ARCAITL JOURNALS

Traditional Journal of Multidisciplinary Sciences (TJMS)

January-June 2023, Vol. 01, No. 01, [40 – 54]

Published: 27th March 2023

https://ojs.traditionaljournaloflaw.com/index.php/TJMS

RESEARCH PAPER

Preliminary Phytochemical Screening and Antimicrobial Study of Mistletoe (Agelanthus dodoneifolius) Growing on Shea Butter Tree (Vitellaria paradoxa)

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ABSTRACT

This study aimed to conduct a preliminary phytochemical screening and antimicrobial investigation of mistletoe (Agelanthus dodoneifolius) that grows on shea butter trees (Vitellaria paradoxa). The objective was to identify the phytochemicals and assess the antimicrobial activity present in the leaves of Agelanthus dodoneifolius using the methods described by Edeoga (2005) and Aliyu et al. (2009). Plants have long been a source of various compounds used in medicine. Agelanthus dodoneifolius is a hemiparasitic plant that typically grows on the branches of host trees, particularly indigenous trees of economic importance. Among the Hausa and Fulani tribes of Northern Nigeria, Agelanthus dodoneifolius is renowned for its ethnomedicinal use in treating ailments such as dysentery, diarrhea, typhoid, and stomachaches. The phytochemical analysis of this plant revealed the presence of phenol (with the highest amount detected at 180.51), tannin, saponin, flavonoid, and terpenoid. The antimicrobial investigation of the plant extract against S. aureus, B. cereus, and A. flavus demonstrated that A. flavus exhibited the highest resistance to the methanolic extract of Agelanthus dodoneifolius, producing the largest diameter zone of inhibition (69mm) at a concentration of 80mg/ml. These findings support the traditional use of Agelanthus dodoneifolius.

Keywords: Agelanthus dodoneifolius, Phytochemicals, Antimicrobial, MIC, Methanolic extract.

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INTRODUCTION

Throughout human history, plants have played a vital role in various aspects of our lives, particularly for survival and medicinal purposes. The utilization of medicinal plants worldwide has contributed to the development of antibiotics and modern pharmaceutical drugs. In essence, a medicinal plant refers to any plant species that contains biologically active compounds within its structures, capable of being utilized for therapeutic purposes or as precursors for synthesizing beneficial medications (Sofowora et al., 2013). Agelanthus, a genus of plants found in the Afrotropical region and belonging to the Loranthanceae family, exhibits a hemi-parasitic nature, growing on trees as shrubs of different sizes. These plants establish a connection with their host

plants through a single haustorium (Smith, 2012). Notably, the stems of Agelanthus often display swollen nodes that produce flowers. These flowers are commonly found in close proximity, with their five petals fused to form a tubular structure. When in bloom, the filaments of the flowers remain spirally rolled inward, while the styles are inconspicuous. The berries of Agelanthus come in varying shades of pink, orange, and red, typically measuring around 1cm in diameter (Smith, 2012). In traditional medicine, the leaves and young twigs of Agelanthus plants have been employed for centuries to treat various ailments, including circulatory and respiratory diseases (Efuntoye et al., 2010). These plants have been held in high regard for their medicinal properties in folklore medicine.

Agelanthus dodoneifolius, an African mistletoe, belongs to the Loranthaceae family and is known for its parasitic nature, which poses a threat to the host plants it attaches to (Dlama et al., 2016). The presence of this mistletoe on host plants restricts their ability to generate branches at the attachment points, depriving them of essential nutrients. As a consequence, the infected trees experience weakened growth, leading to aging and eventual death (Ibrahim, 2009). Despite the detrimental impact on host plants, the African mistletoe is also utilized in ethnomedicine for the treatment of various ailments (Deeni and Sadiq, 2002) A. dodoneifolius specifically thrives by attaching itself to branches or aerial parts of host plants, typically indigenous trees and economically important tree crops such as guava (Psidium guajava), Shea butter (Vitellaria paradoxa), cashew (Anacardium occidentale), mango (Mangifera indica), sugar apple (Annona squamosa), and neem (Azadirachta indica) (Dlama et al., 2016). Medicinal plants, including the African mistletoe, have played a significant role in the lives of people worldwide.

In rural communities, Traditional Medicine Practitioners (TMPs) are commonly recognized as the primary healthcare providers. The prevalent utilization of traditional herbal medicine often arises from the perceived inadequacies of conventional treatments. Africa, being abundantly rich in medicinal plants, stands out as a continent with significant herbal resources (Mgbeahuruikea et al., 2019). Nigeria, situated within the African region, demonstrates regular usage of Complementary and Alternative Medicine (CAM) in conjunction with traditional beliefs for treating various ailments (Shinkafi et al., 2015). The term "antimicrobial" originates from the Greek words "anti" (against), "micros" (little), and "bio" (life), denoting substances that can either eliminate or inhibit the activity of microorganisms. Infections and diseases can be caused by various types of organisms, including bacteria, fungi, and viruses. An antimicrobial agent is a medication used in humans and animals to counteract the pathogenicity of microorganisms (bacteria, fungi, or viruses) (Mgbeahuruikea et al., 2019).

LITERATURE REVIEW

This section provides a literature review of the preliminary phytochemical screening and antimicrobial investigation conducted on mistletoe (Agelanthus dodoneifolius) growing on shea butter trees (Vitellaria paradoxa). Phytochemicals are a diverse group of bioactive compounds naturally found in plants, encompassing a wide range of substances present in various plant parts such as vegetables, fruits, medicinal plants, flowers, leaves, and roots. These compounds play a vital role in plant defense mechanisms against diseases and environmental stressors. While

phytochemicals do not provide direct nutritional value like vitamins and minerals, they have been found to exert significant influence on numerous physiological processes in the human body. The importance of phytochemicals lies in their potential to promote health and protect against various diseases. They work synergistically with essential nutrients and dietary fiber to enhance overall well-being. Their beneficial effects include reducing the risk of chronic diseases and age-related conditions, such as cancer, heart disease, stroke, and high blood pressure. Through their antioxidant and anti-inflammatory properties, phytochemicals help combat oxidative stress, neutralize harmful free radicals, and modulate cellular processes associated with disease development.

Research conducted by Igwenyi et al. (2011) highlights the significance of phytochemicals in health promotion. By incorporating plant-based foods rich in phytochemicals into our diet, we can harness their potential benefits and contribute to the prevention and management of various health conditions. Therefore, emphasizing the consumption of a diverse range of plant-based foods can provide a valuable source of phytochemicals that support optimal health and well-being. Phytochemicals are categorized into two groups based on their roles in plant metabolism: primary and secondary constituents. Primary metabolites encompass essential components like sugars, amino acids, proteins, and chlorophyll. On the other hand, secondary constituents include compounds such as alkaloids, terpenoids, phenolic compounds, as well as additional examples like flavonoids and tannins (Krishnaiah et al., 2009). In Europe, mistletoe is extensively utilized as an anticancer agent, in contrast to the toxic nature of American mistletoe. European mistletoe is renowned for its perceived medicinal properties. It has been historically employed for the treatment of various physical and mental ailments. Currently, it is recognized as a supplementary therapy alongside other drugs and radiation for cancer treatment. Certain HIV/AIDS organizations have also reported its potential in aiding immune system restoration (Hoagy, 2008).

The reputation of mistletoe as an "all healer" has been supported by its effectiveness in medicine. Viscum album, a mistletoe species with white berries, has traditionally been associated with the treatment of diabetes and high blood pressure. In Germany, mistletoe extracts are utilized as an unconventional oncology therapy. Through ethnobotanical surveys conducted in Palestine, Viscum album has been identified as a treatment option for skin diseases and prostate cancer (Khammash, 2005). In Nigeria's traditional medicinal practices, Loranthus bengwensis L. (Loranthaceae), commonly known as African mistletoe, has been extensively employed for the management of Diabetes mellitus (Ibatomi et al., 1994). Another species of African mistletoe, Tapinanthus dodoneifolius, has demonstrated a broad spectrum of antimicrobial activities against specific bacterial and fungal strains that exhibit resistance to multiple drugs. The extract derived from mistletoe has shown potential in inhibiting the growth of bacteria associated with various conditions, including crown gall, gastrointestinal tract infections, and wound infections. Some of the bacterial species affected by the mistletoe extract include Agrobacterium tumefaciens, Bacillus sp., Escherichia coli, Proteus sp., Pseudomonas sp., and Salmonella sp. (Deeni and Sadiq, 2002).

The utilization of African mistletoe in traditional medicine for the treatment of Diabetes mellitus highlights its historical significance and potential therapeutic properties. Furthermore, the antimicrobial activities exhibited by Tapinanthus dodoneifolius against drug-resistant bacterial

and fungal strains suggest its potential as an alternative treatment option for infections that are challenging to manage. The ability of mistletoe extract to inhibit the growth of bacteria associated with various infections indicates its potential in addressing both common and serious health issues. By exploring the antimicrobial properties of African mistletoe, particularly Tapinanthus dodoneifolius, researchers have identified promising avenues for combating microbial infections. These findings contribute to our understanding of the therapeutic potential of natural plant-based compounds and their applications in addressing health challenges. Further studies are warranted to elucidate the specific mechanisms of action and potential clinical applications of mistletoe extracts, paving the way for the development of novel antimicrobial agents derived from this traditional medicinal plant.

Osadebe and Ukwueze (2004) extensively investigated the antimicrobial properties of Loranthus micranthus, a species of African mistletoe found in eastern Nigeria. Their research focused on evaluating the antifungal activity of mistletoe extracts against Candida species, which are significant pathogens known to cause severe illnesses and contribute to mortality rates among critically ill patients in hospital settings (Jahagirdar et al., 2018). Similarly, other studies have explored the antimicrobial potential of various components derived from Viscum album L., another species of mistletoe. For example, researchers investigated the ethanolic extract of Viscum album L. and its activity against Candida species. Notably, the extract demonstrated considerable efficacy against Candida inconspicua, with a minimum inhibitory concentration (MIC) of 5.65 mg/mL (Nacsa-Farkas et al., 2014). These investigations shed light on the antimicrobial properties of African mistletoe species and their potential in combating fungal infections, particularly those caused by Candida species. The findings contribute to our understanding of the therapeutic applications of mistletoe extracts and their potential role in developing novel treatments for fungal-related illnesses. Further research is necessary to uncover the specific mechanisms underlying these antimicrobial effects and to explore their clinical implications in order to enhance our arsenal against infectious diseases.

Furthermore, Assaf et al. (2013) reported that a methanolic extract of Viscum cruciatum Sieber ex Boiss leaves demonstrated effectiveness against Candida albicans, with a minimum inhibitory concentration (MIC) of 1.25 mg/mL. In a similar vein, the n-hexane extract of Viscum album subsp. abietis (Wiesb.) Abrom, along with two fractions obtained through flash column preparation, exhibited activity against Candida albicans. The first fraction displayed activity at a concentration of 1 mg/mL, resulting in a zone inhibition of 11 mm, while the second fraction showed activity at a concentration of 10 mg/mL, resulting in a zone inhibition of 10 mm (Erturk et al., 2003). Additionally, Shah et al. (2017) conducted a comparative study on the antimicrobial activity of different parts of Viscum album L. against Candida albicans.

MATERIALS AND METHODS

Collection of plant material

In September 2022, the leaves of mistletoe (Agelanthus doneifolius) were collected from shea butter trees (Vitellaria paradoxa) located in Lapai, Niger State, Nigeria. The plant material

was identified by the herbarium keeper of the Department of Biology at Ibrahim Badamasi Babangida University, Lapai, Niger State, and assigned a voucher number (IBBU210519).

Extraction of Plant Material

The plant material, specifically the leaves, were gathered and left to air-dry at room temperature for a period of ten days. Once dried, the samples were finely powdered by pounding them with a pestle and mortar. Methanol was chosen as the solvent for the extraction process. Approximately 500g of the powdered plant material (leaves) were accurately weighed using an electronic balance and mixed with 350ml of methanol using the maceration method, also known as the cold extraction method, for a duration of three days. The resulting mixture was then filtered through Whatman No.1 filter paper. The crude extract obtained from the filtration process was subsequently evaporated to dryness using a water bath.

Qualitative Phytochemical Analysis of Agelanthus dodoneifolius leaves

(a) Test for Saponins

In a test tube, approximately 0.5g of the plant extract was mixed with 2ml of distilled water and shaken. The observation of frothing indicated the presence of saponins (Edeoga, 2005).

(b) Test for Terpenoid

In the experiment, around 0.5g of the plant extract was combined with 1ml of chloroform and 1.5ml of concentrated H2SO4, carefully added along the side of the test tube. The appearance of a reddish-brown color at the interface indicated the presence of terpenoids (Chang, 2002).

(c) Test for Flavonoid

A quantity of approximately 0.5g of the plant extract was mixed with 2ml of H2SO4. Upon observation, the presence of a reddish-brown or orange color indicated a positive outcome (Oloyed, 2005).

(d) Test for Alkaloids

To test for the presence of alkaloids in the plant extract, a sample weighing approximately 0.5g was mixed with 2ml of hydrochloric acid (HCl). Subsequently, 1ml of Dragendroff's reagent was added to the mixture. If alkaloids were present, the solution would undergo a chemical reaction resulting in the formation of an orange or red precipitate. This color change serves as a positive indication for the presence of alkaloids in the plant extract, as described by Trease and Evans (2002). Alkaloids are a class of naturally occurring compounds with diverse biological activities and are often associated with medicinal properties in plants.

(e) Test for Tannins

A quantity of approximately 0.5g of the plant extract was mixed with a few drops of neutral 5% ferric chloride solution. The occurrence of a dark green coloration signifies the presence of tannins (Trease and Evans, 2002).

(f) Test for phenol

A measurement of approximately 0.05g of the extract was taken, and then 2ml of 1% lead acetate was added. The formation of a white precipitate indicates the presence of phenols (Sathya, 2013).

Quantitative Phytochemical analysis of Agapanthus dodoneifolius leaves

(a) Determination of Alkaloids

To begin the experiment, a quantity of 0.5g of the plant extract was carefully weighed and dissolved in a mixture of 5ml of 96% ethanol and 20% of H2SO4 (1:1). The resulting solution was then filtered, and 1ml of the filtrate was taken and combined with 5ml of 60% H2SO4. This mixture was allowed to stand undisturbed for a duration of 5 minutes. Following that, 5ml of 0.5% formaldehyde was added to the solution, and the mixture was left to stand for a period of 3 hours.

Subsequently, the reading of the solution was taken at a wavelength of 565nm using the Spectrophotometer method (Trease and Evans, 2002).

(b) Determination of Flavonoid

The experiment began by weighing 0.6g of the plant extract, which was then dissolved in 10ml of distilled water. From this solution, a volume of 0.5ml was transferred into a test tube. To the test tube, 0.5ml of AlCl3, 0.5ml of Sodium Nitrate, and 2.5ml of distilled water were added. Subsequently, the test tube contents were mixed thoroughly and the reading was taken at a wavelength of 450nm using the Spectrophotometer method (Oloyed, 2005). This measurement allows for the evaluation of the reaction or presence of specific components in the plant extract solution.

(c) Determination of Tannins

To initiate the experiment, a quantity of 0.01g of the plant extract was carefully dissolved in 10ml of distilled water. The resulting solution was then filtered to remove any impurities. From this filtered solution, 1ml of the filtrate was transferred into a test tube. In the test tube, the 1ml of filtrate was mixed with 2ml of Socatoa reagent and 2ml of 17% NaCO3 (sodium carbonate) solution. The contents of the test tube were thoroughly mixed to ensure proper reaction and dispersion. Subsequently, the reading of the solution was taken at a wavelength of 750nm using the Spectrophotometer method. This measurement at a specific wavelength allows for the evaluation and analysis of the components present in the plant extract solution (Trease and Evans, 2002). The Spectrophotometer method is a reliable technique for measuring the absorbance or transmission of light through the solution, providing valuable information about the chemical composition and concentration of the analyzed substances.

(d) Determination of Saponin

To commence the experiment, a precise weight of 0.5g of the plant extract was measured and dissolved in 20ml of 1N HCL (hydrochloric acid). The resulting mixture was then subjected to heat in a water bath at a temperature of 80°C for a duration of 4 hours, allowing for proper

extraction of the desired components. After the heating process, the reaction mixture was allowed to cool and subsequently filtered to remove any solid impurities. To further process the filtrate, 10ml of petroleum ether was added. This resulted in the formation of two distinct layers, with the ether layer containing the target compounds of interest. The ether layer was carefully collected and subjected to evaporation using a water bath, causing the solvent to evaporate and leaving behind a dry residue.

Following the evaporation, a solution was prepared by combining 5ml of aceton ethanol (in a ratio of 1:1), 6ml of ferrous sulphate, and 2ml of concentrated sulphuric acid. The resulting mixture was left to stand for a period of 10 minutes, allowing for the necessary reactions to occur. To determine the concentration of the target compounds, the absorbance of the solution was measured at a wavelength of 490nm using the spectrophotometer method. The spectrophotometer is a precise instrument capable of measuring the amount of light absorbed by a solution, providing valuable information about the concentration and characteristics of the analyzed compounds (Edeoga, 2005).

(e) Determination of Terpenoid

To initiate the experiment, an accurate amount of 0.1g of the plant extract was dissolved in a mixture of 9ml of methanol and 10ml of petroleum ether. This solution was prepared in a test tube that had been previously weighed to ensure precise measurements. Following the dissolution, the mixture formed distinct layers, with the ether layer containing the desired terpenoid compounds of interest. Careful separation was conducted to collect only the ether layer, discarding the remaining solution. The collected ether layer was then subjected to evaporation using a water bath, causing the solvent to evaporate and leaving behind a dry residue. The test tube containing the residue was then reweighed to determine the final weight. To calculate the concentration of terpenoids, the initial weight of the test tube (with the plant extract and solvent) was subtracted from the final weight of the test tube (with the dried residue). The difference in weight was then multiplied by 100% to obtain the percentage of terpenoids present in the plant extract. This process allows for the quantitative analysis of terpenoid content in the plant extract, providing valuable information about its composition and potential biological activities (Chang, 2002).

(f) Determination of Phenol

In this experiment, a measured amount of 0.05g of the plant extract was carefully dissolved in 10ml of distilled water. To this solution, 2ml of socatoa and 2ml of sodium carbonate ($NaCO_3$) were added. These reagents played a specific role in the chemical reaction that was about to take place. Following the addition of socatoa and $NaCO_3$, the resulting mixture was thoroughly mixed. Approximately 7.5g of the mixture was then collected and transferred into another test tube. To ensure homogeneity, this portion was dissolved in 5ml of distilled water. To quantify the desired component, a spectrophotometer was employed. A specific wavelength of 750nm was chosen, as it corresponds to the absorption characteristics of the target compound. By measuring the absorbance at this wavelength, the concentration of the compound in the plant extract could be determined.

This analytical method is commonly used to assess the presence and concentration of specific compounds in plant extracts, allowing researchers to gain insights into their chemical composition and potential applications. The study conducted by Sathya in 2013 utilized this approach to investigate the composition of the plant extract and assess its potential bioactive properties.

ANTIMICROBIAL SCREENING

Source of Micro-Organism

The clinical isolated microorganisms, namely Bacillus aereus, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Aspergillus niger, and Aspergillus flavus, were acquired from the stock cultures maintained in the Microbiology Laboratory of the Federal University of Technology in Minna, Niger State. These organisms were chosen for their relevance in microbiological studies and their association with various human infections. To evaluate the effectiveness of the plant extract against these microorganisms, susceptibility testing was conducted following the method described by Aliyu et al. in 2009, with reference to the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI) in 2006. This standardized approach ensures consistency and accuracy in assessing the response of the microorganisms to the plant extract. By subjecting the isolated microorganisms to the plant extract, researchers aimed to determine the extent to which the extract could inhibit their growth or exert antimicrobial effects. The results of this assay provide valuable insights into the potential antimicrobial properties of the plant extract and its possible application in combating infections caused by these specific microorganisms. The study conducted by Aliyu et al. and the adherence to the CLSI guidelines demonstrate the scientific rigor and methodology employed to evaluate the susceptibilities of the clinical isolated microorganisms to the plant extract.

Reconstitution

To prepare different concentrations of the plant extract, weights of 40mg, 60mg, and 80mg were measured. Each weighed amount of the plant extract was then mixed with 5ml of distilled water for dilution purposes. This process resulted in three different dilutions of the plant extract, allowing for further experimentation or analysis at varying concentrations.

Determination of Anti-bacterial Activity

The antibacterial activities of Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, and Salmonella typhi were evaluated in this study. Sterile disposable petri dishes were filled with nutrient agar and left to solidify. The surface of the dried nutrient agar plates was inoculated using a sterile wire loop to streak the pathogens. As a positive control, ampicloxacillin (100mg/ml) was used for all the tested pathogens. Wells were created on the inoculated nutrient agar using a 6mm diameter cork-borer. These wells were then filled with 0.5ml of methanol extracts of leaves from A. dodoneifolius, reconstituted to concentrations of 40mg/ml, 60mg/ml, and 80mg/ml. After allowing the plates to stand for 10 minutes at room temperature, the plates were incubated for 24 hours. The antibacterial activity was assessed by measuring the diameter of

the zones of inhibition formed around the wells. All tests were performed in triplicate, and the average values were calculated to determine the results.

Determination of Antifungal Activity

In this study, the antifungal activity of Aspergillus niger and Aspergillus flavus was investigated. Sterile disposable petri dishes were filled with Sabouraud dextrose agar, which was then allowed to solidify. The surface of the dried Sabouraud dextrose agar plates was inoculated using a sterile cotton swab stick to streak the pathogens. Ketoconazole (40mg/ml) was used as the positive control for all the tested pathogens. Wells were created on the inoculated Sabouraud dextrose agar using a 6mm diameter cork-borer. These wells were then filled with 0.5ml of methanol extracts of leaves from A. dodoneifolius, reconstituted to concentrations of 40mg/ml, 60mg/ml, and 80mg/ml. After allowing the plates to stand for 10 minutes at room temperature, the plates were incubated for 48 hours at ambient room temperature. The antifungal activity was assessed by measuring the diameter of the zones of inhibition formed around the wells. All tests were performed in triplicate, and the average values were calculated to determine the results. The guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI, 2006) were followed in conducting the experiments.

Minimum Inhibitory Concentration (MIC)

The determination of the minimum inhibitory concentration (MIC) of the plant extract against the test microorganisms was conducted using broth dilution techniques, following the protocols outlined by Vollokova et al. (2001) and Wiegand et al. (2008). The test microbes were inoculated and incubated at a temperature of 37°C for a period of 6 hours. To prepare a turbid suspension of the microorganisms, McFarland's turbidity scale number 0.5 was utilized. The test microbes were diluted in normal saline until the turbidity matched that of the McFarland's standard, resulting in a concentration of approximately 1.5 x 108 colony-forming units per milliliter (cfu/ml), as determined through visual comparison. Serial dilutions of the plant extract were prepared in sterile broth to obtain concentrations of 40, 60, and 80mg/ml. The initial concentration was achieved by dissolving 2ml of the extract in sterile broth. A loopful of the test microbes was then inoculated into the different concentrations of the plant extract. Following incubation at 37°C for 24 hours, each test tube was observed for turbidity. The lowest concentration of the extract that showed no turbidity was considered as the Minimum Inhibitory Concentration (MIC), as defined by Osadebe and Ukwueze (2004).

Minimum Bactericidal and Fungicidal Concentration (MBC/MFC)

The determination of the minimum bactericidal and fungicidal concentration of the extract was carried out as follows: Nutrient agar and Sabouraud dextrose agar were prepared and sterilized at a temperature of 121°C for 15 minutes. The sterilized agar media were poured into sterile petri dishes and allowed to solidify. Subsequently, the content of the Minimum Inhibitory Concentration (MIC) from the serial dilution was inoculated onto the respective agar media and the plates were incubated at a temperature of 37°C for a duration of 24 hours. During the incubation period, the plates were carefully observed for any visible growth of bacterial or fungal colonies. The lowest concentration of the extract that demonstrated no microbial growth on the agar plates was

determined as the minimum bactericidal concentration and minimum fungicidal concentration. This was determined in accordance with the study conducted by Ogbulie et al. (2007).

RESULT

The result of preliminary phytochemical analysis of methanol extract of *A. dodoneifolius* is shown in Table 1. Plant constituent including flavonoid, Saponin, Terpenoid were detected while Alkaloid and Tannin were not detected (Table 1).

Table 1: Preliminary phytochemical constituents of methanol extract of *A. dodoneifolius* leaves. (Qualitative analysis)

Chemical constituent	Result
Alkaloid	-
Tannin	-
Flavonoid	+
Saponin	+
Terpenoid	+
Phenol	++

Key: + = present, - = absent, ++ = highly present.

Table 2: Preliminary phytochemical analysis of methanol extract of *A.dodoneifolius* leaves. (Quantitative analysis). The shows the presence and amount of tannin, flavonoid, saponin, phenol and terpenoid (80%).

Chemical constituent (mg/g)	Results	Amount present
Alkaloid	-	0.00
Tannin	++	84.35
Flavonoid	+	13.06
Saponin	+	8.09
Terpenoid(%)	++	80(%)
Phenol	++	180.51

Key: + = present, - = absent, ++ = highly present.

***Zone of inhibition (mm) of extracts of A. dodoneifolius leaves against test microorganism*.**

Table 3 presents the data collected from the antimicrobial activity assessment of the methanol extract derived from the leaves of A. dodoneifolius at various concentrations (40mg/ml, 60mg/ml, and 80mg/ml) against the tested microorganisms. The results demonstrate that the extract displayed significant antimicrobial activity towards Aspergillus flavus, Staphylococcus aureus, Aspergillus niger, Pseudomonas aeruginosa, Bacillus cereus, and Salmonella typhi at different concentrations.

Among the microorganisms tested, Pseudomonas aeruginosa, Salmonella typhi, and Aspergillus niger exhibited sensitivity to the extract, indicating that they were not resistant. Aspergillus flavus showed antimicrobial activity at all three concentrations (40mg/ml, 60mg/ml, and 80mg/ml). Staphylococcus aureus displayed antimicrobial activity at concentrations of 60mg/ml and 80mg/ml, while Bacillus cereus exhibited antimicrobial activity at a concentration of 80mg/ml. These findings suggest that the methanol extract of A. dodoneifolius leaves possesses antimicrobial properties, inhibiting the growth of various microorganisms, and the degree of inhibition varies depending on the concentration and specific microorganism tested.

Test Organisms	40mg/ml	60mg/ml	80mg/ml	CB) 100mg/ml) (CF) 40mg/ml)
S. aureus	-	24.00mm	28.00mm	52.00mm
B. cereus	-	-	18.00mm	57.00mm
P. aeruginosa	-	-	-	39.00mm
S. typhi	-	-	-	44.00mm
A. niger		-	-	62.00mm
A. flavus	34.00mm	34.00mm	69.00mm	72.00mm

Key; -= no zone of inhibition, CB= control for bacterial (Ampicloxacillin), CF= control for fungi (Ketoconazole).

Table 4: The MIC result exhibited by the plant extract against *S. aureus, B. cereus* and *A. flavus* at different concentration (mg/ml). The MIC result shows *A. flavus* at different concentration (40, 60 and 80mg/ml) followed by *S.aureus* at concentration (60 and 80mg/ml).

Test Organisms	40mg/ml	60mg/ml	80mg/ml
S. aureus	-	2.4mg/ml	0.0256mg/ml
B. cereus	-	-	16.0mg/ml
A. flavus	1.6mg/ml	0.096mg/ml	0.0021mg/ml

Key: -= No inhibition.

Table 5: The result of Minimum bacterial and fungal concentration inhibited by the plant extract against *S. aureus, B. cereus* and *A. flavus* at different concentration (mg/ml). It shows the result of MBC and MFC, the inhibition of different concentration was observed in *A. flavus,* followed by *S. aureus* (60 and 80mg/ml) then *B.cereus* (80mg/ml).

Test Organisms	40mg/ml	60mg/ml	80mg/ml
S. aureus	-	2.4mg/ml	0.64mg/ml
B. cereus	-	-	3.2mg/ml
A. flavus	3.2mg/ml	0.48mg/ml	0.0051mg/ml

Key: -= no inhibition.

DISCUSSION

The presence of antibacterial and antimicrobial properties in plants has been documented in previous studies (Habtamu and Melaku, 2018). Specifically, the mistletoe plant has demonstrated a broad range of antimicrobial activity, including against certain drug-resistant strains (Deeni and Sadiq, 2002). Table 1 provides qualitative results from preliminary phytochemical analysis, revealing the presence of flavonoids, saponins, and tannins. These secondary metabolites are known to be active constituents in many plant-derived drugs, emphasizing the importance of conducting basic phytochemical investigations to identify major phytoconstituents. The presence of phenols, saponins, tannins, and flavonoids in A. dodoneifolius can partially account for its antiplasmodial effects. The results obtained from this study indicate that the extract derived from Agelanthus dodoneifolius exhibited inhibitory effects on the growth of microorganisms, including Aspergillus flavus, Staphylococcus aureus, and Bacillus cereus. However, it did not exhibit inhibitory effects on Pseudomonas aeruginosa, Salmonella typhi, and Aspergillus niger. Therefore, the presence of inhibitory substances in the plant extract has been demonstrated, indicating its potential to impede the growth of various microorganisms. This finding aligns with the research conducted by Osadebe and Ukwueze (2004), which supports the antimicrobial effects of the plant extract at different concentrations. The observed antimicrobial activity can be attributed to the presence of tannins, flavonoids, phenols, and terpenoids, as these compounds are known to possess antimicrobial properties, as described by Cowan (1999). Moreover, the presence of phenols in the extract can explain its potential anti-cancer activity and its ability to combat diseases associated with oxidative stress, as noted by Art and Hollman (2005). Phenols play a beneficial role in the intestinal tract by acting as antioxidants and protecting cells from oxidative damage caused by free radicals. Additionally, phenols contribute to the enhancement of the anti-inflammatory capacity in humans. The presence of tannins in the extract accounts for its wide-ranging activity against diseases caused by the tested organisms in this study, as tannins are recognized for their strong antiviral and antibacterial properties. These findings are consistent with the research conducted by Tijjani et al. (2011). Furthermore, tannins and flavonoids have been reported to contribute to the anti-diarrheal effects, as stated by Okoli (2009). The antimicrobial and phytochemical properties exhibited by the extracts further highlight the significance of plants as valuable sources of potentially beneficial compounds for the control of fungal and bacterial diseases.

CONCLUSION

The African mistletoe (A. dodoneifolius) has been scientifically proven to possess significant medicinal properties. Experimental findings have confirmed its antimicrobial activity, thus supporting its traditional use in ethnomedicine for treating fungal and bacterial infections. The phytochemical analysis conducted on the plant extract offers preliminary insights into its chemical composition and suggests the potential biological activities exhibited by the extract.

Recommendation

There is a need for both public and private organizations to promote research focused on identifying the active components responsible for the antimicrobial properties of the mistletoe

plant. It is also important to characterize the bioactive compounds present in the plant and explore their potential use as a herbal medicine in combating common infections prevalent in our society.

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