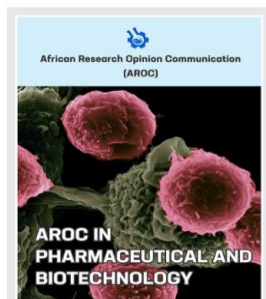




RESEARCH ARTICLE

Phytochemical and safety evaluations of *Diospyros mespiliformis*, and *in silico* evaluations of drug-likeness, pharmacokinetics and acute toxicity of its bioactive compound (Diospyrin)

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ABSTRACT

Diospyros mespiliformis is among the popular multipurpose tropical fruit trees, commonly used as herbal medicines. However, due to the lack of adequate scientific data on the safety of this plant, the present study was conducted to determine the phytochemical compositions and acute toxicity profile of the crude methanol extract of *D. mespiliformis*. In addition, diospyrin, a bioactive compound from the plant was evaluated for *in silico* drug-likeness, pharmacokinetics (PKs) and acute toxicity. The phytochemical contents of the plant were quantified using standardized protocols while the 50% lethal dose (LD₅₀) was evaluated using Lorke's methods. Results revealed that flavonoids (265.46±0.32 mg/g) are the most abundant phytochemical in methanol leaf extract of *D. mespiliformis*, followed by alkaloids (224.56±0.19 mg/g) and phenols (191.82±0.04 mg/g) while saponins (7.90±0.32 mg/g) was the least abundant phytochemical. The plant extract has LD₅₀ of > 5000 mg/kg in rats. No death was recorded throughout the study period. Similarly, no behavioural changes were observed in animals dosed with the crude extract at 10 -2900 mg/kg BW. Animals administered 5000 mg/kg BW were hyperactive, restless, and displayed profused breathing which lasted only for 30 minutes after administrations. Diospyrin a bioactive compound from *D. mespiliformis* demonstrated good druglike candidates and exhibited a high safety profile as revealed by *in silico* study. In conclusion, the crude methanol extract of *D. mespiliformis* and its bioactive compound is well-tolerated and non-toxic to rats, and thus could be considered a safe medicinal plant for acute oral remedies.

Keywords: *Diospyros mespiliformis*; *in silico* stud; *in vivo* study; phytochemical, acute toxicity;

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1.0 Introduction

Medicinal plants are commonly used in developing countries for the treatment of various diseases, this practice being an alternative way to compensate for some perceived deficiencies in conventional pharmacotherapy [1].

Medicinal plants have increasingly become an integral part of human society with regards to their therapeutic uses. Thus, phytomedicine research is now being promoted, as shown by the resolutions and recommendations given by the World Health Organization, which advocates the application of

scientific criteria and methods for proof of safety and efficacy of medicinal plants [2]. Acute toxicity test is a test in which a single dose of the drug is used in each animal on one occasion only for the determination of gross behaviour and LD₅₀ (the dose which has proved to be lethal (causing death to 50% of the tested group of animals) [3]. It is usually the first step in the assessment and evaluation of the toxic characteristics of a substance. It is an initial assessment of toxic manifestations, providing information on health hazards likely to arise from short-term exposure to drugs [4]

The LD₅₀ for a particular substance is the amount that can be expected to cause death in half (i.e. 50%) of a group of some particular animal species, usually rats or mice when administered by a particular route [5]. It is usually expressed as the amount of chemical administered (e.g. Milligrams) per 100 g (for small animals) or per kilogram (for bigger subjects) of the bodyweight of the test animal [6]. LD₅₀ obtained at the end of a study is reported in relation to the route of administration of the test substance e.g. LD50 (oral), LD50 (dermal) etc. The most frequently performed lethal study is the oral LD50. Generally, the smaller the LD50 value, the more toxic the substance is and vice versa [7]

Diospyros mespiliformis Hoschst ex A. DC commonly known as African ebony is a large deciduous tree belonging to the family *Ebenaceae* in the order *Ebenales* found mostly in Tropical and Sub Saharan Africa [8]. It is a tall tree that grows up to 25 meters in height. It has a dense evergreen canopy [9]. *D. mespiliformis* has been used in Traditional Medical systems including Ayurveda, Chinese and African [10]. The plant is widely used in parts of Africa and a number of chemical constituents of therapeutic importance have been isolated [11]. *D. mespiliformis* has a large range of medicinal uses [12-15]. A traditional food plant in Africa, the fruit has the potential to improve nutrition. It is used as astringent, febrifuge, hemostatic, mild laxative, stimulant and vermifuge and to facilitate childbirth [16].

According to the literature review, *D. mespiliformis* has numerous pharmacological activities including; anticancer, analgesic, anti-inflammatory, hypoglycemic, antiparasmodial and anti-oxidant [17]. Since *D. mespiliformis* is used for the treatment of many diseases in traditional medicinal systems, hence it is important to evaluate its phytochemical compositions and acute toxicity. However, due to the side effect demonstrated by some medicinal plants, World Health Organization has recommended that safety evaluation should be the overriding criterion for the selection of medicinal plants for therapeutic purposes [2].

The aim of the present study is to evaluate the phytochemical compositions, in silico and in vivo safety profile of *Diospyros mespiliformis* and its bioactive compound, Diospyrin, upon acute administration to rats. The present study is therefore justifiable as it will provide us with

valuable scientific evidence on the safety profiles of *D. mespiliformis* upon acute administration

2.0 Materials and Methods

2.1 Collection and identification of plant material

The leaves of *D. mespiliformis* were collected from the Kacha Local Government area of Niger State, Nigeria. The plant was identified and authenticated at the Department of Plant Biology, Federal University of Technology, Minna.

2.2 Experimental animals

A total of twenty-one (21) rats weighing 125.65 ± 3.89 g were obtained from Animal Holding Unit, Department of Biochemistry, Federal University of Technology Minna, Nigeria. They were housed in clean cages with wood shavings as beddings under standard environmental conditions of temperature and relative humidity, 12 hrs daylight/night cycle) with access to commercial feed pellets (growers) and water *ad libitum*. The cages were cleaned regularly throughout the experimental periods (their beddings were changed every two days). Animals were kept in compliance with internationally accepted principles for human handling and use of laboratory animals in the Canadian Council on Animal Care Guidelines and Protocol Review [18].

2.3 Preparation and Extraction of plant material

The carpels were rinsed under clean running water and air-dried for four weeks in the laboratory. The dried carpels were pulverized into a coarse powder with mortar and pestle, milled into fine powder with an electric miller and stored in a clean container till ready for use. Three hundred grams (300 g) of powdered *D. mespiliformis* leaf was weighed into a reflux flask (100 g per turn), 2.5 liters of methanol was used in succession and the extraction step was exhaustively carried out for two hours with reflux extractor. The mixture was sequentially filtered with chess cloth and Whatman's paper (No.1). The final filtrate was first concentrated in a rotary evaporator and then later in a water bath at 65°C. The dried extract was stored in a sample bottle in the refrigerator at 4°C.

2.4 Analysis of Phytochemical compositions

The total flavonoids contents of the crude extract were estimated using a spectrophotometer based

on the formation of a flavanoid-aluminium complex that absorbs maximally at 415 nm [19]. Total phenol was estimated using the Folin-Ciocalteu reagent protocol [20]. The total alkaloids were quantitatively estimated spectrophotometrically at 565 nm using vincristine as standard [21]. The tannin content was determined using the Folin Denis reagent. The amount of tannin was calculated as tannic acid equivalent from the standard curve [22]. A gravimetric method of AOAC [23], was used for saponin determination in the samples.

2.5 In vivo Acute toxicity

The acute toxicity of the extract was determined in 2 phases according to Lorke's [24]. In Phase 1, a total of 9 rats were grouped into 3 of three (3) rats each and were given a single dose of 10, 100 and 1000 mg/kg BW of the fractions respectively. A control group was also set up comprising of 3 rats and was given 2 ml/kg BW normal saline. The absence of death after 24 hours of extract administration led to the initiation of Phase II which was set up with another 3 groups of 3 rats each and were given a single dose of 1600, 2900 and 5000 mg/kg BW of the fractions respectively. All fractions were administered orally using an oesophageal cannula. The rats were observed for any adverse effect and mortality within 24 hours of treatment and after a week.

2.6 In silico evaluation of the drug-likeness, pharmacokinetics (PKs), acute toxicity, of diospyrin

The canonical smile of the compound CC1=CC2=C(C(=C1)O)C(=O)C=C(C2=O)C3=C(C4=C(C=C3C)C(=O)C=CC4=O)O was obtained from the PubChem (<https://pubchem.ncbi.nlm.nih.gov/compound/308140>) with ID 308140. The in silico analysis was conducted according to the protocols reported in previous studies [25-27].

Briefly, We used the SwissADME software [28], and computer-aided Prediction of Biological Activity Spectra (PASS) web resources (<http://way2drug.com/dr>) [29] to analyse the drug-likeness, PKs, medicinal chemistry, and toxicity of the compound. We used the blood-brain barrier (BBB) Prediction Server (<https://www.cbligand.org/BBB/>) to analyze the BBB-permeation ability of the compound [30] In addition, we also used the Brain Or Intestinal Estimated permeation method (BOILED-Egg)

model [31] to further analyze the brain- and intestinal-permeation abilities of the compounds based on their lipophilicity and polarity.

2.7 Data analysis

All analysis was conducted in triplicate and analyzed using statistical package for social science (SPSS) version 16 and presented as means $\pm$ standard error of the mean. One-way analysis of variance (ANOVA) at $p < 0.05$ were used for comparing the significant differences between treatment groups ($p < 0.05$).

3.0 Results

3.1 Phytochemical composition

The qualitative phytochemical composition of *D. mespiliformis* is shown in table 1. The crude methanol leaf extract of *D. mespiliformis* was found to contain alkaloids, flavonoids, tannins, phenols, saponins, anthraquinone, steroids and phlobatannins. Quantitatively, flavonoids (265.46 ± 0.32 mg/g) is the most abundant phytochemical in methanol leaf extract of *D. mespiliformis*, followed by alkaloids (224.56 ± 0.19 mg/g) and phenols (191.82 ± 0.04 mg/g) while saponins (17.90 ± 0.32 mg/g) was the least abundant phytochemicals (Table 2).

Table 1: Qualitative phytochemical composition of crude methanol leaf extract of *Diospyros mespiliformis*

Phytochemicals	Inference
Alkaloids	+
Total Flavonoids	+
Total Phenols	+
Saponins	+
Tannins	+
Anthraquinone	+
Phlobatannins	+
Steroids	+

+; presence

Table 2: Quantitative Phytochemical composition of crude methanol leaf extract of *Diospyros mespiliformis*

Phytochemicals	Quantity (mg/g)
Phenol	191.82 ± 0.04^c
Flavonoids	265.46 ± 0.32^e
Tannins	23.46 ± 0.04^b
Saponins	17.90 ± 0.32^a
Alkaloids	224.56 ± 0.19^d

3.2 Acute oral toxicity of crude methanol leaf extract of *D. mespiliformis* in rats

The LD₅₀ of the crude methanol leaf extract of *D. mespiliformis* in rats were > 5000 mg/kg BW. No death was recorded throughout the study period. Animals administered 2900 and 5000 mg/kg BW of the crude methanol leaf extract of *D. mespiliformis* showed some behavioural changes including; hair erection, accelerated heart rate, hyperactivity, but no death was recorded (Table 3)

3.3 Bioactive compound from *D. mespiliformis*

Diospyrin (IUPAC Name: 5-hydroxy-6-(5-hydroxy-7-methyl-1,4-dioxonaphthalen-2-yl)-7-

methylnaphthalene-1,4-dione) (figure 1) is a bioactive compound isolated from *D. mespiliformis*. We evaluated the pharmacokinetics, drug-likeness and medicinal chemistry friendliness of the diospyrin using the SwissADME algorithm. Interestingly we found that the compound is a good druglike candidates that satisfies all the Lipinski, Ghose, Veber, Egan, and Muegge rules of drug-likeness. It has a good bioavailability score (0.55) and good synthetic accessibility (3.57) (table 4). Furthermore, it is highly absorbed by the gastrointestinal tract (GIT) but is poor BBB permeant. The detailed drug-likeness, pharmacokinetics (PKs) and physicochemical properties of diospyrin are presented in Table 4

Table 3: Acute oral toxicity of crude methanol leaf extract of *Diospyros mespiliformis*

Dosage (mg/kgbw)	Mortality	Sign of Toxicity
Phase1		
10	0/3	Normal behaviour of rats after gavage
100	0/3	Normal behaviour of rats after gavage
1000	0/3	Normal behaviour of rats after gavage
Phase2		
1600	0/3	Normal behaviour of rats after gavage
2900	0/3	Hair straightening, Drowsiness, No death
5000	0/3	Hair Straightening, Accelerated heart rate, Drowsiness, Slow activity, No death

LD₅₀=> 5000 mg/kg bw

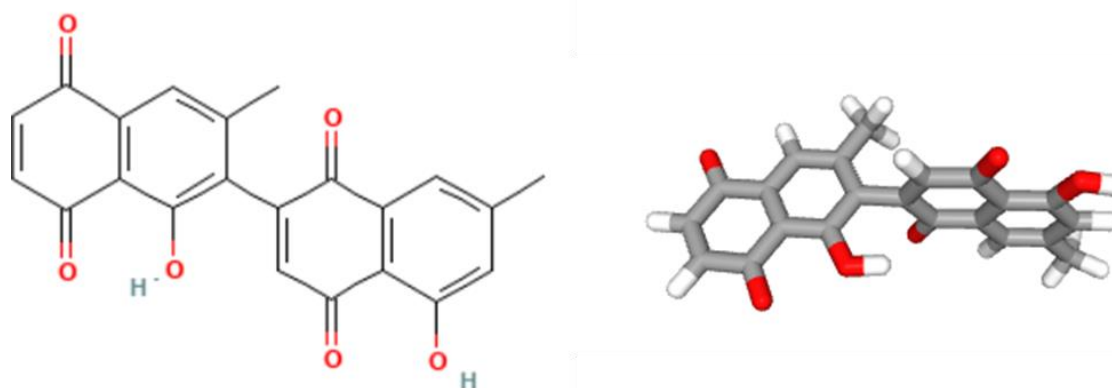


Figure 1: The two (2) Dimensional and three (3) Dimensional Structures of diospyrin

Table 4: Drug likeness, pharmacokinetics (PKs), and physicochemical properties of diospyrin

Physicochemical Properties	
Formula	C ₂₂ H ₁₄ O ₆
Molecular weight	374.34 g/mol
Num. heavy atoms	28
Num. arom. heavy atoms	12
Fraction Csp ³	0.09
Num. rotatable bonds	1
Num. H-bond acceptors	6
Num. H-bond donors	2
Molar Refractivity	101.30
TPSA	108.74 Å ²
Lipophilicity	
Log P _{o/w} (iLOGP)	2.53
Log P _{o/w} (XLOGP3)	3.73
Log P _{o/w} (WLOGP)	3.11
Log P _{o/w} (MLOGP)	0.62
Log P _{o/w} (SILICOS-IT)	4.21
Consensus Log P _{o/w}	2.84
Water Solubility	
Log S (ESOL)	-4.76
Solubility	6.48e-03 mg/ml ; 1.73e-05 mol/l
Class	Moderately soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K _p (skin permeation)	-5.94 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55
Medicinal Chemistry	
Synthetic accessibility	3.57

3.4 *In silico* assays for BBB permeability and acute rat toxicity of diospyrin

The BBB analysis revealed that diospyrin has a poor BBB permeability score of 0.0005 (figure 2) on a BBB permeant threshold of 0.02, suggesting that diospyrin is a poor BBB permeant compound (figure 2). QSAR modelling of acute toxicity in rats revealed that diospyrin had a very high LD₅₀ value in different routes of administration (Table 5), suggesting a high safety profile of the compound. In addition, diospyrin demonstrated high

environmental safety as measured by the bioaccumulation factor, *Daphnia magna*, fathead minnow, and *Tetrahymena pyriformis*.

Table 5: *In silico* Acute rodent toxicity assays for diospyrin

LD₅₀			
Route of administration	Log10 (mmol/kg)	mg/kg	OECD classification
Intraperitoneal (i.p)	0.654	1698,000	Non Toxic
Intravenous (i.v)	-0.299	189,300	Class 4
Oral gavage (o.p)	0.609	1529,000	Class 4
Subcutaneous (s.c)	0.345	833,700	Class 4
Environmental Toxicity			
Bioaccumulation factor Log10(BCF)			1.397
Daphnia magna LC ₅₀ -Log10(mol/L)			5,655
Fathead Minnow LC ₅₀ Log10(mmol/L)			-3,284
Tetrahymena pyriformis IGC50 - Log10(mol/L)			2,100

The toxicity classification was based on the acute rodent toxicity classification of chemicals by the OECD Project. LD₅₀, 50% lethal dose.

4.0 Discussion

Plant extracts contain different phytochemicals with various biological activities that can be of value in both medical and veterinary practice. Different plant extracts may therefore contain different phytochemicals each of which has its biological activity [32]. In this study, phytochemical screening of *D. mespiliformis* revealed that the extract contains very important phytochemicals of therapeutic application. Previous phytochemical screening of *D. mespiliformis* also revealed the presence of several secondary metabolites namely; anthraquinones, tannins, triterpenes, saponins, steroids [12], triterpenes and naphthoquinones [33]. Shagal *et al.* [34] also revealed the presence of saponins, tannins, volatile oils, alkaloids and phenols. *D. mespiliformis* is used in traditional medicine as a diuretic mild laxative treatment for cardiac and nerve diseases, cancer, hypertension, hypolipidemic effect [13] and has promising activities against atherosclerosis, liver disease, cancer, diabetes, antimicrobial [12], antimalarial [15]. The medicinal properties of this plant may be in connection with the bioactive constituents including alkaloids, flavonoids, saponins, tannins, anthocyanins, anthraquinone, terpenoids and glycoside that were identified in the plant extract.

Flavonoids have been known to have antibacterial, antifungal, antiviral, antioxidant and hepatoprotective properties activity [35]. Tannins precipitate proteins of the wound, forming a

protective layer on the wound, thus assisting in the arrest of bleeding and therefore promoting wound healings activity [20].

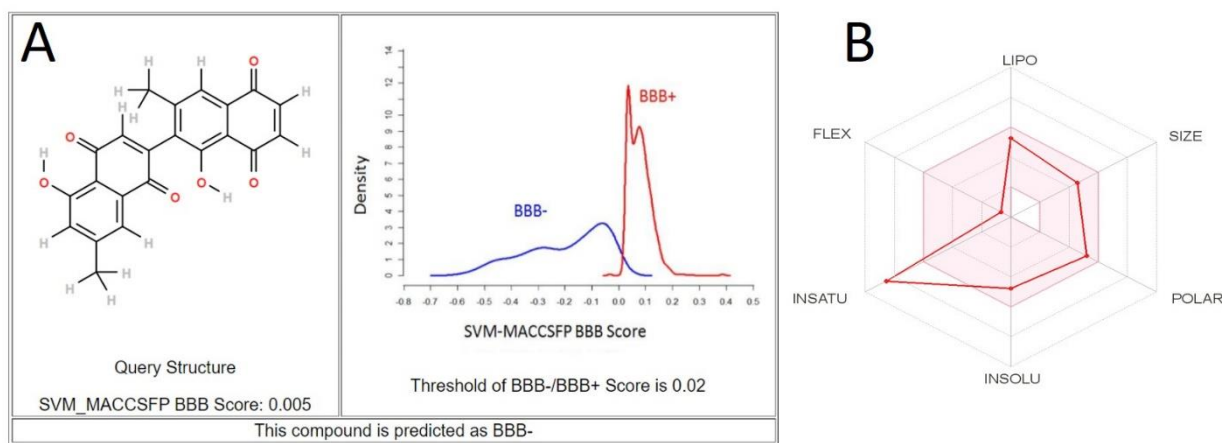


Figure 2: The blood-brain barrier (BBB) (A) and bioavailability radar (B) of diospyrin

Tannins have also been reported to be used in the treatment of diarrhoea 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 [36]. Saponins have been linked with decreased cholesterolemia. They are also thought to aid in the absorption of calcium [37]. The plant extract therefore could serve as a candidate extract for management or prevention of atherosclerosis which is a condition precipitated by cholesterol. The presence of the above phytochemicals in *D. mespiliformis* is an indication that this plant if properly screened could yield a drug of pharmacological importance.

Investigation of acute toxicity is the mainstay in the toxicological investigation of unknown substances. In the present study, acute toxicity testing was carried out in order to ascertain the LD₅₀ which helps in apportioning the dose at which no mortality or lethal effect of the extract is observed [5]. The results obtained from the acute toxicity study showed that the extract of *D. mespiliformis* demonstrated a high degree of safety since the animals tolerated up to 5000 mg/kg body weight of the extracts with no mortality recorded and this adhered to the guideline by the chemical labelling and classification of acute systemic toxicity based on oral LD₅₀ value which was recommended by the Organization for Economic Co-operation and

Development (OECD) [38]. Therefore, the high safety attributes of this extract through oral administration justifies the widespread use of this plant by traditional healers. According to the Guidelines recommended by OECD on the acute oral toxicity testing based on LD₅₀, *D. mespiliformis* extract could be assigned to the class 5 (LD₅₀ > 2000 mg/kg body weight), as designated to be the lowest toxicity class. This is, however, contrary to the study of Luka *et al.* [16] who reported that the LD₅₀ of 570mg/kg for *D. mespiliformis*.

The BBB is a limiting factor in the therapeutic effects of most drugs [25,39]. Unfortunately, the in silico studies indicated that diospyrin is not a BBB permeant molecule and hence cannot be used for the treatment of brain-related diseases, however, the good druglike properties and the low toxicity profile of diospyrin suggest that the compound possesses good translational relevance for the treatment of several other diseases.

5.0 Conclusions

In conclusion, the crude methanol extract of *Diospyros mespiliformis* contains a significant amount of phytochemicals that could be responsible for the biological activities of the plants as reported in the literature. The plant is well tolerated and non-toxic to the experimental rats upon acute administration. Diospyrin a bioactive compound

from *D. mespiliformis* demonstrated good druglike candidates and exhibited a high safety profile as revealed by in silico study. Thus, *D. mespiliformis* could be considered a safe medicinal plant for acute oral remedies.

Competing interests

The authors declared no conflict of interest exist

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