

Full length Research Paper

Preliminary phytochemical investigation and antimicrobial evaluation of roots, stem-bark and leaves extracts of *Diospyros mespiliformis*

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The roots, stem-bark and leaves 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 studied. The Ethanol and water extracts of the afore mentioned parts of the plant were subjected to phytochemical screening and antimicrobial activity against *Shigella sp*, *Salmonella typhii*, *Escherichia coli*, *Streptococcus sp*, *Staphylococcus aureus* and *Klebsella pneumonia*. From the tests carried out, the result revealed the presence of saponins, tannins, volatile oils, alkaloids and phenols in both the leaves extract of the plant. The antimicrobial activity revealed that both the extracts of the plant parts were active against *Shigella sp*, *Escherichia coli*, and *Streptococcus sp*. This supports the claims of efficacy reported in folk use of the plant in the treatment of disease caused by some pathogens and if further purified can be used to source novel antibiotics.

Key words: *Diospyros mespiliformis*, drugs, shigella sp, alkaloids, dysentery

INTRODUCTION

Oxford Dictionary (2004) defined medicinal plants as a living thing that grows on the earth and usually has a stem, leaves and roots and has healing properties and usually used for healing. Plants parts, which have one or more of its organs containing substances that can be used for therapeutic purpose, are called medicinal plants (Sofowora, 1982). Plants have been used as medical agents from the earliest days of man's existence (Hansch, 1990; Shellard, 1987) and has made it necessary to study them in details in order to discriminate the kinds employed for different purposes (Ghani, 1986).

The exact number of medicinal plant is not yet known, but it quite obvious that the number is large (Kanabirank, 2006). Many of these medicinal plants were discovered by the end of 18th century although in the process some useful ones were eliminate i.e. those with toxics constituents which are taken in minute doses otherwise considered poisonous. The plants were brought to popular use in the nineteenth century, when active principle were first isolated, more efficient method of plants analysis have been developed and put to the use

effectively for the isolation and characterization of ever increasing number of medicinal substances from plant sources, with the technology advancement, these therefore, has led to the discoveries of large medicinal plants with medicinal constituents. (Shellard, 1979). For example, phenols are widely distributed in natural product, anthocyanins and polyhydric phenol have a strong reducing action (Godwin and Mercer, 1993). A number of unsaturated alcohols occur in volatile oils (Myers, 1982). Tannins act as chemical defense substance and for prevention and treatment of plastic and duodenal ulcer (Gruelack, 1973).

Plant materials have remained central to tradomedical practices and have remained useful sources of new drugs. Although orthodox medical practice is generally acceptable, alternative healthcare is still relied on all over the world (Ozulua and Alonge, 2008). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of compounds for pharmaceutical purposes has gradually increased in different parts of the world. According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed

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countries use drugs, which has compounds derived from medicinal plants, while 80% of developing countries rely directly on crude concoctions, infusions or decoctions of plants in traditional medicine for their health remedies. Ethnobotanical studies carried out throughout Africa confirm that indigenous plants are the main constituents of traditional African medicines (Sofowora, 1981; Adesina and Sofowora, 1992; Mann et al., 2008). Globally, only a small proportion, out of the several thousand plant species has been investigated both phytochemically and pharmacologically, when one considers that a single plant may contain up to thousands of constituents, the possibilities of making new discoveries become evident (Hostettmann et al., 1995; Bansa and Adeyemo, 2007). Plants should therefore be investigated for better understand of their properties, safety and efficiency (Nascimento et al., 2000). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatment.

Diospyros mespiliformis (Ebenaceae) is one of the most widely distributed African trees and it grows in Nigeria under wider range of condition than any other, this specie is widespread north up to Sahara (Keay, 1989). Some native name of *D. Mespiliformis* include; Hausa: Kanya, Fulan: Nelbi, Kanuri Bergem, Arabic: Jukham, Isala: Obiudu, Yoruba: Kanram.

D. mespiliformis commonly called African ebony or jackal tree is a tall, upright tree that can be of 25 m, with a trunk circumference of >5 m. Leaves are simple, alternate, leathery, and dark green. The fruit is a fleshy berry, with an enlarged calyx, orange when ripe. It is found to be growing in woodlands, Savannas, along river banks and it is also found in Ethiopia, North to South Swaziland, Sudan, Tanzania, Uganda, Zimbabwe, and Senegal. *D. mespiliformis* is confined to tropical and sub tropical regions notably in central Africa (Dalziel, 1935; Watt and Brandwijk, 1962). Fallen fruits are eaten by, among others, Kudu, Impala, Nyala and Jackal (Palgrave, 2002). The plant possess a number of medicinal uses; the leaves are used to treat fever, as wound dressings, and as antidote to variety of poisonous substances. The roots and bark are used to treat diseases such as malaria, syphilis, leprosy, stop purging. The antihelminthic and insecticidal properties have also been reported. The methanol extracts of stem and roots have also been reported to show broad spectrum of antibacterial activity (Esimone et al., 2009). Several ethnopharmacological applications have been reported for *D. mespiliformis*, which include the use of leaf decoction as extraordinary remedy for fever, whooping cough and for wounds (Dalziel, 1935; Adzu et al., 2002) anthelmintic and to facilitate delivery. Different parts of the tree are used against diarrhea, skin infections, headache, toothache and similar pains and as a psychopharmacological drug. (Watt and Brandwijk, 1962). A decoction of roots is ingested to get rid of internal

parasites such as worms. Extracts of various parts of the plants are believed to have antibiotic properties (Smith, 1996).

The plant of study is being used locally for the treatment of some disease such as dysentery, fever; it is also considered a remedy for leprosy. Thus, testing the extracts of this plant against these organisms known to be the causes of these diseases become vital, 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 sample of roots, stem-bark and leaves of the indigenous plant were collected from Ribadu District of Fufore Local Government Area of Adamawa State and were identified by Dr. C. A. Amadi of Forestry Department, School of Agriculture and Agricultural Technology, Modibbo Adama University of Technology, P.M.B. 2076, Yola, Nigeria. 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. The powdered samples were each collected into sterile cellophane bags and labelled to prevent mix up. The samples were kept in cool dry place till further use. The powdered samples were used for extraction purposes.

Extraction procedure

250ml of distilled water was added to 50g each of the powdered samples in a flask. Each of the soaked sample was stirred, sealed with aluminium foil and allowed to stand for 72 hrs. The content was then filtered with Whitman No. 1 filter paper. The filtrates were concentrated using rotary evaporator at 40°C. The extracts were stored in a universal bottle and refrigerated at 4°C prior to use.

The same procedure was followed with fresh samples as described above except that ethanol was used as solvent in place of distilled water.

Phytochemical screening

Phytochemical screening for major constituents was undertaken using standard qualitative method as described by (Odebiyi and Sofowora 1990) and (Fadeyi et al, 1989). Alkaloids, tannins, flavonoids, phenols, saponins and volatile oils tests were conducted in all the fractions. Results are shown in Table 1 and 2.

Table 1. Phytochemical analysis of ethanol extracts of roots, stem-bark and leaves of *Diospyros mespiliformis*

Bioactive compounds	Leaves extract	Stem- bark extract	Roots extract
Saponins	+++	+	–
Tannins	+++	+	+
Volatile oils	+	–	–
Alkaloids	++	++	–
Flavonoids	++	–	++
Phenols	++	–	+

+++ = highly present; ++ = present; + = fairly present; - = absent

Table 2. Phytochemical analysis of the water extract of roots, stem-bark and leaves of *Diospyros mespiliformis*.

Bioactive compounds	Leaves extract	Stem- bark extract	Roots extract
Saponins	+	+++	+++
Tannins	++	++	++
Volatile oils	++	–	+
Alkaloids	+	+	+++
Flavonoids	++	–	+
Phenols	+++	–	–

+++ = highly present; ++ = present; + = fairly present; - = absent

Antimicrobial investigation

Clinical isolates of *Salmonella typhi* (ST12011FMC), *Escherichia coli* (EC22011FMC), *Staphylococcus aureus* (SA32011FMC), *Streptococcus* spp (SS42011FMC), *Shingella* sp (SS52011FMC) and *Klebsiella pneumonia* (KP62011FMC) were obtained from the Microbiology laboratory of Federal Medical Centre, Yola.

The stocks were maintaining on nutrient agar slant and sub-culture in nutrient both for incubation at 37°C 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 what-man 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 (Fatope, 1993). The plates were then turned upside-down and inoculate at 37°C for 24 hours in an incubator. After incubation, the inoculated plates were observed for zones of inhibition (in mm diameter).

The result was taken by considering the zone of growth and inhibition of the organisms by the test fractions (Mackie and McCartney, 1989). Activity and inactivity were observed in accordance with the standard and

acceptable method. Results are shown in Table 2 and 3.

RESULTS AND DISCUSSION

From the results obtained, phytochemical analysis revealed that most of the bioactive compounds tested for were present in both ethanol extracts and water extracts: tannins, saponins, alkaloids, flavonoids, Volatile oils and phenols were present but volatile oils, flavonoids and phenols were absent in the stem- bark of the ethanol extract also saponins, volatile oils, and alkaloids were absent in the root of the ethanol extracts. Similarly volatile oils, flavonoids, and phenols were absent in the stem-bark of the water extracts. Phenols are also absent in the root of the water extracts. This shows the generality of the component in the medicinal plants. To large extent, the age of the plant, percentage humidity, climatic condition, soil condition, geographical location, time of harvesting and method of extraction are possible for the variation in chemical composition.

Table 3 and 4, shows the zone of inhibition (mm) of the various plant parts extracts against the microorganisms, both showed antimicrobial activity against *Shigalla* sp, *Escherichia, coli* and *Streptococcus spp* respectively but did not show any activities against *Salmonella typhi* and *Klebsiella pneumonia* with the water extracts.

Table 3. Antimicrobial activity of ethanol extracts of roots, stem- bark, and leaves of *Diospyros mespiliformis*.

Test organisms	Diameter of zone of inhibition (mm) of the various plant parts extracts on different isolate			Ampicillin (control)
	Roots	Stem- bark	Leaves	
<i>Escherichia coli</i>	8	11	4	25
<i>Streptococcus spp</i>	8	14	16	25
<i>Shigella sp</i>	16	9	10	24
<i>Salmonella typhi</i>	R	R	R	21
<i>Staphylococcus aureus</i>	R	R	4	20
<i>Klebsilla pneumonia</i>	R	R	R	26

R=Resistance

Table 4. Antimicrobial activity of water extracts of roots, stem-bark and leaves of *Diospyros mespiliformis*.

Test organisms	Diameter of zone of inhibition of the various plant parts extracts on different isolate			Ampicillin (control)
	Roots	Stem- bark	leaves	
<i>Escherichia coli</i>	7	6	4	25
<i>Streptococcus spp</i>	7	9	5	25
<i>Shigella</i>	9	7	8	24
<i>Salmonella typhi</i>	R	R	R	21
<i>Staphylococcus aureus</i>	R	R	4	20
<i>Klebsilla</i>	R	R	3	26

R = Resistance

Staphylococcus aureus was sensitive to only leaves extract of the ethanol. Infact both the methanolic extract and water extract of *Diospyros mespiliformis* shown significant antimicrobial activity against the tested microorganism. Especially in the case of *Shigella sp* and *Streptococcus spp* and this imply it efficiency in the treatment of dysentery and remedy for leprosy.

This study has provided some biochemical basis of the medical use of the extracts from *Diospyros mespiliformis* in the treatment of infection, as a potential source of useful drugs and phytochemicals.

Identification of specific phytochemical from class of bioactive agent found in the plant is therefore crucial towards exploiting the enormous benefits of *Diospyros mespiliformis* to mankind especially with regard to the growing resistance of most pathogens to the activities of the common antimicrobial.

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