

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF LEAF AND STEM-BARK AQUEOUS EXTRACTS OF *DIOSPYROS MESPILIFORMIS*

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Authors' contributions

This work was carried out in collaboration between all authors. Author GYS designed the study, wrote the protocol, wrote the first draft of the manuscript. Author SD carried out all laboratory work, analysis of the study. Authors SD, AE, YYB and IJ managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

ABSTRACT

Objective: To investigate the phytochemical composition and antimicrobial activities of the leaf and stem-bark extracts of *D. mespiliformis* against some pathogenic microorganisms.

Methods: The leaf and stem-bark extracts of *Diospyros mespiliformis* from Ebanaceae family, which is used as herbal remedies for the cure of many ailments by natives in northern part of Nigeria, were collected from Mubi in Mubi North Local Government area of Adamawa State, air dried, pulverised, extracted by simple overnight maceration techniques and analyzed. Aqueous extracts of the aforementioned parts of the plant were screened phytochemically for its chemical constituents and subjected to antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* spp, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Streptococcus pyogenes* and *Salmonella typhi*.

Results: The results show that alkaloids, anthraquinones, carbohydrates, flavonoids, phlobatannins, saponins, steroids, tannins and terpenoids are present in both the leaf and the stem-bark extracts of the plant while glycosides are present in the leaf extract but absent in the stem-bark extract. The antimicrobial activity reveals that both the leaf and the stem-bark extracts of the plant, showed high sensitivity to *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* spp, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Streptococcus pyogenes* and *Salmonella typhi*.

Conclusion: *The phytochemical constituents and the antimicrobial potential of the plant part may account for varied ethnobotanical uses of the plants in traditional medicine in Nigeria, which if further purified can be used to source novel antibiotics.*

Keywords: *Diospyros mespiliformis*, Phytochemical Screening, Antimicrobial, Ethnobotanical

1. INTRODUCTION

Phytomedicines have been an integral part of traditional health care system in most parts of the world for thousands of years. Phytochemicals are compounds that are produced by plants ("phyto" means "plant"). They are found in fruits, vegetables, grains, beans, and other plants, each of which may cure or prevent a disease singly or in synergism with some others [1]. Some of these phytochemicals are believed to protect cells from damage that could lead to cancer [1]. Phytochemical compounds have curative and prophylactic properties against a wide range of human and animal diseases [2]. Phytochemical screening is of great importance in providing information about chemicals found in a plant in term of their nature and range of occurrence [3].

According to the World Health Organization (WHO), the definition of traditional medicine may be summarised as the sum total of all the knowledge and practical, whether explicable or not, used in the diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing. The WHO has described traditional medicine as one of the surest means to achieve total health care coverage of the world's population [4].

Traditional medicine might also be considered as a solid amalgamation of dynamic medical know-how and ancestral experience. In Africa, traditional healers and remedies made from plants play an important role in the health of millions of people. Numerous medicines have been derived from the knowledge of tropical forest people and clearly, there will be more in the future [4]. It has been estimated that in developed countries such as the United States, despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants, while in fast-developing countries such as China and India, the contribution is as much as 80% [5]. Out of about 2,500,000 higher plant species on earth, more than 80,000 are medicinal. India is one of the world's 12 biodiversity centres with the presence of over 45,000 different plant species. Thus, the economic importance of medicinal plants is much more to countries such as

India than to rest of the world. These countries provide two third of the plants used in the modern system of medicine, and the healthcare system of rural population depend on indigenous systems of medicine. In Nigeria, application of medicinal plants especially in traditional medicine is currently well acknowledged and established as a viable profession [6].

According to the WHO, about 80% of the world's people depend on traditional medicine for their primary healthcare needs. Studying medicinal plants with ethnobotanical importance and folklore reputation has become the more important need in recent times in order to promote the use of herbal medicines and to determine their potential as a source of new drugs [7, 8, 9]. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology studies leading to the synthesis of a more portend drugs with reduced toxicity [10].

Medicinal plants are plants and plant parts found to be rich in one or more individual elements thereby providing a possible link to the medicinal value of the plant. Plants which naturally synthesise and accumulate some secondary metabolites and vitamins, possess medicinal properties [11]. Medicinal plants contain both organic and inorganic constituents and serve as an important therapeutic agent as well as important raw materials for the manufacture of both traditional and modern medicine [12, 10].

The use of crude extracts of plants parts and phytochemicals of known antimicrobial properties can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in various countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesised by the plants. These products are known by their active substances like, phenolic compounds which are part of the essential oils, as well as in tannins.

Antimicrobial agents are substances that interfere with the growth and metabolism of microbes. In common usage, the term denotes inhibition of growth, and with reference to specific groups of organisms, terms as antibacterial, antifungal, antiviral and antiprotozoa are frequently employed. Antimicrobial agents may either kill microorganisms or inhibit their growth. Those that inhibit growth are called bacteria static. These agents depend on the normal host defenses to kill or eliminate the pathogens after its growth has been inhibited. While those

antimicrobial agents that kill are bactericidal and are particularly useful in situations in which the normal host defenses cannot be relied on to remove or destroy pathogens. A given antimicrobial can be bactericidal in one situation, yet bacteria static in another, depending on the concentration of the drug and the growth stage of the microorganism [13].

Antimicrobial agents used to treat infection are called chemotherapeutic agents. Chemotherapeutic agents are chemicals substance used for the treatment of infectious diseases or diseases caused by the proliferation of malignant cells. These substances are prepared in the chemical laboratory or obtained from microorganisms and some plants and animals in general, naturally occurring substances are distinguished from synthetic compounds by the name antibiotics. Some antibiotics are prepared synthetically, but most of them are prepared commercially by microbial biosynthesis [6].

In the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects [14] and the increasing resistance of most synthetically derived antimicrobial agents are of utmost concern [15]. Therefore, there is need to search for suitable plants of medicinal value to be effective in the treatment of diseases, which must be harmless to human tissue.

In recent years, use of antimicrobial drugs in treatment of infectious diseases has developed multiple drug resistance and with the increase in production of new antibiotics, by the pharmaceutical industry, resistance to these drugs has also increased [16]. Hence, scientists are shifting their attention to folk medicine in order to find new leads for better drugs against microbial infections. Plant materials are known as source of new antimicrobial agents, as a result search has been to discover new antibacterial drugs of plant origin.

The plant *Diospyros mespiliformis*, commonly known as “Kanya” in hausa and “Igidudu” in yoruba is a tall, evergreen tree 15-20 m high, with dense, rounded and buttressed stem belonging to the family *Ebenaceae*. It is called Jackal-berry or African ebony. It is found in Savannah and Northern low land forest [1]. The leaves are simple, alternate dark green with small hairs on the underside of older leaves. *D. mespiliformis* is dioecious, pollinated by bees and flowers in the rainy season while fruit ripening, which coincides with the dry season takes place 6-8 months after flower fertilization [17]. The fruit of this plant is a traditional food of high nutritive value in Africa [16]. *D. mespiliformis* is reportedly one of the most important genera of

Ebenaceae which species have been used over the millennia in traditional medicinal systems [17].

The leaf, roots, stem-bark and fruits contained antibiotic qualities and posses many medicinal uses. Roots and stem-bark are used to facilitate childbirth and are used for infections such as pneumonia, syphilis, leprosy, dermatomycoses, as an anthelmintic [18] while the leaf decoction is used as a remedy for fever, otitis and wound dressing agent [17]. The leaf is also used for treatment of headache, arthritis and skin infections. The leaf and fruits are chewed or applied as infusion for treating gingivitis, toothache and for wound dressing to prevent infection [19]. Barks and roots are used as psycho-pharmacological drug and to treat tumor [7, 8, 11].

The aim of this study was to investigate the phytochemical composition and antimicrobial activities of the leaf and stem-bark aqueous extracts of *D. mespiliformis* against some pathogenic microorganisms. Selection of the medicinal plant for the present study was based on its ethno-medicinal usages. Since the plant of study is known to possess a number of medicinal uses of different purposes. Testing the extracts of this plant against organisms known to be the causes of these diseases become vital, so as to see if their growth can be inhibited in other to prove there folk uses and claims by traditional healers in Nigeria.

2. MATERIALS AND METHODS

2.1 Sampling and sample preparation

Fresh leaves and stems-bark of *D. mespiliformis* were collected in April, 2013 from Mubi in Mubi North Local Government area of Adamawa State and were identified by Prof. Mohammed S. of Department of Biological Sciences, Adamawa State University Mubi, Adamawa State, Nigeria where a voucher number 00012PDSM was issued and deposited for future use. The identified plant parts were washed with tap water and air-dried. The dried parts were chopped into pieces, milled into fine powder by pounding manually with a clean and sterile pestle and mortar for the extraction. The powdered samples were each collected into sterile cellophane bags and labelled to prevent mix up.

2.2 Preparation of the plant extract

Extraction was carried out for the leaf and stem-bark of *D. Mespiliformis* by using overnight maceration techniques [20]. About 50 g each of leaf and stem-bark material were

macerated in 250 ml of water in a flask. Each of the soaked samples was stirred, sealed with aluminium foil and allowed to stand for 72 hours at room temperature and the supernatant decanted. Thereafter, the supernatant was filtered using Whatman No. 1 filter paper followed by evaporation at 45°C using a rotary evaporator at a minimum pressure. The extracts were kept and stored in a refrigerator at 4°C until further analysis [9].

2.3 Phytochemical screening

Qualitative phytochemical analysis of the leaf and stem-bark extract of *D. mespiliformis* was carried out using standard qualitative procedures [20, 21]. Alkaloids, anthraquinones, carbohydrates, flavonoids, glycosides, phlobatannins, saponins, steroids, tannins and terpenoids tests were conducted in all the extracts and the results are shown in Table 1.

2.4 Test Organism

The bacterial and fungal strains used for the investigation are:- *Escherichia coli* (EC114ADSUC), *Pseudomonas aeruginosa* (PA214ADSUC), *Shigella spp* (SS314ADSUC), *Staphylococcus aureus* (SA414ADSUC), *Streptococcus pneumonia* (SP514ADSUC), *Streptococcus pyogenes* (SP614ADSUC) and *Salmonella typhi* (ST714ADSUC).

2.5 Antimicrobial investigation

The microorganisms were clinically isolated from Adamawa State University Clinic, Mubi. The stocks bacterial cultures were maintained at 4°C on nutrient agar slant and sub-culture in nutrient broth for incubation at 37°C for 24 hours prior to each antimicrobial testing. Inoculation of the test organisms on nutrient agar prepared plates was achieved by flaming a wire loop on a spirit lamp, cooling the wire loop (air cooling) and fetching the test organisms.

The discs were prepared using a Whatman No. 3 filter paper and putting in vials-bottles and sterilizing in an oven at 150°C for 15 minutes. Prepared discs containing the various extracts were carefully placed on the inoculated plates using a sterilized forceps in each case. The plates were then turned upside-down and inoculate at 37°C for 24 hours in an incubator [22]. After incubation, the inoculated plates were observed for zones of inhibition (in mm diameter). Ampiclox (10µg/mL) was used as the **positive control**. The result was taken by considering the zone of growth and inhibition of the organisms by the test fractions [23]. Activity and inactivity

were observed in accordance with the standard and accepted method. Results are shown in Table 2.

3 RESULTS AND DISCUSSION

Phytochemical screening conducted on leaf and stem-bark aqueous extracts of *D. mespiliformis* revealed that alkaloids, anthraquinones, carbohydrates, flavonoids, phlobatannins, saponins, steroids, tannins and terpenoids were present in both the leaf and the stem-bark aqueous extract of the plant while glycosides are present in the leaf extract but absent in the stem-bark extract as shown in table 1.

It is evident that ethanolic extracts have significantly higher activity when compared to aqueous extracts, but due to some environmental issues, like non destructive nature of water to both man and the environment, non inflammability of water, operational simplicity, easy to obtain (low cost), etc, it was chosen to make the aqueous extract which has no negative impact on the result as all the bioactive compounds tested in the aqueous extracts of the leaf and stem-bark were present. However, in the stem-bark aqueous extracts, glycosides were absent.

Phytochemical screening of the extract varies from one plant part to another as shown in the results. It could also vary from place to place due to geographical location, climatic conditions and soil condition of a particular area. This may explain why it could be possible to have differences in chemical composition of the same plant of study in other areas.

The presence of some of these compounds has been demonstrated previously by other researchers. For example, the presence of alkaloids, saponins and tannins in the leaf and stem-bark of *D. mespiliformis* has been demonstrated [24, 25]. Similarly the absence of glycosides in the stem-bark of *D. mespiliformis* has also being demonstrated [26]. However some of the results obtained are not in agreement with the previous findings. For example alkaloids were found to be absent in the stem-bark of *D. mespiliformis* [26] which is contrary to other findings [24, 25]. This might be due to climatic and environmental factors.

Alkaloids, steroids, tannins and terpenoids were found present in both the leaf and stem-bark extracts of *D. mespiliformis*. This suggests that both the leaf and the stem-bark extracts of the plant may possess some antibacterial potential most especially for antimalarial, antidiarrhea and antihemorrhagic potentials because of the presence of alkaloids and tannins in the leaf and

stem-bark extracts [27, 28]. Fruroquinolines and acridones have been reported to be compounds contained in plant alkaloids which are capable of curing malaria [29].

Phytochemicals constituents such as flavonoids, alkaloids and saponins present in the leaf and stem-bark extracts were reported to possess biological activity against microbes [20, 24]. Flavonoids and tannins are proven to induce an important antimicrobial activity due to their possession of ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins and so forth [30]. Furthermore, the leaf and stem-bark extracts of *D. mespiliformis* may have some antioxidant properties due to the presence of flavonoids and terpenoids in the extracts. Flavonoids have been known to have antioxidant, antibacterial, antifungal and antiviral activity [29]. Other studies confirmed that flavonoids and terpenoids produce antidiabetic activity, possibly by their antioxidant effects [2, 29].

Antimicrobial activities of the leaf and stem-bark aqueous extract of *D. mespiliformis* were studied by measuring the zone of inhibition formed around the disc and the results are given in table 2 and figure 1. The result revealed that the stem-bark aqueous extract of the plant showed high sensitivity to *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella spp*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Streptococcus pyogenes* and *Salmonella typhi* than the leaves extracts. *D. mespiliformis* shows significant antimicrobial activity against the tested microorganism as shown in figure 1. Especially in the case of *Shigella spp*, *Streptococcus pyogenes* and *Salmonella typhi* and this imply it efficiency in the treatment of dysentery, diarrhea and remedy for typhoid fever. However, statistical analysis is an obvious limitation for this study. This study has provided some biochemical basis of the medical use of the extracts from *D. mespiliformis* in the treatment of infection, as a potential source of useful drugs and will help to develop new drug compositions.

Table 1: Phytochemical analysis of aqueous extracts of leaves and stem-bark of *D. mespiliformis*

Phytochemicals	Test	Inference	Result	
			Leave Extract	Stem-bark Extract
Alkaloids	Mayer's test	Appearance of yellow cream precipitate	+	+
Anthraquinones	Borntrager's Test	Ammoniacal layer turns pink or red	+	+
Carbohydrates	Molisch's Reagent	Formation of violet ring	+	+
Flavonoids	Alkaline Test	Intense yellow colouration	+	+
Glycosides	Liebermann-Burchard's Test	Brown ring	+	-
Phlobatannins	Hydrochloric acid test	Formation of red precipitate	+	+
Saponins	Frothing test	Produce foam or emulsion	+	+
Steroids	Liebermann-Burchard's Test	Brown ring at the junction, green at the upper layer	+	+
Tannins	Ferric chloride test	Formation of blue or green colour	+	+
Terpenoids	Liebermann-Burchard's Test	Brown ring at the junction, deep red at the upper layer	+	+

Key: + = Presence of Phytochemical substance, - = Absence of Phytochemical substance

Table 2: Antimicrobial activity of aqueous extracts of leaves and stem-bark of *D. mespiliformis*

Strains of Microorganisms	Zone of inhibition (mm)		Ampiclox (Control)
	Leaves 50 mg/mL	Stem-bark 50 mg/mL	
<i>Escherichia coli</i>	12	17	30
<i>Pseudomonas aeruginosa</i>	12	20	25
<i>Shigella spp</i>	15	19	22
<i>Staphylococcus aureus</i>	14	18	26
<i>Streptococcus pneumonia</i>	5	8	20
<i>Streptococcus pyogenes</i>	28	29	35
<i>Salmonella typhi</i>	24	26	32

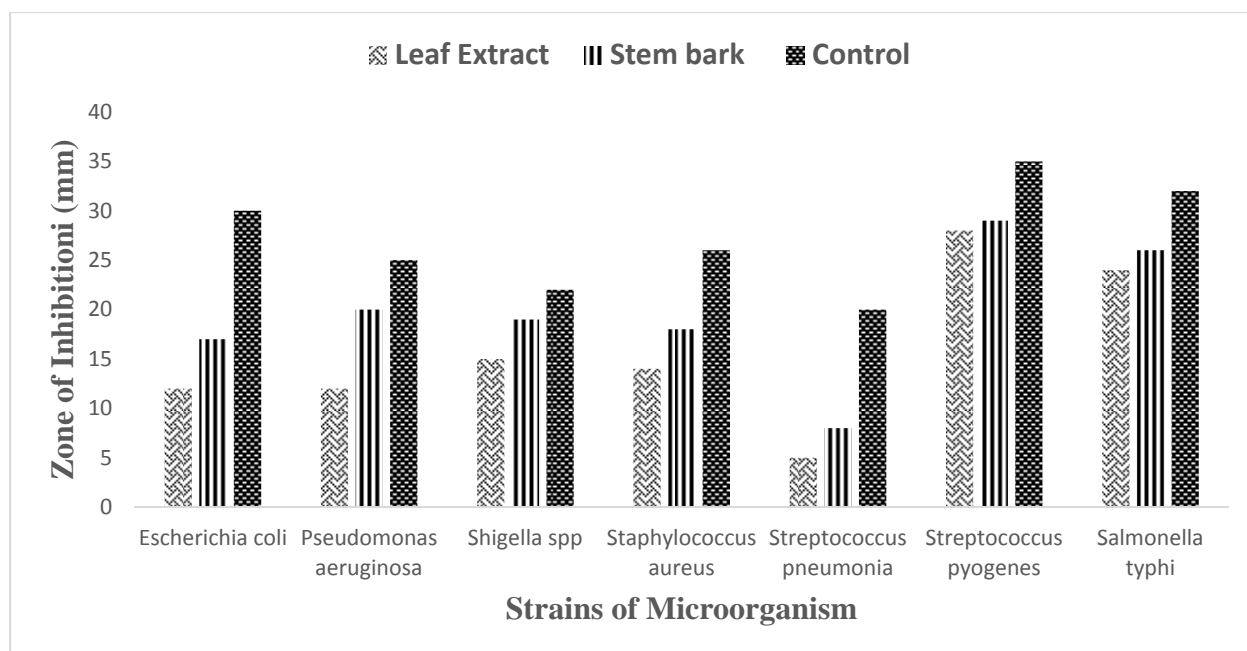


Fig. 1: Antimicrobial activity of aqueous leaf and stem-bark extracts of *D. mespiliformis*, measured by average inhibition zone values in mm

4. CONCLUSION

The result of the study reveals that the aqueous extracts of the leaf and stem-bark extract of *D. mespiliformis* plant contained some primary bioactive compounds that inhibit the growth of the test microorganisms, thereby proving to have significant antimicrobial potential. Therefore, the results of this study provide a rationale for the use of the plant parts in traditional medicine practice in Nigeria. With growing resistance of most pathogens to the activities of the common antibiotics, further work is required to isolate specific active constituents of the plant responsible for the antimicrobial activity and antioxidant activity. Other related pharmacological studies such as in vivo investigation, drug formulation and clinical trials are highly recommended.

Ethical approval and consent: NA

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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