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Research Article

Ethnobotanical Survey, Phytochemical Screening, Growth Inhibitory Effects and Cytotoxicity Evaluation of Medicinal Plants used for Cancer Management in Ilorin Metropolis, Nigeria.

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Abstract
Cancer is a leading cause of death globally and in many local communities, medicinal plants are employed by traditional medicine practitioners (TMP's) to manage cancer patients. This study was designed to access information on medicinal plants that are locally employed for cancer management in Ilorin metropolis and evaluate growth inhibitory and cytotoxic potentials of selected plants mentioned from the survey.An ethnobotanical survey was conducted among TMP's in Ilorin metropolis. Plants selected from the survey were collected at Ilorin and authenticated at the Herbarium Unit Department of Plant Biology University of Ilorin before they were air-dried, pulverised and extracted into aqueous-methanol (30:70%). Phytochemical constituents of the plants were determined and thereafter the extracts obtained were subjected to growth inhibitory assays using *Sorghum bicolor* radicle and *Allium cepa* root. Cytotoxicity of the plant extracts using brine shrimp lethality and MTT colorimetric assays was evaluated.Fifteen (15) respondents comprising of 9 herb-sellers and 6 herbalist gave their consent to participate in the ethnobotanical study. Nineteen plants belonging to twelve families were mentioned for the management of cancer. The plants were observed to contain secondary metabolites including saponins, tannins, alkaloids, free and combined anthraquinones, flavonoids and terpenoids. Extracts of *Detarium microcarpum* stem bark and *Prosopis africana* whole fruit displayed the highest growth inhibitory and cytotoxic effects, with *Detarium microcarpum* displayed the higher cytotoxicity against Hep-2C cancer cells (IC_{50} value of 0.08±0.00 µg/mL) compared to cyclophosphamide (2.69±0.25 µg/mL).This study reveals that *Detarium microcarpum* and *Prosopis africana* used traditionally in Ilorin for the management of cancer possess growth inhibitory and cytotoxicity effects. These plants might be further explored for anticancer drug discovery.

Key Words: Ethnobotanical survey, Ilorin metropolis, *Detarium microcarpum*, *Prosopis africana*, cytotoxicity

INTRODUCTION

Cancer is a global cause of death and accounted for about 10 million deaths in the year 2020 alone. Sadly, the number of cancer incidences and death are expected to rise by 47% from what it was in 2020 to about 28.4 million cases in 2040 (Haokip *et al*., 2021). This is because of the increasing number of people involved in excessive alcohol consumption and sedentary life, and higher number of people living with chronic inflammatory diseases (Sung *et al*., 2021).

In Nigeria, about a hundred thousand new cancer cases and over seventy thousand cancer related death are reported annually. Female breast cancer (46%) is the most common form of cancer that is diagnosed in Nigeria while prostate cancer is the second most diagnosed cancer and the most diagnosed among Nigerians males (32%). About 92% of all tumors diagnosed here in Nigeria are malignant and most patients (97%) are symptomatic at the point of diagnosis. Sadly because of high cost of cancer therapy up to 67% of patients who had commenced cancer therapy are unable to complete their treatment (Fapohunda *et al*., 2020).

Over the years, cancer has become a major cause for health and economic concern requiring urgent intervention that can proffer innovative treatment approaches such as the development of new and effective chemotherapeutic drugs. Since nature has endowed humanity with limitless biodiversity and novelty, it is logical that scientists may further their search for newer anticancer medicines from unexploited natural products. As it stands today, over 47% of all anticancer medicines in use own their origin to natural source. Bioactive compounds from plants, animals, fungi, micro-organisms and marine organisms are being explored for innovative anticancer

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agents (Sidra *et al*., 2014).There is need to exam medicinal plants that are locally used for the management of cancer in an effort to identify plant(s) that may serve as source for new anticancer compounds. Currently, there is a growing demand for effective and safe anticancer medicine(s) because most of the clinically useful anticancer drugs have narrow therapeutic index and are often toxic to normal healthy cells, thereby resulting in life threatening adverse effects such as fatal myelosuppression (Khan *et al*., 2020).

Hence, this study was designed and focused to collect information on medicinal plants that are used for treatment of cancer in Ilorin metropolis. The selected plants were subjected to growth inhibitory and cytotoxicity assays

MATERIALS AND METHODS

Ethnobotanical survey

Study area: An ethnobotanical survey was conducted within Ilorin metropolis. Ilorin is the state capital of Kwara and it is located in north central part of Nigeria. There are three Local Government Areas in Ilorin, namely Ilorin East, Ilorin West, and Ilorin South. Kwara state is situated on latitude 8º 24'N and 8º 36'N and longitude 4º 10'E and 4º 36'E and covers an area of about 100 km^2. Ilorin is located strategically amid the heavily populated South Western and the lightly populated Middle Belt of Nigeria. The vegetation of Ilorin is a mix of the rainforest of the south and dry savannah of northern of Nigeria and the major occupations of the people include cloth weaving, farming, hunting and trade.

Ethnobotanical survey: Ethnobotanical survey was conducted in May, 2017 among herbs sellers, herbalists and traditional healers within Ilorin metropolis. An oral interview was conducted by a semi structured informal method of data collection. Questionnaires were administered in "*Yoruba*" (local language) to encourage cooperation. A total of 15 respondents gave their consent to provide information catalogue of plants that are employed in their practices for the management of malignancy. All communication was done in "*Yoruba*". A total of 19 medicinal plants were mentioned out of which 4 plants were selected based on their frequency of mention (over 80% frequency of mention) and citation in literature for the treatment of cancer.

Literature validation: The medicinal plants mentioned from the survey were arranged in order of their frequency of mention. The most frequently mentioned plants were crossed checked from available literature at the time of this study to identify those plants whose cytotoxic activity has not been reported in literature with the aim of identifying new plants with cytotoxic activity. Four plants were selected for preliminary cytotoxicity studies following literature review. The selected plants include *Detarium microcarpum*Guill. & Perr., *Prosopis africana*(Guill. & Perr.) Taub., *Parinari polyandre* Benth., and *Phyllanthus muellerianus* (Kuntze) Exell .

Plant selection, collection and authentication

The selected plants were collected from Ilorin and authenticated at the herbarium unit in the Department of Plant Biology, University of Ilorin by Mr Bolu Ajaiye.

Phytochemical screening of selected active plant extracts

Air-dried and powdered *Detarium microcarpum*(stem bark), *Prosopis africana*(whole fruit, stem bark and root bark), *Parinari polyandre* (stem bark), and *Phyllanthus muellerianus* (stem bark) were screened to identify the class of secondary metabolites present in the plants. The technique employed was described by Sofowora (2008).

Preparation of Plant Extracts

Plant materials collected were shade dried at ambient temperature (32˚C). Dried plant materials were pulverised. Two hundred grams (200 g) of each plant materials were extracted into aqueous-methanol (30:70) by maceration at ambient temperature (32˚C) for 72 h. The solvent was removed from the plant materials. Thereafter, the resulting marc was re-extracted successively by means of redistilled aqueous-methanol (30:70) until the extract became colourless. The extracts collected were concentrated *in vacuo* at 45˚C. Dried aqueous-methanol extracts were obtained and kept at 4˚C until needed. Percentage yield of the extracts were calculated.

Growth inhibitory assays

***Sorghum bicolor* radicle growth inhibitory assay:** *Sorghum bicolor* seeds were purchased from *Ipata* market in Ilorin. Viable seeds were selected by introducing 100 g of the seeds into distilled water. The floating seeds and husk were decanting off while the submerged seeds were collected, washed with 95% ethanol for a minute and later rinsed with distilled water before they were dried and stored in dried dark place until needed. Only viable seeds were used for *Sorghum bicolor* radicle growth (SBRG) inhibitory assay. Ten milliliters (10 mL) of 39.06, 156.25, 625, 2500 and 10000 µg/mL of plant extracts were prepared by four fold dilution of the stock solution (40 mg/mL) prepared in 5% DMSO. The same concentrations as above were prepared for cyclophosphamide (positive control). A volume of 10 mL graded concentrations of the plant extracts (39.06 - 10000 µg/mL) were transferred into pre-labelled petri-dish of 10 cm lined with filter paper underlie with wool, thereafter ten (10) seeds were placed on each filter paper in the petri-dishes. The petri-dishes were incubated in a dark cupboard at ambient temperature (32 ˚C) and the lengths of emerging radicle were measured after 48 and 96 h of incubation. The negative control seeds were treated with equal volume of 5% DMSO in distilled water. The experiment was repeated in three replicates for all test concentrations and controls. The radicle new lengths were measured by the aid of the scaled ruler. The mean change in radicle length per concentration was calculated. The percentage radicle growth inhibition at 48 and 96 h and IC_{50} were determined (Ayinde and Agbakwuru, 2010).

Percentage inhibition of the root growth was calculated using formula below;

$$\%\ \text{inhibition} = \frac{A - B}{A} \times 100\%$$

Where;

A = length of untreated root.

B = length of treated root with plant extracts/ cyclophosphamide

***Allium cepa* root growth inhibitory assay:** *Allium cepa* root growth (ACRG) inhibitory assay was conducted using method described by Akinboro and Bakare (2007). Moderate size onion bulbs (50 ± 10 g) were also purchased from *Ipata* market in Ilorin. *Allium cepa* bulbs (50 ± 10 g) were rinsed with distilled water and grown with the root submerged in water in the dark at ambient temperature (32 ˚C) for 24 - 48 h until the roots have grown up to 3 cm length.

Twenty (20) millilitre of graded concentrations (39.06, 156.25, 625, 2500 and 10000 µg/mL) of each plant extract was prepared by four fold dilution of the stock solution (40 mg/mL) prepared in 5% DMSO. Twenty (20 mL) of the plant extract concentrations were transferred into the petri-dishes of 10 cm diameter and the base of each of three *A. cepa* bulbs were placed on a petri-dishes containing each extract (39.06 - 10000 µg/mL). The same concentrations as above were prepared for cyclophosphamide (positive control), while 5% DMSO in water served as the negative control. New root growth lengths were measured at 0, 48, 96 h and the percentage root growth inhibition were estimated by the formula below;

Percentage inhibition was calculated as:

$$\% \text{ inhibition} = \frac{A - B}{A} \times 100\%$$

Where;

A = length of untreated radicle.

B = length of treated radicle with plant extracts/ cyclophosphamide

Cytotoxicity assays

Brine shrimp lethality (BSL) assay: The eggs of *Artemia salina* were incubation in seawater for 48 h to hatch the eggs. The BSL assay was carried out using a modified method illustrated by Sharififar *et al*., (2009). Ten milligrams of the extract was dissolved in 2 mL 5% DMSO in sea water and resulted in 5 mg/mL stock solution. Further dilutions of 1, 10, 100, 500 and 1000 µg/mL were made in microplates in triplicates. Ten nauplii was added to each well. The plates were incubated at room temperature (RT = 25-33°C) for 24 h. 0.5% DMSO in sea water was used as the negative control and thereafter the number of dead nauplii in each well was counted.

The percentage cytotoxicity was estimated by the formula below;

$$\% \text{ inhibition} = \frac{A - B}{A} \times 100\%$$

Where: A = the mean number of shrimp in negative control wells.

B = the mean number of shrimp in the wells with plant extracts / cyclophosphamide.

Methanol plant extracts with LC_{50} values < 100 µg/mL are regarded as active and are selected for further cytotoxicity studies.. Extracts that demonstrate strong brine shrimp lethality are regarded as promising candidate for anticancer drug discovery (McLaughlin *et al*., 1991).

Cytotoxicity screening of selected extracts on Rd and Hep-2C cancer cell lines: Cytotoxicity of the selected plant extracts were determined by MTT (3-(4, 5-dimethyl thiazole-2yl)-2, 5-diphenyl tetrazolium bromide) assay using the method described by (Kritsanawong *et al*., 2016). A stock solution (10 mg/mL) of plant extract in DMSO was prepared and further diluted in culture media to obtain a working solution of 1000 µg/mL. Thereafter, a ten-fold serial dilutions of the plant extract was prepared to obtain graded concentrations (0.01, 0.1, 1, 10, 100 and 1000 µg/mL). Fifty microliter (50 µL) of each plant extract solution was dispensed into 96-well microtitre plates already seeded with monolayer of Rd or Hep-2Ccells in triplicates. The plates were incubated at 37˚C in a carbon-dioxide environment and the cells observed under microscope after 72 h. Supernatants were removed from all wells after 72 h incubation and 25 µL of 2 mg/mL MTT solution in phosphate buffer saline (PBS) was added to each well and the plates were further incubated for 2 h at 37˚C. Thereafter, 125 µL of DMSO was added to the wells to dissolve the MTT crystals formed. The absorbance of the test plate was measured using a UV microplate reader (Titertek Uniskan) at wavelength of 570 nm against a background control as blank. The UV absorbance of each plate directly correlates to the number of viable cells in each well.

Percentage inhibition of cancer cell proliferation was calculated by the formula below;

$$\% \text{ cytotoxicity} = \frac{A - B}{A} \times 100\%$$

Where; A = the optical density of untreated cells.

B = the optical density of cells treated with plant extracts

The IC_{50} was determined from a non-linear regression curve plotted using Graphpad prism® version 6.0

Statistical analysis

Data obtained were expressed as means ± SEM of values obtained in triplicates from three independent experiments. Statistical differences between treated groups and standards were evaluated using one way analysis of variance (ANOVA). A p value <0.05 was considered to be significantly. Data were analysed using GraphPad Prism®version 6.0 Software.

RESULTS

Ethnobotanical survey and plant collection: From the ethnobotanical survey, a total of fifteen (15) respondents comprising of 9 (60%) herb-sellers and 6 (40%) herbalist gave their consent to participate in the ethnobotanical study (Table 1). Most of the traditional medicine practitioners were above forty (40) years of age, it was observed that those above the age of 60 constitute only 20%, while most of the respondent are between the age of 41-60 (53.3%) and only 4 (26.7%) are between the ages of 21-40 years. Most of the respondents were female (73.3%) while only 26.7% of the respondents were male. Most of the respondent (66.7%) had no formal education while only 26.7% had primary school education and 6.7% had secondary school education and none of them had university education as shown in Table 1. Majority of the respondents are married (93%) and are Muslim (86%) while only 13.3% practice traditional system of believe. About 60% of the respondents claimed that the source of their knowledge is from their ancestral heritage while 6.7% claimed that they acquired their traditional knowledge by apprenticeship and 26.7% of them claimed that they enhanced their ancestral knowledge with apprentices. Forty-six percent (46%) of the respondents have been in practice between 21-30 years while 26.6 % each

claimed less than have been in practice for over 31years and most (86%) of the respondents are completely devoted into herbal practice while the other 13.3% do part time herbal practice. The motivation for practicing traditional medicines by the respondents is different, majority (60%) of the respondents mentioned spiritual calling as their motivation for practicing herbal medicine, while 26.7% are into the practice because they have no other skills or training. However, few (13.3%) of the respondents claimed that they are into herbal practices for financial gain as shown in Table 1.

Table 1:
Demographic data of respondents

Demographic Parameters		Number	Percentage (%)
Profession	Herb-seller	9	60.0*
	Herbalist	6	40.0
Age	< 20	0	0.0
	21-40	4	26.7
	41-60	8	53.3*
	> 60	3	20.0
Gender	Female	11	73.3*
	Male	4	26.7
Education level	None	10	66.7*
	Primary	4	26.7
	Secondary	1	6.7
	Tertiary	0	0.0
Marital status	Single	0	0.0
	Married	14	93.3*
	Divorced	0	0.0
	Widow	0	0.0
	Widower	1	6.7
Religion affiliation	Christianity	0	0.0
	Islam	13	86.7*
	Traditional	2	13.3
Source of knowledge	Ancestral heritage	9	60.0*
	Apprenticeship	1	6.7
	Ancestral heritage and apprenticeship	4	26.7
Duration of practices	< 20	2	13.3
	21-30	7	46.7*
	31-40	2	13.3
	> 40	4	26.6
Mode of practices	Part time	2	13.3
	Full time	13	86.7*
Motivation for practice	Profit making	2	13.3
	Spiritual calling	9	60.0*
	Has no other means of livelihood	4	26.7

***P<0.05**

Nineteen (19) plants belonging to twelve (12) families were mentioned by respondents for the management of malignant diseases as shown in Table 2. The most frequently mentioned plants include;Citrus aurantifolia fruit, *Detarium microcarpum* stem bark and Parinari polyandra stem bark. Allium cepa leaf and Prosopis africana were among the second most frequently mentioned plants along with *A. barteri*, *A. meleguata* and *P. muellerianus*. Plants of Leguminosae, Euphorbiaceae and Zingiberaceae family (15.8% each) are the most mentioned for the management of cancer and painful sores. Plants mentioned belongs to other families such as Liliaceae, Rutaceae, Bignoniaceae, Rubiaceae, Amaryllidaceae, Meliaceae, Phytolaccaceae, Guttiferae and Rosaceae together constitute 53 % of the medicinal plant mentioned as shown in Figure 1. The selected plants were collected from Ilorin and authenticated at the herbarium unit in the Department of Plant Biology, University of Ilorin and the voucher number of UILH/002/1272, UILH/001/163, UILH/001/582, and UILH/001/886 were issued for *Detarium microcarpum*, *Parinari polyandre*, *Prosopis africana* and *Phyllanthus muellerianus*, respectively.

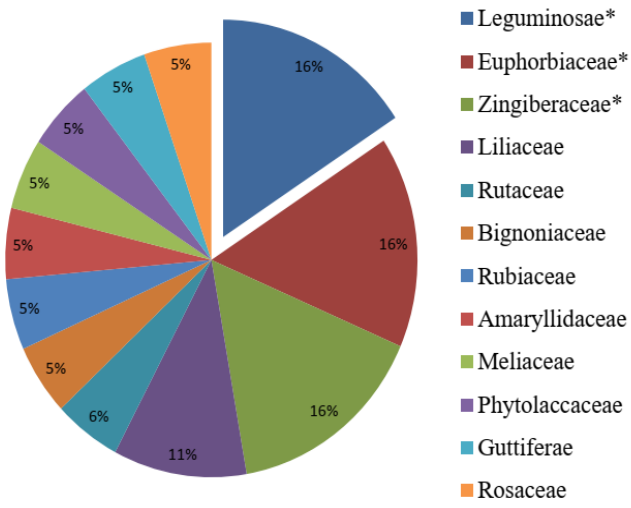

Figure 1. Family of medicinal plants mentioned by respondents

Phytochemical composition and Percentage yield of the selected plant: Phytochemical evaluations of the selected plants led to identification of various secondary metabolites including saponins, tannins, alkaloids, free and combined anthraquinones, flavonoids and terpenoids as shown in Table 3. All the selected plants gave positive test for saponins, tannins free and combine anthraquinones and flavonoids while only P. meullerianus gave a positive test for alkaloids.
The methanol extract of the whole fruit of P. africana had the highest percentage yield of 17%. In addition, methanol extract of *Detarium microcarpum* stem bark and Parinari polyandra stem bark had the lowest percentage yield of 6% (Table 4).

***Allium cepa* root and *Sorghum bicolor* radicle growth inhibitory effects:** The ability of the plant extracts to inhibit growth of rapidly dividing cells of S. bicolor radicle and A. cepa root were determined. The methanol extract of *D. microcarpum* stem bark (DMS) displayed the highest (IC_{50} = 2.11±0.22 µg/mL) radicle growth inhibition that is significantly better than cyclophosphamide (IC_{50} = 4.51±0.53 µg/mL) in S. bicolor (Table 4). The methanol extracts of Prosopis africana whole fruit (PAF), The methanol extract of stem bark of *Detarium microcarpum* and *Parinari*

*polyandra*displayed similar A. cepa root growth inhibitory activity with IC_{50}ranging from 1.93±0.12 to 1.96±0.94µg/mL

Table 2:
Plants use in treatment of cancer in Ilorin metropolis

S/N	Botanical Name	Family	Local Names	Part used	Frequency of Mention
1	*Citrus aurantifolia*	Rutaceae	Orombo	Fruit Juice	10
2	*Detarium microcarpum**	Leguminosae	Ogbogbo	Stem bark	10
3	*Parinari polyandra*	*Rosaceae*	*Aboidefin / Abere*	Stem Bark	10
4	*Kigelia africana*	Bignoniaceae	Pandoro	Fruit, and Stem Bark	9
5	*Aloe barteri*	Liliaceae	Eti erin	Leaves	9
6	*Aframomum melegueta*	Zingiberaceae	Atare	Seed	9
7	*Prosopis africana**	Leguminosae	Ayan	Fruit, Stem and Root Back	9
8	*Phyllanthus meullerianus**	Euphorbiaceae	Egungun eja	Stem Bark	9
9	*Securinega virosa*	Euphorbiaceae	Iranje	Leaves	8
10	*Bridelia ferruginea*	Euphorbiaceae	Ira	Stem Bark	8
11	*Nauclea latifolia*	Rubiaceae	Egbesi	Root	8
12	*Zingiber officinale*	Zingiberaceae	Atale pupa	Rhizomes	8
13	*Curcuma domestica*	Zingiberaceae	Atale funfun	Rhizomes	7
14	*Crinum jagus*	Amaryllidaceae	Lsu merin	Bulb	6
15	*Khaya grandifoliola*	Meliaceae	Oganwo	Stem Bark	6
16	*Hilleria latifolia*	Phytolaccaceae	Ogo,akaato	Whole plant	5
17	*Bandeiraea simplicifolia*	Leguminosae	Kporekpo	Leaves and Bark	5
18	*Garcinia kola*	Guttiferae	Orogbo	Root	5
19	*Allium cepa*	Liliaceae	Alubosa onisu	Leave	4

* = Plants selected for preliminary evaluation

Table 3:
Phytochemical constituents of selected plants used in the management cancer in Ilorin

S/N	Selected Plants	Saponins	Tannins	Alkaloids	Cardiac Glycosides	Free Anthraquinones	Combined Anthraquinones	Flavonoids	Terpenoids
1	*Prosopis africana* fruit (PAF)	+++	++	++	-	+	+	++	++
2	*P. africana* stem bark (PAB)	+++	++	+++	-	+	+	+	+
3	*P. africana* root bark (PAR)	+++	+	+++	-	+	+	+	+
4	*Detarium microcarpum*stem bark (DMS)	++	+++	-	-	+	+	+++	+++
5	*Parinari. polyandra* stem bark (PPS)	++	++	++	-	+	++	+++	+
6	*Phyllanthus meullerianus* stem bark (PMS)	+++	+++	+	+	+	++	++	+

- Absence of component; + Trace presence of component; ++ moderate amount of component, +++ Copious amount of component

Table 4:
Percentage Yield, growth inhibitory and cytotoxic activities of selected plant extracts

Selected Plants	Percentage yield	IC_{50} (µg/mL)				
		***S. bicolor* RGI**	***A. cepa* RGI**	**BSL**	**Rd**	**Hep-2C**
DMS	6.0	2.11±0.22 ¥	1.96± 0.02	69.74±9.12	0.32±0.10¥	0.08±0.00¥
PAR	16.0	3.09±0.05¥	2.65± 0.70	166.00±8.41	6.64±0.37	9.42±1.71
PAS	14.5	4.26±0.34¥	3.21±0.06	232.50±6.61	26.25±4.89	21.69±0.25
PAF	17.0	3.49± 0.19 ¥	1.93±0.12¥	80.36±6.59	1.84±0.11¥	0.89±0.18¥
PMS	8.0	4.34±0.35	3.27±0.35	349.40±10.12	35.73±3.94	27.89±5.69
PPS	6.0	4.44±0.22	1.96±0.94¥	105.90±2.65	3.47±0.25	1.42±0.14
Cyclophosphamide		4.51±0.53	1.96±0.48	106.00±5.78	2.25±0.28	2.69±0.25

DMS= *Detarium microcarpum* stem bark, PAR= *Prosopis africana* root bark, PAS= *Prosopis africana* stem bark, PAF= *Prosopis africana* whole fruit, PMS= *Phyllanthus meullerianus* stem bark, PPS= *Parinari polyandra* stem bark, * =Positive Control, ¥ significantly better than the positive control

which is similar to that of cyclophosphamide (IC_{50} = 1.96±0.48µg/mL).

Cytotoxic effects of selected plant extracts: Classification of the toxicity of the extracts was done according to the classification reported by Padmaja *et al*. (2002). Extracts with LC_{50}> 1000 µg/mL are non-toxic, LC_{50}>500 and < 1000 µg/mL are weakly toxic, LC_{50}> 100 and < 500 µg/mL are moderately toxic and LC_{50}< 100 µg/mL are strongly toxic. The methanol extract of *D. microcarpum* stem bark and Prosopis africana whole fruit (PAF) showed strong cytotoxic effect with LC_{50}of69.74±9.12 and 80.36±6.59 µg/mL, respectively in the brine shrimp lethality (BSL) assay. Cyclophosphamide had $LC_{50}$of 106.00±5.78 µg/mL (Table 4).

In addition, cytotoxicity of the plant extracts on cancer cells are shown on Table 4.As a strategy to selecting medicinal plant extract for discovery of anticancer molecules, the American National Cancer Institute (NCI) issued criteria for selecting plant extracts and noted that extract with IC_{50} lesser than 30 µg/mL can be selected for further anticancer studies. Interestingly, all the extracts except *Phyllanthus meullerianus* stem bark extract displayed IC_{50}<30 µg/mL as shown in Table 4. The most active extracts on Rd cell line were *Detarium microcarpum* stem bark (IC_{50} = 0.32±0.10 µg/mL) and *Prosopis africana* whole fruit (IC_{50} = 1.84±0.11 µg/mL) that displayed comparable cytotoxic effect to cyclophosphamide (IC_{50} = 2.25±0.28 µg/mL). Similarly methanol extract of *Detarium microcarpum* stem bark was the most active on Hep-2c cancer cell line with IC_{50} of 0.08±0.99 µg/mL (Table 4)

DISCUSSION

Defining criteria for selecting medicinal plant(s) for drug discovery activity is an important step in order to achieve good results. There are three or four possible criteria for selecting medicinal plants and they include; ethnobotanical, chemotaxonomical and random selection approaches or combination of the three approaches. In this study, plants were selected after literature review of medicinal plants that were mentioned during and ethnobotanical survey of medicinal plants used for the management of cancer.

Ethnobotanical survey involves collection of information on how indigenous people of a selected area utilize their indigenous knowledge, skills and natural products in the management of a particular ailment. Unfortunately, the indigenous knowledge of local people is often underrated and under documented and at risk of extinction over time (Cheikhyoussef *et al*., 2011). Thus this study interviewed selected Traditional Medicine Practitioners (TMP's) in Ilorin for information on the medicinal plants use for the management of cancer. Since most people in low income countries like Nigeria depend on plant based medicines that are often sourced from herbalist and herb sellers for the treatment of various ailments. It appears that the herbalist and herb seller maybe custodians of knowledge of herbal medicines. Many investigators often consult herbalist and herb sellers to source information about medicinal plant and the ethnobotany of the local people (Hassan *et al*., 2020). The demographic characteristic of the TMP's obtained from our current study is similar to those obtained in a more recent survey at Ilorin metropolis, it was observed that most of the TMP's were married Muslims women older than 40 years of age (Ibrahim *et al*., 2021).

The domination of women in herbal practices could be because women are more involved with sales of herbal medicines while men are more involved with herbalism (Boudjelal *et al*., 2013). Interestingly, women have been reported to possess more indigenous knowledge of herbal medicine than men probably because of their domestic role in caring for their families particularly children. Most of the TMP's obtained their knowledge of herbal medicine from ancestral heritage and practice full-time because they believed that they are spiritually called into herbalism. Females are generally more involved with the selling of herbal medicines while men are more involved with herbalism. Interestingly, women have been reported to possess more indigenous knowledge of herbal medicine than men, this is probably because of the role women play in caring for her family, particularly her children who often require special health needs.

Botanicals are the most common ingredients used in traditional medicines, sometime animal parts and minerals are employed in preparation of herbal remedy. Plants are mostly used because they possess diverse and potent biological activities (Kozlowska *et al*., 2019). Nineteen (19) plants belonging to twelve (12) families were mentioned by respondents for the management of malignant diseases. The most frequently mentioned plants include *Citrus aurantifolia* fruit, *Detarium microcarpum* stem bark and *Parinari polyandra* stem bark. The leaf of Allium cepa and Prosopis africana were among the second most frequently mentioned plants along with *A. barteri*, *A. meleguata* and *P. muellerianus*. The vegetation of Ilorin is similar to those obtained in the northern and southern parts of Nigeria hence, the medicinal plants employed in cancer treatments are similar to those from both regions. Other plants like *Crinum jagus*, *Kigelia africana* and *Aframomum melegueta* mentioned in this study have been previously mentioned in an ethnobotanical survey conducted in south-west Nigeria (Soladoye *et al*., 2010). In addition, plants like *Detarium microcarpum* and *Parinari polyandra* are often employed in North-West Nigeria (Ngulde *et al*., 2015).

Biological activities of medicinal plants lies primarily with type of bioactive secondary metabolites present in the plants. Secondary metabolites such as saponins, tannins, alkaloids, terpenoids have been associated with anticancer activities (Hussein and El-Anssary, 2018). Phytochemical evaluations of the selected plants led to identification of various secondary metabolites including saponins, tannins, alkaloids, free and combined anthraquinones, flavonoids and terpenoids. All the plant materials tested were rich in Saponins, Tannins, Alkaloids, flavonoids and terpenoids. However, cardiac glycosides are absent in selected plants except *P. muellerianus* stem bark. Phytochemicals such as alkaloids, glycosides, flavonoids, phenolics, saponins, terpenes and steroids have been reported to demonstrate anticancer activities (Vaghora *et al*., 2016).The stem bark, root bark and whole fruit contained similar phytochemical similar to those reported in an earlier study where Prosopis africana stem bark was found to contain flavonoids, tannins, glycosides, carbohydrates, saponins and alkaloids (Ayanwuyi *et al*., 2010). The stem bark of *Detarium microcarpum* used in this study was observed to contain similar phytochemical constituents to those observed in a recent study, the phytochemicals such as saponins, tannins, alkaloids, flavonoids and terpenes (Sanusi *et al*., 2020).

Detarium microcarpum methanol extract displayed the best activity in *Sorghum bicolor* radicle growth inhibitory assay while *P. africana* displayed the highest *Allium cepa* root growth inhibitory effect. Growth inhibitory activity is regarded as a good indicator for anticancer activity and extracts that have shown good growth inhibitory activities have been reported to also have potent anticancer effects (Ayinde and Agbakwuru, 2010). Methanol extract of *D. microcarpum* stem bark and *P. africana* fruit extracts showed the highest cytotoxicity against cancer cell lines and Artemia salina nauplii. Methanol extract of *D. microcarpum* has been previously reported to be cytotoxic against *Artemia salina* nauplii (Zakari *et al*., 2016). The extract of *P. africana* whole extract also displayed potent cytotoxicity on nauplii of *Artemia salina*, similar to previous study (Idris and Idris, 2019).

Detarium microcarpum stem bark and P. africana whole fruit extracts displayed the highest activity against the human rhabdomyosarcoma and Laryngeal cancer cell lines. However in an earlier report methanol and aqueous extracts of *D. microcarpum* were reported to demonstrate some antiproliferative effects on MCF7 breast cancer cell lines (Adebayo *et al*., 2019). There are limited information on cytotoxic activity of P. africana against human cancer cell lines. Although, *P. africana* had been reported to possess cytotoxic effect against Artemia salina nauplii and antimalarial activity (Idris and Idris, 2019).

CONCLUSION

This study revealed that *Detarium microcarpum* stem bark and *Prosopis africana* whole fruit that are employed for the management of cancer in Ilorin ethnobotany possess growth inhibitory and cytotoxic potentials and also justify that medicinal plants traditionally employed in cancer management may contain cytotoxic compounds. Based on this promising result, the isolation of cytotoxic compounds from stem bark of *Detarium microcarpum* and whole fruit of *P. africana* will be pursued.

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