COMPARISON OF THE ALKYLATING ACTIVITY OF AQUEOUS AND ETHANOL EXTRACTS OF *MORINDA LUCIDA* ROOT BARK WITH CHLORAMBUCIL

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ABSTRACT

Plants have long been used to treat and manage non-communicable diseases. *Morinda lucida* also known as 'Oruwo' in the South-western part of Nigeria is a medium-sized tree with a crooked hole and rather short twisted branches. It belongs to the family *Rubicaeae*. The anticancer activity of *Morinda lucida* has been reported in literature. However, the mechanism of the anticancer activity has not been fully elucidated, especially its alkylating property. Alkylating drugs transfer alkyl groups to DNA and cause a disruption in structure, leading to breakage of DNA. This research aims to compare the alkylating activity of aqueous and ethanolic root bark extracts of *M. lucida* with a reference drug, chlorambucil. Aqueous (AML) and ethanol (EML) extract of *M. lucida* root bark were prepared by maceration in water and ethanol respectively. Alkylating activity of varied concentration of aqueous and ethanolic extracts of *M. lucida* root bark and chlorambucil was assessed using the 4-(4-nitrobenzyl) pyridine (NBP) as a DNA model. Absorbance was read at 540nm at different time intervals (5, 10, 30 and 60 min), and this corresponds to the extent of alkylation of extracts and drug. The results indicate that EML had comparable alkylating activity with chlorambucil. EML and chlorambucil at 1000µg/ml showed the highest alkylating activities after 60 minutes with absorbance of 0.890 and 0.929 respectively. EML showed more potent alkylating activity compared to chlorambucil and AML.

Keywords: alkylating, chlorambucil, *Morinda lucida*, nitrobenzyl pyridine, root
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INTRODUCTION

Cancer is a term used to describe a group of diseases characterized by the abnormal and uncontrolled growth of cell in any part of the body [1]. It is known to be the second leading cause of death worldwide, causing about 10 million deaths, or a sixth of all deaths recorded worldwide, in 2020 [2]. According to WHO, chemotherapy, using natural or synthesized biological therapies, is a key component of the treatment and management of cancer. Most of the drugs approved for the treatment of cancer by the US Food and Drug Administration are expensive [3] and have known side-effects. Hence, there is a gradual increase in the patronage of herbal drugs of medicinal plant origin, especially in low-income countries like Nigeria [4].

Morinda lucida, otherwise known as Brimstone tree, is a medium-sized tree with crooked branches reported to possess several medicinal uses [5]. *M. lucida* whole plant is a nutrient-rich plant. *M. lucida* has been shown to attenuate acetaminophen-induced oxidative stress and hepatotoxicity in rats [6]. The plant is known to have potential cytotoxic effect, as demonstrated by its confirmed anti-malarial activity [7]. Secondary metabolites isolated from the stem of *M. lucida* have been reported to possess anti-malarial effect [7]. Appiah-Opong *et al*. [8] demonstrated the anti-proliferative, antioxidant and pro-apoptotic effect of stems and leaves of *M. lucida*. Alkylating agents are used majorly in anticancer chemotherapy to treat hematological and solid malignancies, mostly in combination with other drugs [9]. Alkylating agents are known to exert their anti-tumour and anticancer effects by blocking all phases of the cell cycle. Alkylating agents are electrophilic groups that covalently bind to electron-rich functional groups in DNA and cause DNA fragmentation leading to a disruption in the replication and hence may lead to apoptosis, or programmed cell death [10]. Alkylating agents act by the adding an alkyl group, most often at the N7 position of guanine residues. The mechanism of anti-cancer action of *M. lucida* has not been fully elucidated. Hence, this present study was designed to determine the alkylating activities of aqueous and ethanolic extracts of *M. lucida* using the NBP alkylating assay.

MATERIALS AND METHODS

Plant collection

The root bark and leaves of *M. lucida* were collected from Osogbo, South-West, Nigeria in 2010. The leaves of the plant were used for the identification and authentication by Mr. Odewo. A voucher specimen was deposited in the University Herbarium, University of

Lagos, Lagos, Nigeria with herbarium number LUH 8392.

Preparation of aqueous root-bark extract of *Morinda lucida*

The root-bark was rinsed and shade dried for three days and pulverized into powdery form. The aqueous root-bark extract was prepared by maceration of 1 kilogram of the coarsely powdered root-bark in 1 Litre of distilled water for 48 h. The ethanolic root-bark extract was prepared by maceration of 1 kilogram of the coarsely powdered root-bark in 1 L of distilled water for 48 h. Both macerates were concentrated using a Rotary Evaporator (Rotavapor® R-300, BUCHI, Switzerland). The aqueuous root bark extract was further lyophilized to constant weight in vacuo using a lyophilizer (Lyotrap, LTE, England).

Alkylating activity assay

The alkylating activity of aqueous and ethanolic root-bark extract of *M. lucida* and chlorambucil was determined using 4-(4-nitrobenzyl)pyridine (NBP) as described by Thomas *et al.* **[11]** and Lawal *et al.* [**12**]. Briefly, 0.1 ml of varying concentrations (0.1, 1, 10, 100 and 1000µg/ml) of the aqueous and ethanol extracts of *M. lucida* and chlorambucil were added to 0.1 ml phosphate buffer (pH 4.0) solution and incubated at 70^0C for 30 minutes with a 0.1 ml solution of 5% w/v Nitrobenzyl pyridine in test tubes. The resultant solutions were mixed thoroughly. The mixture was added (in a 1:1 ratio) to test tubes in an ice bath containing 0.5 ml absolute ethanol and 0.5 ml 0.1 N NaOH. The absorbance of the blue colour of the alkylated product was read in triplicates at 540 nm with a UV-Vis spectrophotometer () after 5, 10, 30 and 60 min.

Statistical analysis

Results were expressed as Mean ± standard error mean of 3 values. SPSS version 17.0 software was used for the analysis of results.

RESULTS

The absorbance of the mixtures from the NBP assay is considered to be directly proportional to alkylating activities and hence used to define alkylating activities for the different extracts and drugs used in this study. The results of the alkylating activities of aqueous, ethanol root bark extract of *M. lucida* and chlorambucil at time intervals of 5, 10, 30 and 60 min are shown in Figure 1-5. A concentration of 0.1µg/ml ethanol extract of *M. lucida* root bark had more alkylating activity (0.031, 0.098, 0.257, 0.29) than 0.1µg/ml chlorambucil (0.097, 0.118,0.126,0.129) and 0.1µg/ml aqueous root bark extract of *M. lucida* (0.025, 0.026, 0.044, 0.03) at 5, 10, 30 and 60 min respectively (Figure 1). The alkylating activity at 0.1µg/ml of aqueous, ethanol extract of *M. lucida* root bark and chlorambucil all had time-dependent increases after 5, 10, 30 and 60 min except aqueous root bark extract of *M. lucida* that decreased after 60 min.

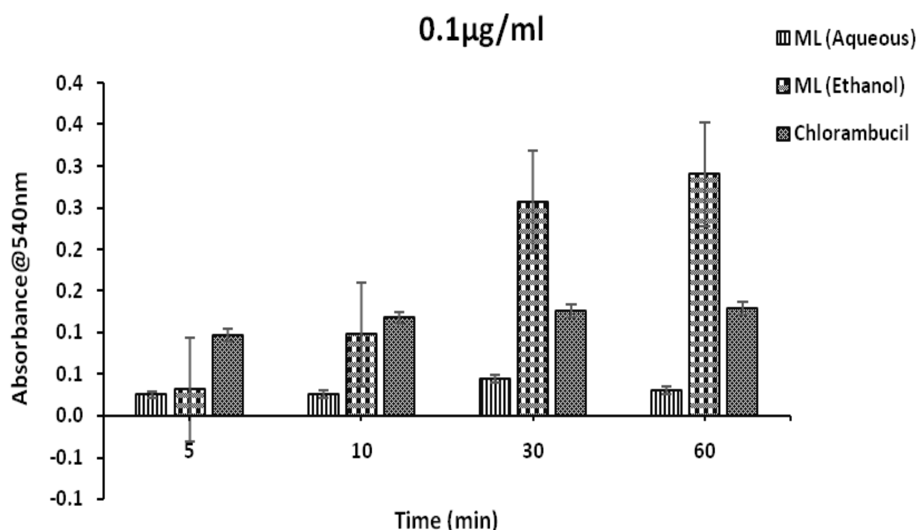

Figure 1: Nitrobenzyl pyridine alkylating activity of 0.1µg/ml *M. lucida* root bark extracts and chlorambucil at different time intervals.

At concentration of 1µg/ml, ethanol extract of *M. lucida* root bark had more alkylating activity (0.046, 0.099, 0.173, 0.333) than chlorambucil (0.086, 0.102, 0.135, 0.201) and aqueous root bark extract of *M. lucida* (0.04, 0.039, 0.042, 0.101) at 5, 10, 30 and 60 min respectively (Figure 2). The alkylating activity of 1µg/ml concentration of aqueous, ethanol extract of *M. lucida* root bark and chlorambucil had time-dependent increases after 5, 10, 30 and 60 min.

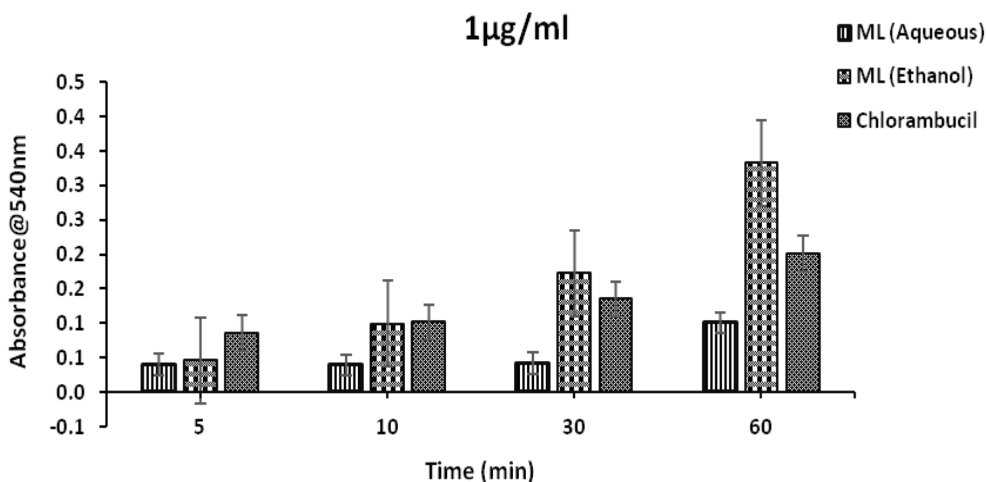

Figure 2: Nitrobenzyl pyridine alkylating activity of 1µg/ml *M. lucida* root bark extracts and chlorambucil at different time intervals.

At concentration of 10µg/ml, ethanol extract of *M. lucida* root bark had more alkylating activity than chlorambucil and aqueous root bark extract of *M. lucida* at 5, 10, 30 and 60 min respectively (Figure 3). The alkylating activity of 10µg/ml aqueous (0.091, 0.072, 0.126), ethanol extract of *M. lucida* root bark (0.225, 0.476, 0.565) and chlorambucil (0.184, 0.315, 0.49) had time-dependent increases after 5, 10, 30 min. However, after 60 min, the alkylating activity of aqueous root bark extract of *M. lucida* root bark and chlorambucil decreased to 0.099 and 0.479 respectively (Figure 3).

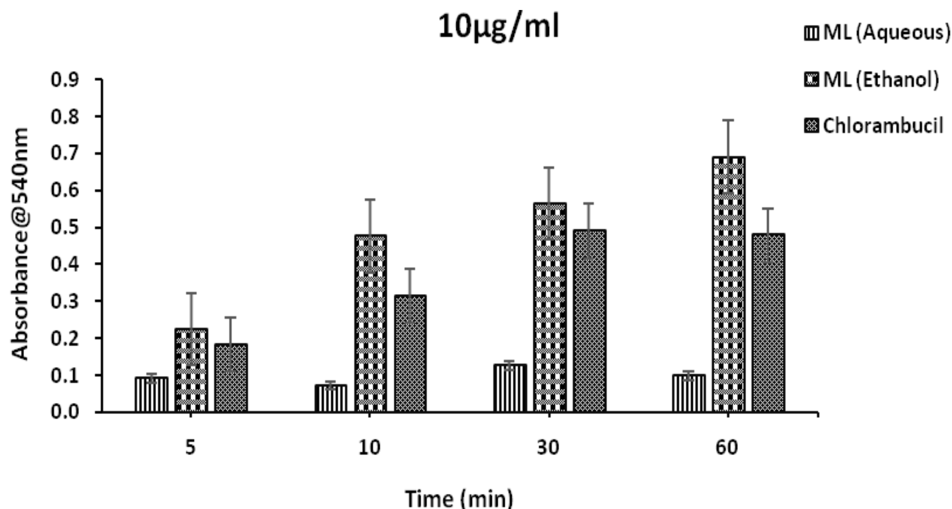

Figure 3: Nitrobenzyl pyridine alkylating activity of 10µg/ml *M. lucida* root bark extracts and chlorambucil at different time intervals.

At concentration of 100µg/ml, ethanol extract of *M. lucida* root bark had more alkylating activity (0.652, 0.707, 0.788, 0.77) than chlorambucil (0.529, 0.603, 0.711, 0.7) and aqueous root bark extract of *M. lucida* (0.116, 0.231, 0.097, 0.177) at 5, 10, 30 and 60 min respectively (Figure 4). The alkylating activity of 100µg/ml aqueous, ethanol extract of *M. lucida* root bark and chlorambucil had time-dependent increases after 5 and 10 min. However, the alkylating activity of aqueous extract of *M. lucida* root bark decreased after 30 min. Ethanol extract of *M. lucida* root bark and chlorambucil also showed a decrease in activity after 60 min (Figure 4).

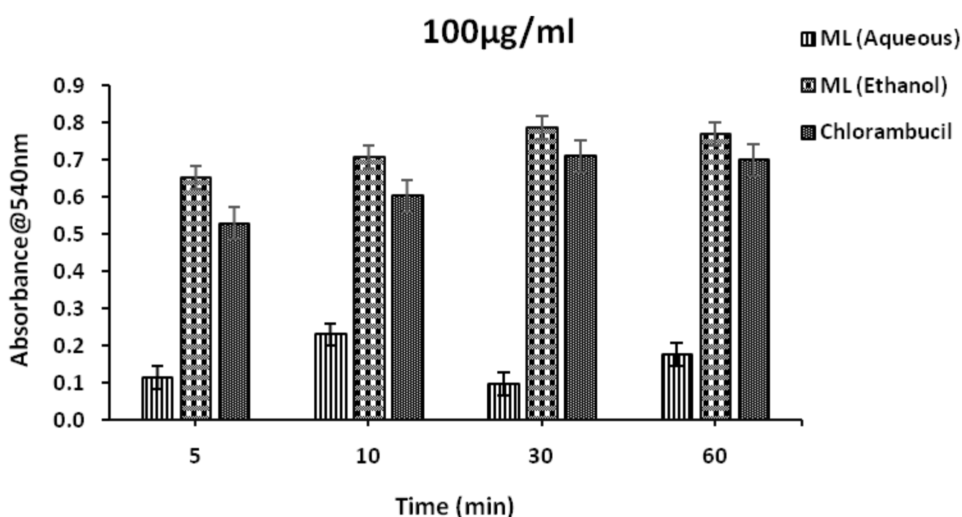

Figure 4: Nitrobenzyl pyridine alkylating activity of 100µg/ml *M. lucida* root bark extracts and chlorambucil at different time intervals.

At concentration of 1000µg/ml, chlorambucil showed similar activity (0.76, 0.793) with 1000µg/ml of ethanol extract of *M. lucida* root bark (0.757, 0.788) after 5 and 10 minutes. After 30 and 60 min, chlorambucil had more alkylating activity (0.782, 0.929) than ethanol extract of *M. lucida* root bark (0.699, 0.89) (Figure 5).

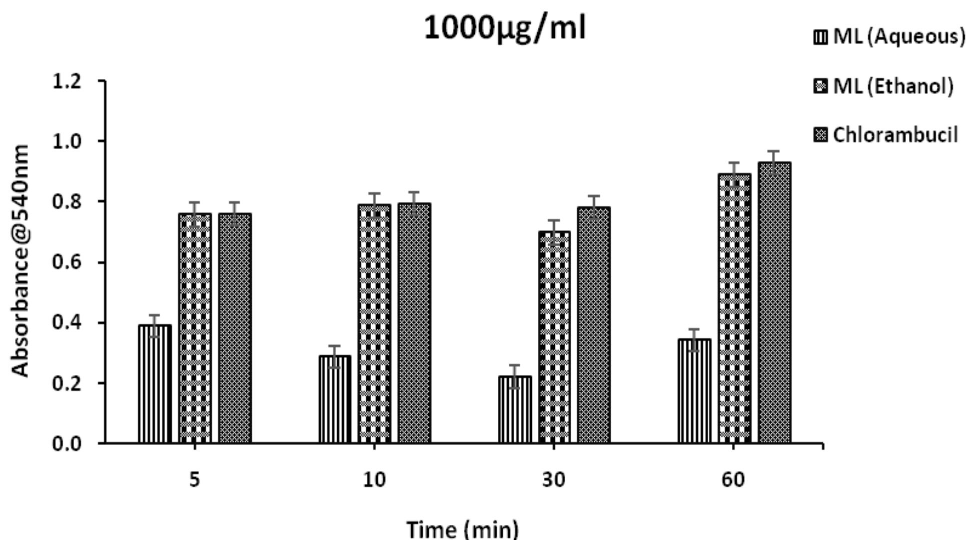

Figure 5: Nitrobenzyl pyridine alkylating activity of 1000µg/ml *M. lucida* root bark extracts and chlorambucil at different time intervals.

DISCUSSION

The present study was designed to compare the alkylating activity of aqueous and ethanolic extracts of *M. lucida* with a reference drug, chlorambucil, using the NBP alkylating assay. According to Gómez-Bombarelli *et al*. [13], the NBP assay is a suitable method used for the evaluation of alkylating activity of various compounds with suspected alkylating properties. The NBP alkylating activity assay method is based on the formation of colour in a reaction between the alkylating agent and a nucleophile; 4-(p-nitrobenzyl) pyridine, with nucleophilic characteristics similar to DNA bases [11, 12]. NBP has been shown to react with strong and weak alkylating agents [12, 14]. The NBP assay is currently being used to estimate the alkylating activity of medicinal plants with potential anticancer activity [12]. *M. lucida* is reported to be a rich source of phyto-constituents with immense bioactivities that have therapeutic effect on man and has been used traditionally to enhance the healthcare needs of populations across tropical Africa [15]. Cytotoxic activity of *M. lucida* on Ehrlich ascites carcinoma cells has been confirmed in a previous study [16] and its potential anticancer activity had been reported [17]. Machana *et al*. [14] reported that the NBP assay was used to estimate the mechanism of DNA damage of anticancer agents from medicinal plants, *in vitro*. In this study, the NBP assay was used to estimate the alkylating activity of medicinal plants with potential anticancer activity and the extent of colour development is known to be proportional to the strength and concentration of the alkylating agent [12]. The alkylating activity of ethanol extract of *M. lucida* was observed to be comparable to the activity of the known nitrogen-based alkylating agent, chlorambucil [9]. The ethanol extract of *M. lucida* showed more colour with the NBP than obtained for the aqueous extract of *M. lucida* and the known alklyating drug indicating the presence of more alkylating agents in the ethanol extract. Machana *et al*. [14] categorized alkylating agents into very high, high or low according to the absorbance obtained on reacting with NBP. Medicinal plants with absorbance values greater than

0.50 were considered very high, values between 0.15 and 0.50 as high, while absorbance values below 0.15 were considered to be low alkylating. In the previous study [14], a known alkylating agent (melphalan) was considered a very high alkylating agent due to its extremely high absorbance values. In our study, chlorambucil recorded absorbance values above 0.5 at high concentrations indicating its very high alkylating activity. The results of this study may vary from the studies conducted by other authors [12, 14] due to shortcomings associated with the NBP model used for alkylating activity or the concentration of the NBP used in this study. Also, the use of NBP as a DNA model suffers from the compound's low water solubility, its lack of reactive oxygen sites, and dissimilar steric encumbrance compared to DNA [18]. The results obtained from this study suggests that constituents found in the plant ethanol extracts might directly alkylate DNA, thereby causing strand breakage and damage, leading to the death of cancer cells.

CONCLUSION

The results of this study show that ethanol and aqueous root-bark extract of *M. lucida* has alkylating activity. However, the ethanol root bark extract of *M. lucida* has high alkylating activity comparable to chlorambucil, indicating its potential use as an anti-cancer plant.

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