Original Research Article

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

ABSTRACT

The leaves 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, pulvurised, 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. The results show that alkaloids, anthraquinones, carbohydrates, flavonoids, phlobatannins, saponins, steroids, tannins and terpenoids are present in both the leaves and the stem-bark extracts of the plant while glycosides is present in the leaves extract but absent in the stem-bark extract. The antimicrobial activity reveals that, both the leaves 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. The phytochemical constituents and the antimicrobial potential of the plant part may account for varied enthnobotanical 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

INTRODUCTION

Diospyros mespiliformis is a tall, evergreen tree 15-20 m high, with dense, rounded and buttressed stem belonging to the family *Ebenaceae*. It is known in Hausa as “Kanya” and in Yoruba as “Igidudu”. It is commonly 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 [2]. The edible fruit of this plant is a traditional food of high nutritive value in Africa that is either used fresh in fermented drink or dried and stored for later use [3]. *D. mespiliformis* is reportedly one of the most important genera of *Ebenaceae* which species have been used over the millennia in traditional

medicinal systems [4]. The leaves, roots, stem-bark and fruits contain antibiotic qualities and posses many medicinal uses in West Africa. Roots and stem-bark is used to facilitate childbirth, and are used for infections such as pneumonia, syphilis, leprosy, dermatomycoses, as an anthelmintic [5] while the leaves decoction is used as a remedy for fever, otitis and wound dressing agent [2]. Different parts of the plant are used against diarrhea, headache, toothache and as a psycho-pharmacological drug. The leaves are eaten by elephants, giraffe, black rhino, baboons while the wood, which is hard, strong, fungal and termite resistant, is used for construction purposes, furniture, carvings, walking sticks, stamping blocks, pestles and makes good firewood and charcoal [6, 7].

Plant parts, which have one or more of its organs containing substances that can be used for therapeutic purpose, are called medicinal plants [8]. Ethnobotanical studies carried out throughout Africa confirm that indigenous plants are the main constituents of traditional African medicines [9, 10].

Phytochemical is a non - nutritive plant chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators or pathogens [11] and protect them against environmental stresses like cold and heat [1]. Phytochemical compounds have curative and prophylactic properties against a wide range of human and animal diseases [12]. There are many phytochemical in fruits, leaves, stem-bark and roots of plants, each of which may cure or prevent a disease singly or in synergism with some others [1]. Phytochemical screening is of great importance in providing information about chemicals found in a plant in term of their nature and range of occurrence [13].

The plant *D. mespiliformis* possess a number of medicinal uses, hence the need to phytochemically screen the plant extracts to establish its phytochemical profile for the better prediction and easier confirmation of the medicinal value of this plant and to test the plant extract against organisms known to be the causes of these diseases, so as to see if their growth can be inhibited in order to prove their folk uses and claims by traditional healers in Nigeria.

MATERIALS AND METHODS

Sampling and sample preparation

Fresh leaves and stems-bark of *D. mespiliformis* were collected from Mubi in Mubi North Local Government area of Adamawa State and identified by Prof. Mohammed S. of Department of Biological Sciences, Adamawa State University Mubi, Adamawa State, Nigeria. The identified plant parts were cleaned under a running tap water before being air-

dried at room temperature and pulverized, using a clean, sterile mortar and pestle into fine particles suitable for extraction. The pulverized leaves and stem-bark samples were each collected into sterile labeled cellophane bags to prevent mix up.

Preparation of the plant extract

Extraction was carried out for the leaves and stem-bark of *D. Mespiliformis* by using overnight maceration techniques according to the method reported in [14]. About 50 g each of leaves 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^{o}C using a rotary evaporator at a minimum pressure. The extracts were kept and stored in a refrigerator at 4°C until further analysis.

Phytochemical screening

Qualitative phytochemical analysis of the leaves and stem-bark extract of *D. mespiliformis* was carried out using standard qualitative methods as described by [14,15]. 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.

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).

Antimicrobial investigation

The microorganisms were clinically isolated from Adamawa State University Clinic, Mubi. The stocks bacterial cultures were maintained at 4^{o}C on nutrient agar slant and sub-culture in nutrient broth for incubation at 37^{o}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^{o}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^{o}C for 24 hours in an incubator [16]. After incubation, the inoculated plates were observed for zones of inhibition (in mm diameter). Ampiclox (10µg/mL) was used as the control. The result was taken by considering the zone of growth and inhibition of the organisms by the test fractions [17]. Activity and inactivity were observed in accordance with the standard and accepted method. Results are shown in Table 2.

RESULTS AND DISCUSSION

Phytochemical screening conducted on leaves and stem-bark extracts of *D. mespiliformis* revealed that alkaloids, anthraquinones, carbohydrates, flavonoids, phlobatannins, saponins, steroids**,** tannins and terpenoids were present in both the leaves and the stem-bark extract of the plant while glycosides is present in the leaves extract but absent in the stem-bark extract. The plant extracts contain different phytochemical, with various biological activities that can be of value in both medical and veterinary practice. Thus, the component of these chemicals suggests that these plants could posses some pharmacological behavior.

Alkaloids like morphine and codeine have been reported to have analgesic properties. However, some of them like the morphine have addictive tendencies [18]. Anthraquinones have been associated with anticancer, laxative and anti-arthritic properties [19]. Phytochemical constituents such as flavonoids, alkaloids and saponins present in the leaves and stem-bark extracts were reported to possess biological activity against microbes [14, 20].

Tannin a compound that is able to react with protein to appear as stable water insoluble compound, precipitate proteins of the wound, forming a protective layer on the wound, thus assisting in the arrest of bleeding and therefore promoting wound healing. Both the leaves and the stem-bark extracts of *D. mespiliformis* in this study contain tannin and could all be investigated for wound healing activity. Tannins have also been reported to be used in the treatment of diarrhea as an effective astringent medicine that does not only stop the flow of the disturbing substance in the stomach rather controls the irritation in the small intestine [21]. In addition, phlobatannin a subtype of tannin is also confirmed to be present in all the extracts.

Besides tannin and phlobatannin, all of the investigated plant extracts also contained flavonoids and terpenoids. Flavonoids have been known to have antioxidant, antibacterial,

antifungal and antiviral activity [22]. Other studies confirmed that flavonoids and terpenoids produce antidiabetic activity, possibly by their antioxidant effects [12, 22].

Saponins have been associated with decreased cholesteraemia. They are also thought to aid in the absorption of calcium [23]. The two extracts analyzed in this study contain saponin as one of their phytochemical. The leaves and the stem-bark extracts of *D. mespiliformis* are therefore candidate extracts for the prevention of artherosclerosis, which is a condition precipitated by cholesterol. Glycosides were only observed in leaves extracts of the plant. A recent study [22] revealed that glycoside is a phytochemical which contributes to one of the hypoglycaemic effects. These phytochemical particularly tannins and flavonoids 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 [24].

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

Bioactive compounds	Leaves extract	Stem- bark extract
Alkaloids	+	+
Anthraquinones	+	+
Carbohydrates	+	+
Flavonoids	+	+
Glycosides	+	-
Phlobatannains	+	+
Saponins	+	+
Steroids	+	+
Tannins	+	+
Terpenoids	+	+

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

Antimicrobial activities of the leaves and stem-bark 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 display in figure 1. The result revealed that the stem-bark 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. 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 2: Antimicrobial activity of aqueous extracts of leaves and stem-bark of *D. mespiliformis*

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

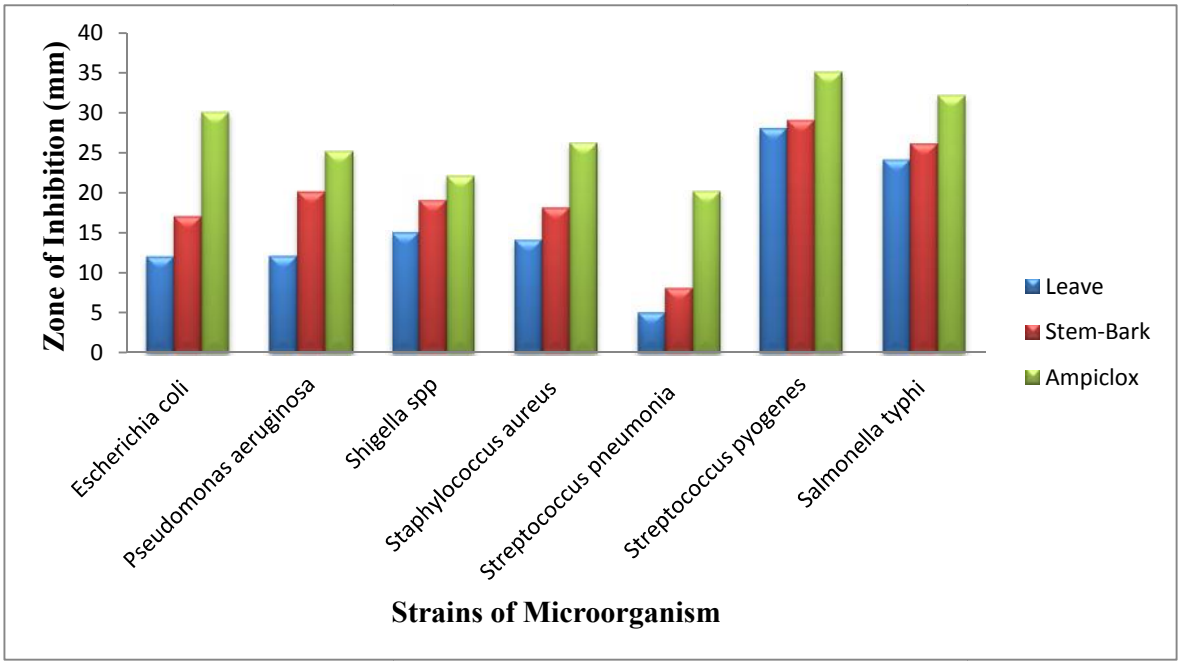

Fig. 1: Antimicrobial activity of aqueous extracts of leaves and stem-bark of *D. mespiliformis*

CONCLUSION

The result of study reveals that, the aqueous extracts of the leaves and stem-bark part of *D. mespiliformis* plant contains some major bioactive compounds that inhibit the growth of

the test microorganisms, thereby proving to have significant antimicrobial potential. The studies provided some biochemical basis of the medical use of the extracts from *D. mespiliformis* in the treatment of infectious diseases, particularly those causes by the test organism susceptible to the extracts. Further work on the identification of specific phytochemical from class of bioactive components identified in the leave and stem-bark extract of the plant is required in order to explore more enormous benefits of *D. mespiliformis* to mankind, especially with regard to the growing résistance of most pathogens to the activities of the common antibiotics. Other related pharmacological studies such as in vivo investigation, drug formulation and clinical trials are highly recommended.

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