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PHYTOCHEMICAL SCREENING AND ANTIPROLIFERATIVE EFFECTS OF METHANOL EXTRACT OF STEM BARK OF *Diospyros mespiliformis* Hochst (EBENACEAE) AGAINST GUINEA CORN (*Sorghum bicolor*) SEEDS RADICLES LENGTH

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ABSTRACT
The plant Diospyros mespiliformis Hochst (Ebenaceae) is commonly known as Kanya in Northern Nigeria which has been used widely in treating various ailments such as fever, whooping cough, wounds. Malaria, Pneumonia, Syphilis, Leprosy, Dermatomycoses, Diarrhea without scientific validation. Preliminary Phytochemical screening. Thin layer chromatographic profile (TLC) of methanol crude extract and antiproliferative studies were carried out in this research. Phytochemical screening revealed the presence of carbohydrate, glycoside, anthraquinone, steroid, triterpenes, saponin, tannins, flavonoids and alkaloid. TLC profile of the crude extract gave four sports with good R_f values. Antiproliferative evaluation were carried out using Guinea corn (Sorghum bicolour) seeds spread in a 9 cm wide petridish laid with cotton wool and Whatman filter paper which was treated with 1-30 mg/ml of methanol extract in 24-96 hr period of incubation. At 24 hrs of incubation, the methanol extracts had 24.771 ± 0.526 mm length of growth for the controls whereas the seeds treated with 10, 20, and 30 mg/ml of the extract produced a length total of 2.772 ± 0.494 mm, 2.150 ± 0.490 mm and 2.257 ± 0.489 mm respectively, while at the end of 96 hours of incubation period, the radicles length of the control seeds measured 93.77 ± 9.730 mm while those treated with 10, 20, and 30 mg/ml were observed to be 37 ± 3.297 mm, 17.023 ± 2.802 mm and 16.086 ± 1.976 mm. This reduction in the growth implied 60.54, 81.87 and 82.83% respectively compared to the controls. This study has scientifically justified the traditional uses of Diospyros mespiliformis stem bark extracts asantiproliferative agent against radicles of a Guinea corn (Sorghum bicolour) which may relate to its use as anticancer agent.
Keywords: Antiproliferative effects, Diospyros mespiliformis, Phytochemical

INTRODUCTION

Diospyros mespiliformis Hochst (Ebenaceae) has a good mutualism and symbiotic network with many living organisms, from human beings to small insects. There is a complex ecological system revolving around this tree. It is one of the savanna giants that can live for more than 200 years. It is a tall, upright tree that can reach a height of 25 m, with a trunk circumference of more than 5 m. It has a dense evergreen canopy(Belemtougri *et al*., 2006). The bark is black to grey, with a rough texture. The fresh inner skin of the bark is reddish. Leaves are simple, alternate, leathery and dark green. The margin is smooth and new leaves in spring are red, especially in young plants. Flowers are cream-coloured and bell-shaped(Belemtougri *et al*., 2006). Among Hausa Fulani people of Jigawa in Nigeria, *Diospyros mespiliformis* Hochst (Ebenaceae) happens to be one of such plant used in the treatment of tumor-related disease. In local Nigerian language (*Hausa*) the plant is known as *Kanya*. This plant is not used only to treat tumor related disease but also to treat diseases such as fever, malaria, pneumonia, syphilis, leprosy, dermatomycoses (Mohamed *et al*.,2009). It is also used in the treatment of diarrhea, whooping cough, wounds(Adzu *et al*., 2002). This studies were designed to test the inhibitory effects of methanol crude extract against tumor related cells. However a model was designed to use by (Ayinde andAgbakwuru 2010), where cancer cell lines are not available in order to mimic the situation.Phytochemical screening which revealed the presence of carbohydrate, glycoside, anthraquinone, steroid, triterpenes, saponin, tannins, flavonoids and alkaloid. Thin layer chromatographic profile of crude extract and antiproliferative studies were carried out in this research.

MATERIAL AND METHODS

Collection and authentication of plant material

The plant sample for the study were collected from Ringim local government Area of Jigawa State, Nigeria in January 2013. It was authenticated by a Taxonomist of Biological Sciences Department, Ahmadu Bello University, Zaria with voucher specimen number 901431.

Extraction of plant materials

About 200g of powdered plant material were macerated in a separating funnel with 600ml of Methanol for one day (24hr) at room temperature with occasional shaking. The content was then filtered with cotton plug. The filtrate were then concentrated to dryness using a water bath at 60^0C.

Preliminary Phytochemical Studies

This procedure was carried out on the Methanol extracts according to Harboune (1973), Brain and Turner (1975), Evans (2008) and Sofowora (2008) as outlined below.

Molisch test; to 2 ml of the extract in a test tube, few drops of Molisch reagent and sulphuric acid was added and the colour reactions was recorded.

Fehling test; 5 ml of Fehling solution A and B was added to 2 ml of the extract in a test tube and the colour reaction was recorded. This was passed over hot water bath for 15 minutes and the result was also observed and recorded.

Ferric chloride test; 0.5 ml of the extracts were dissolved in 10 ml of water each and filtered. Few drops of ferric chloride were added to the filtrate and the colour reaction was observed and recorded.

Lead sub-acetate test; 3 drops of lead sub-acetate solution was added to the extract solution and reaction was observed and recorded.

Frothing test; about 2 ml of the extract was dissolved in 10 ml of water and shaken vigorously for 30 seconds and allowed to stand for 30 minutes before observing and recording the reaction.

Lieberman-Burchard test; 1 ml of acetic anhydride was added to 1 ml of the extract. Few drops of sulphuric acid were carefully then added to the solution above and the reaction was observed and recorded.

Salkowski test; 2 ml of chloroform and few drops of sulphuric acid were added to about 2 ml of the extract and the reaction was observed and recorded.

Bontrager test; 2 ml of the extracts was added to 10 ml of benzene and shaken. This was then filtered and 5 ml of 10% ammonia solution was added to the filtrate and stirred and the reaction was observed and recorded.

Shinoda test; about 0.5 g of the extract was dissolved in 2 ml of 50% methanol. Few drops of magnesium fillings and 3 drops of hydrochloric acid were added and the reaction observed and recorded.

Sodium hydroxide test; few drops of sodium hydroxide was added to 5 ml of the extract and the reaction was observed and recorded.

Keller-Killiani test; 2 ml of the extract was dissolved in glacial acetic acid containing ferric chloride and 1 ml of sulphuric acid was added to the solution. The reaction were observed and recorded.

Test for alkaloids; Mayers reagent, Wagners reagent and Drangendoff reagent were added to different test tubes containing the extract solution and each of the reaction was observed and recorded.

Experimental Material (*Sorghum bicolor*)

Guinea corn (*Sorghum bicolor*)were obtained from the local market of Samaru Zaria which was cleaned with absolute alcohol after which the seeds were dried before use. The seeds viability was determined by their ability to remain submerged in water. Those that have remained submerged in water were chose and dried for use in accordance to Ayinde and Agbakwuru (2010).

Determination of growth inhibitory effects of Methanol extracts on guinea corn Seeds radicles length.

About 10ml of 1 – 30 mg/ml of each of the extracts dissolved in 5% dimethyl sulphoxide in water was poured in to 9cm wide petri dishes laid with cotton wool and filter paper (Whatman no.1). Twenty (20) viable seeds were spread on each plate and incubated in dark environment. The lengths (mm) of radicles emerging from the seeds were taken at 24, 48, 72 and 96 hr. The control seeds were treated with distilled water containing no extracts. The experiment were carried out in triplicates, (Ayinde and Agbakwuru, 2010).

Statistical Analysis

All data were expressed as mean±SEM and one way Analysis of Variance (Anova) statistical test using SAS Version to test the significance. $P<0.05$ was considered Significance.

RESULTS AND DISCUSSION

The 200g of the powdered stem bark of *D. mespiliformis* were observed to have yielded 15.83% (w/w). The stem bark of *D. mespiliformis* was observed in this work to contain Alkaloids, Flavonoids, Saponins, Steroids and triterpenes, Tannins and Anthraquinones, which are likely to be some of the constituents that contributes to the plants uses in ethnomedicine. The activities of plant extracts in effecting any therapeutic or biological changes in ailing of animals suffering from diseases or living tissues are direct functions of the chemical constituents inherently present in them,(Ayinde and Agbakwuru, 2010). In this research, Methanol extract were tested for the inhibitory effect on the seeds radicles of the Guinea corn (*Sorghum bicolour*). These problems have led to the establishment of various methods which have been reportedly used by scientists as indicators of potentially promising anti-tumour phytochemicals (Shogbaike *et al.,* 2002: Obuotor and Onajobi, 2000) and Mclaughlin *et al*., 1999), used radicles of germinating seeds. This method is of tremendous value is simple, rapid, reproducible, time and material saving. It is an experimental procedure which can be carried out in laboratories where appropriate human cells lines are not available. This method can be used to test and evaluate many medicinal plants that may be claimed to treat tumor related diseases. In this research, at 24 hours, the stem bark extract had 4.771 ± 0.526 mm for the controls whereas the seeds treated with 10, 20, and 30 mg/ml of the extract produced a length total of 2.772 ± 0.494 mm, 2.150 ± 0.490 mm and 2.257 ± 0.489 mm respectively as seen in Fig. 2. Above. At the end of 96 hours of incubation period, the radicles length of the control seeds measured 93.77 ± 9.730 mm, while those treated with 10, 20, and 30 mg/ml were observed to be 37 ± 3.297 mm, 17.023 ± 2.802 mm and 16.086 ± 1.976 mm respectively as shown in the fig. below.This reduction in the growth implied 60.54, 81.87 and 82.83% respectively compared to the controls as shown in Fig.2 above. This activity might be due to genera relationship between this plant and other species in the same genus as in the *D. undulata* have found to possess the antiproliferative activity against various cancer cells (Rungrojtrakool *et al*., 2012). Another species e.g *D. peregrine* hasfound to possess antitumor and anti-inflammatory activity (Gopal *et al*., 2011).

Table 1: Phytochemical Studies of *Diospyros mespiliformis* Stem Bark which reveals the presence of carbohydrate, glycoside, anthraquinone, steroid, triterpenes, saponin, tannins, flavonoids and alkaloid

Test	Inference
Test for carbohydrate	
Molisch test	+
Test for tannins	
Ferric chloride test	+
Lead sub-acetate test	+
Test for saponins	
Frothing test	+
Test for sterols	
Salkowski test	+
Test for triterpenes	
Liebermann-burchard test	+
Test for anthraquinones	
Bontragers test	+
Test for flavonoids	
Shinoda test	+
Sodium hydroxide test +	
Test for deoxy sugars	
Keller-kellani test	+
Test for alkaloids	
Drangendoff's reagent	+
Wagners reagent	+
Mayers reagent	+

Key: (+) indicates presence of secondary metabolites

Figure 1: Chromatogram of Methanol extract developed in Chloroform:Methanol (4:1)sprayed with Anisaldehyde/ H_2SO_4.

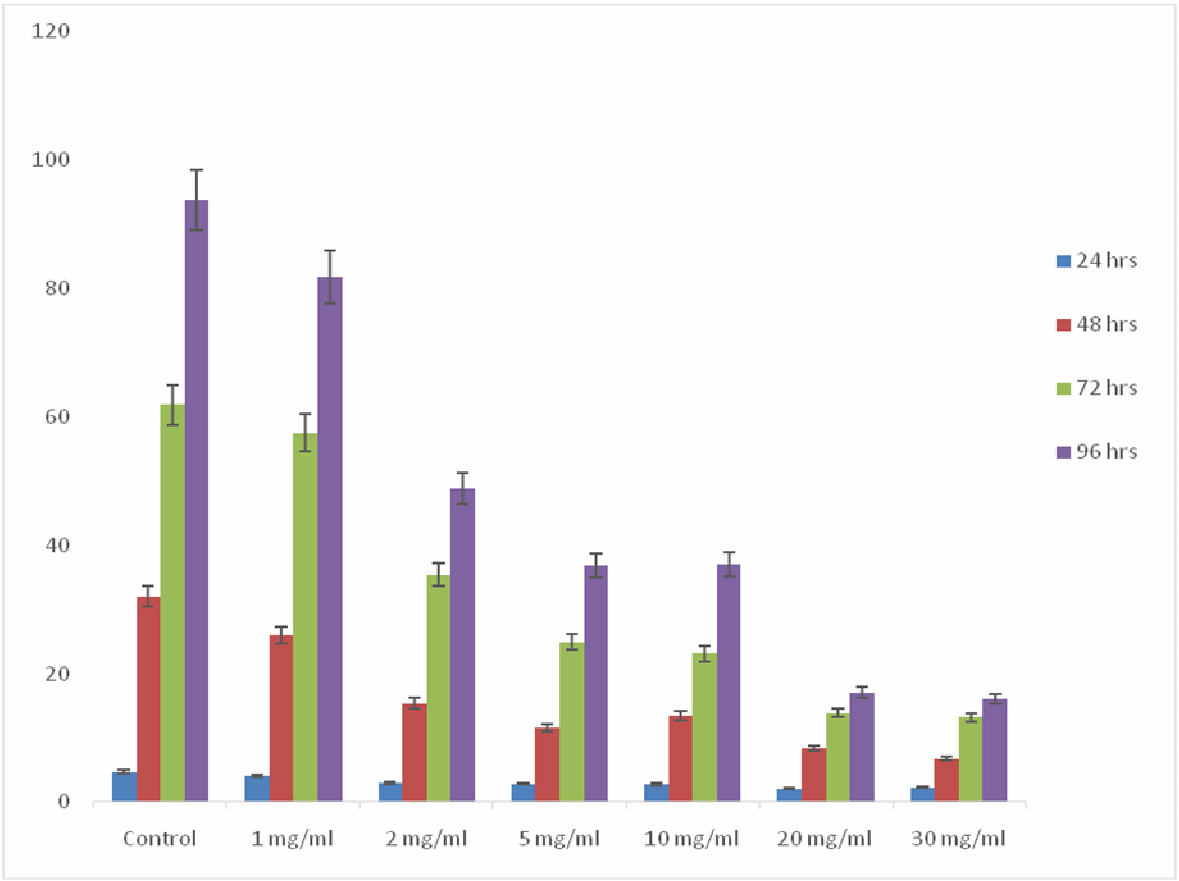

Figure 2: AntiproliferativeEffects of the Methonol Extract of the *D. mespiliformis* on seeds radicles length of Guinea corn (*Sorghum bicolor).*

CONCLUSION

This study has justified the traditional uses of *Diospyros mespiliformis* stem bark extracts for it has an antiproliferative property against radicles of a Guinea corn *(Sorghum bicolour)* which may be attributed the presence of some phytochemical constituents in the plant such as triterpenes and flavonoids which may relate to its use as anticancer agent. However, use of cancer cell lines will further confirm this claim.

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