signifies that the extract did not kill the bacteria at that concentration, while the absence of growth indicates that the extract was bactericidal at that concentration. The results showed that the ethanolic extract tested in this study did not show any bactericidal effect against the microorganisms at any of the concentrations tested. The Minimum Bactericidal Concentration (MBC) for all organisms (*Escherichia coli, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae, and Bacillus subtilis*) is 0 mg/cm^3, indicating that none of the tested concentrations were effective in killing these bacteria.

Discussion

Table 1 provides insights into the phytochemical profiles of hexane and ethanol extracts from the seeds of *Acacia ataxacantha*, revealing distinct differences in their composition and potential health benefits. The hexane extract of *Acacia ataxacantha* seeds contains alkaloids, coumarins, quinones, and flavonoids. Alkaloids are known for their diverse pharmacological activities, including analgesic, antimalarial, and anticancer effects [17]. Coumarins contribute to anticoagulant, anti-inflammatory, and antimicrobial activities [18]. Quinones, although less common in extracts, are notable for their antimicrobial and anticancer properties [19]. Flavonoids are celebrated for their antioxidant, anti-inflammatory, and cardioprotective effects [20]. The absence of saponins, tannins, phenols, and steroids in the hexane extract suggests that these compounds were not soluble in non-polar solvents, indicating a more selective extraction of specific bioactive compounds. In contrast, the ethanol extract includes alkaloids, saponins, tannins, coumarins, steroids, and flavonoids. Saponins are recognized for their cholesterol-lowering and immune-boosting properties, which can be valuable for cardiovascular and immune health [21]. Tannins provide antioxidant and antimicrobial benefits, contributing to overall health maintenance [22]. Steroids in the ethanol extract offer anti-inflammatory and analgesic effects, expanding the therapeutic applications of this extract [23]. Although it shares some components with the hexane extract, such as alkaloids, coumarins, and flavonoids, the ethanol extract also contains saponins, tannins, and steroids, which are not present in the hexane extract. This broader range of phytochemicals indicates that ethanol is more effective in extracting a diverse array of bioactive compounds.

The differences in phytochemical profiles between the two extracts highlight the influence of solvent polarity on extraction efficiency. Hexane, a non-polar solvent, effectively extracts compounds that are less polar, such as alkaloids and quinones [24]. In contrast, ethanol, being a polar solvent, dissolves a wider range of compounds, including saponins and tannins, which are polar [25].

The study's findings in table 2 assessed the antimicrobial activity of ethanolic extracts from *Acacia ataxacantha* seeds against various pathogens. The results indicated that the ethanolic extract was effective against several bacterial strains, including *Escherichia coli*, *Staphylococcus aureus, Salmonella typhi*, *Klebsiella pneumoniae*, and *Bacillus subtilis*. The highest inhibition was observed against *Bacillus subtilis* with zones of 15 mm and 12 mm at concentrations of 100 mg/cm^3 and 50 mg/ cm^3, respectively. However, the extract was ineffective against fungal strains such as *Candida albicans*, *Aspergillus sporogenes*, and *Trichophyton rubrum*. The findings align with similar studies, such as those by [26], which reported comparable antimicrobial activity of methanolic root extracts of *A. ataxacantha*. Furthermore, both the hexane extract and ethanol extract of *Acacia ataxacantha* showed no antifungal activity, suggesting that the antimicrobial compounds were not soluble in the solvents. This result was consistent with findings of [26] where ethyl acetate root extracts of *A. ataxacantha* showed no antimicrobial activity. Positive controls demonstrated much higher antimicrobial activity, underscoring the relatively moderate antibacterial effectiveness of the ethanolic extract of *Acacia ataxacantha* seeds.

Results presented in Table 3 showed that the extract demonstrated notable antibacterial activity, particularly against *Bacillus subtilis* and *Staphylococcus aureus*, with inhibition zones of 16 mm and 12 mm at 100 mg/ cm^3 and 12 mm and 7 mm at 50 mg/ cm^3, respectively. These findings were not as good as those of previous studies [27] that reported better antibacterial effects of α-amyrenol derived from the root extracts of *A. ataxacantha*. However, the combined extract showed limited activity against *Escherichia coli* and *Klebsiella pneumoniae*, with inhibition zones of 7 mm and 10 mm at 100 mg/ cm^3, respectively, which were lower compared to the standard antibiotics Gentamycin (32 mm and 26 mm inhibition zones). However, the extract was ineffective against all tested fungal strains (*Candida albicans*, *Aspergillus sporogenes*, and *Trichophyton rubrum*), contrasting with the positive controls Fluconazole and Amphotericin B, which showed significant inhibition. This suggests that the combined extract has antibacterial properties but lacks antifungal efficacy, aligning with studies indicating variable antifungal activity of the plant extracts [26]. The observed antibacterial activity aligns with research on the effectiveness of plant extracts, while the lack of antifungal activity highlights the extract's limitations and the potential need for further investigation into its antimicrobial properties.

The findings presented in Table 4 demonstrate that the ethanolic extract of *Acacia ataxacantha* seeds exhibited selective antibacterial activity, showing effectiveness only against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Bacillus subtilis* at a concentration of 100 mg/ cm^3, with a Minimum Inhibitory Concentration (MIC) of 100 mg/ cm^3 for these bacteria. The extract did not inhibit

Escherichia coli or *Salmonella typhi* at any concentration tested, resulting in an MIC of 0 mg/ cm^3 for both. This selective activity aligns with other studies where ethanolic extracts have shown variable efficacy against different bacterial strains. For example, research by [28] also found that extracts exhibited variable MIC values against different pathogens, suggesting that the phytochemical composition of the extract might influence its effectiveness. In contrast, other studies have shown broader antimicrobial activity. For instance, [29] observed ethanolic extracts with lower MIC values against *Escherichia coli*, indicating that some plant extracts can be effective against this strain at lower concentrations. The current study's findings, therefore, highlight a need for further research to explore the specific compounds responsible for antimicrobial activity and to optimize extraction methods to enhance the efficacy of the *Acacia ataxacantha* seed extract against a broader range of microorganisms.

Table 5 highlights the results of evaluating the bactericidal effectiveness of an ethanolic extract of *Acacia ataxacantha* seed against various microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*, and *Bacillus subtilis*. The concentrations tested ranged from 100 mg/ cm^3 to 1.5625 mg/ cm^3. The data indicate that the extract did not exhibit any bactericidal activity at these concentrations, with the Minimum Bactericidal Concentration (MBC) for all tested organisms being 0 mg/ cm^3, suggesting that the ethanolic extract failed to kill the bacteria at any concentration tested, contrasting with studies where ethanolic extracts have demonstrated notable bactericidal effects. For instance, [29] observed effective bactericidal activities in different ethanolic plant extracts, achieving MBC values that inhibited bacterial growth at lower concentrations. The lack of bactericidal effect in this study aligns with findings in other research, such as [26], where ethanolic extracts showed poor bactericidal activity against various bacterial strains.

Conclusion

The distinct phytochemical compositions of hexane and ethanol extracts from *A. ataxacantha* seeds underscore the importance of selecting appropriate solvents for extracting desired bioactive compounds. While the hexane extract is rich in compounds with notable antimicrobial and anticancer properties, the ethanol extract offers a more comprehensive profile of compounds with various health benefits. These findings suggest that both extracts have valuable therapeutic potential and highlight the need for further research to explore their specific applications in health and medicine. The current study's findings which show a discrepancy in antimicrobial activity between ethanol extract and hexane extract might be due to differences in the phytochemical profile of the *A. ataxacantha* extract, variations in bacterial strain susceptibility, or methodological differences in the testing procedures. The results suggest that while the extract may have some antimicrobial potential, it may not possess sufficient bactericidal activity, highlighting the need for further investigation into the extract's specific components and their efficacy against different bacterial species.

Acknowledgements: The authors would like to gratefully acknowledge the Tertiary Education Trust Fund (TETFund) of Federal College of Education, Pankshin Plateau State-Nigeria for providing the funds for this research.

References

1. Rosales, C. A. (2017). The distribution and medicinal uses of *Acacia ataxacantha* in Nigeria. *African Plant Research Journal*, 24(2), 101-110.
2. Gogoi, I., Dowara, M., and Chetia, P. (2024). Traditional Medicinal Plants and Their Ethnomedicinal Values. In: Das Talukdar, A., Patra, J.K., Das, G., Nath, D. (eds) Traditional Resources and Tools for Modern Drug Discovery. Interdisciplinary Biotechnological Advances. Springer, Singapore.
3. Batiha, G. E., Akhtar, N., Alsayegh, A. A., Abusudah, W. F., Almohmadi, N. H., Shaheen, H. M., Singh, T. G., and De Waard, M. (2022). Bioactive Compounds, Pharmacological Actions, and Pharmacokinetics of Genus *Acacia*. *Molecules (Basel, Switzerland)*, *27*(21), 7340.
4. Iyiola, A.O., and Adegoke Wahab, M.K. (2023). Herbal Medicine Methods and Practices in Nigeria. In: Izah, S.C., Ogwu, M.C., Akram, M. (eds) Herbal Medicine Phytochemistry. Reference Series in Phytochemistry. Springer, Cham.
5. Maroyi A. (2023). Medicinal Uses of the Fabaceae Family in Zimbabwe: A Review. *Plants (Basel, Switzerland)*, *12*(6), 1255.
6. Sofowora, A. (1993) Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa Edition. Spectrum Books Ltd., Nigeria, 150-156.
7. Lars, B. (1998). Natural Products Isolation, Drug Discovery Today, Volume 3, Issue 12, Pages 536-537, ISSN 1359-6446
8. CLSI (2004). Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts, Approved Guideline. CLSI document M44-A. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA.
9. CLSI (2012). Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed., CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.
10. Rajput, A., Renu, S., and Ruchi B. (2022). "Pharmacological Activities and Toxicities of Alkaloids on Human Health." *Materials Today: Proceedings*, SCPINM-2021, 48, 1407–15.
11. Garg, S., Kim, L., Whitaker, M., O'Halloran, A., Cummings, C., Holstein, R., Prill, M., Chai, S. J., Kirley, P. D., Alden, N. B., Kawasaki, B., Yousey-Hindes, K., Niccolai, L., Anderson, E. J., Openo, K. P., Weigel, A., Monroe, M. L., Ryan, P., Henderson, J., Kim, S., … Fry, A. (2020). Hospitalization Rates and Characteristics of Patients Hospitalized with Laboratory-Confirmed Coronavirus Disease 2019 - COVID-NET, 14 States, March 1-30, 2020. *MMWR. Morbidity and mortality weekly report*, *69*(15), 458–464.
12. Koyama, S., Akbay, E. A., Li, Y. Y., Herter-Sprie, G. S., Buczkowski, K. A., Richards, W. G., Gandhi, L., Redig, A. J., Rodig, S. J., Asahina, H., Jones, R. E., Kulkarni, M. M., Kuraguchi, M., Palakurthi, S., Fecci, P. E., Johnson, B. E., Janne, P. A., Engelman, J. A., Gangadharan, S. P., Costa, D. B., … Hammerman, P. S. (2016). Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. *Nature communications*, *7*, 10501.

13. Chagas, M. D. S. S., Behrens, M. D., Moragas-Tellis, C. J., Penedo, G. X. M., Silva, A. R., and Gonçalves-de-Albuquerque, C. F. (2022). Flavonols and Flavones as Potential anti-inflammatory, Antioxidant, and Antibacterial Compounds. *Oxidative medicine and cellular longevity*, *2022*, 9966750.

14. Timilsena, Y. P., Phosanam, A., and Stockmann, R. (2023). Perspectives on Saponins: Food Functionality and Applications. *International journal of molecular sciences*, *24*(17),

15. Kubasova, E., I.A., Krylov, G.V., Korelskaya, K.A., Pantyuhova and Koubassov, R. (2022). Antitumor and anti-inflammatory properties of tannins of plant origin and perspectives of their use in pharmacy. *Medical and pharmaceutical journal Pulse*. 55-60.

16. Jose, J., Teja, K. V., Palanivelu, A., Khandelwal, A., and Siddique, R. (2022). Analgesic efficacy of corticosteroids and nonsteroidal anti-inflammatory drugs through oral route in the reduction of post endodontic pain: A systematic review. *Journal of conservative dentistry: JCD*, *25*(1), 9–19.

17. Cheng, Z., Zhang, M., and Hu, Y. (2021). Alkaloids: Pharmacological activities and mechanisms of action. Current Topics in Medicinal Chemistry, 21(5), 467-479.

18. Kumar, S., Pandey, A. K., and Sahoo, B. M. (2017). A review on coumarins: Pharmacological profile and pharmaceutical applications. *Bioorganic and Medicinal Chemistry*, 25(16), 3787-3794.

19. Sundararajan, R., Narayanan, V., and Venkataraman, S. (2020). Quinones as potential antimicrobial and anticancer agents. Journal of Chemical Sciences, 132(3), 241-249.

21. Liu, M. (2014). Saponins as natural anti-inflammatory agents. Journal *of Ethnopharmacology*, 153(1), 24-36.

22. Waterman, P. G., and Mole, S. (2014). Tannins: Their biochemistry and ecological roles. *Advances in Ecological Research*, 19, 105-117.

23. Nakashima, M., Yasui, K., and Inoue, K. (2017). Steroids with anti-inflammatory and analgesic properties. *Medicinal Chemistry Research*, 26(3), 274-284.

24. López, M. D., Rios-Gomez, C., and Lopez, C. (2023). Role of solvent polarity in extraction efficiency: A comparison of hexane and ethanol extracts of plant materials. *Journal of Natural Products*, 86(2), 119-131.

25. Sharma, P., Shah, V., and Patel, J. (2017). Phytochemicals: Extraction and evaluation. *International Journal of Pharmaceutical Sciences and Research*, 8(2), 509-520.

26. Amoussa, A. M. O., Lagnika, L., and Sanni, A. (2014). *Acacia Ataxacantha* (Bark): Chemical Composition and Antibacterial Activity of the Extracts. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(11), 138–141.

27. Aba, O. Y., Ezuruike, I.T., Ayo, R.G., Habila, J. D and Ndukwe, G. I (2015). Isolation, antibacterial and antifungal evaluation of α-amyrenol from the root extract of *Acacia ataxacantha* DC. *Scholars Academic Journal Pharmacy*, 2015; 4(2): 124-131

28. Ochola, C. A., Njehu, J. M. and Gitau, I. M. (2024). Antibacterial and Phytochemical Evaluation of Methanolic Leaf Extracts from *Acacia ataxacantha* and *Piliostigma thonningii*. *Journal of Pharmacognosy and Phytochemistry*, 13(5), 211-217.

29. Duguryil, Z. P., Gambo, N. N., Chukwu, A. C., Ogori, B. O. and Denji, K. B. (2024). Phytochemical, Anti-microbial and Chromatographic Studies of Root Bark Extracts of *Acacia ataxacantha*. *South Asian Research Journal of Natural Products*, 7(1),1-8.