# *Original Research Article*

## Ethnobotanical Survey, Physiochemical Composition and Preliminary Cytotoxic Evaluation of Some Medicinal Plants with Anticancer Potential from Certain Areas In South-West Nigeria


**ABSTRACT**

**Aims:** Medicinal plants used by traditional medical practitioners (TMP) to treat cancers are considered safe when used alone or in combination with conventional therapy to ensure their effectiveness and eliminate the toxic effects of orthodox medicines. Using cytotoxic and antioxidant studies, the study attempted to assess some of the commonly used medicinal plants used to cure cancer among Yoruba people in Ogun, Oyo, Osun, and Lagos (South-West, Nigeria).
**Study design:** Samples of commonly utilized anticancer plants obtained from the chosen areas using physical and virtual oral seminars were studied.
**Methodology:** To identify the already-exploited anticancer plants, online academic literature searches were done on the cited plants. Mineral and proximate studies of the cited unexploited plants were performed. The ethanolic extracts of the plant were examined for the presence of bioactive components and their total flavonoid content, with a focus on quercetin detection using thin layer bioautography (TLB) and brine shrimp lethality assay (BSLA) for cytotoxicity. In comparison to quercetin and ascorbic acid, the scavenging of superoxide radical (SOR), hydrogen peroxide, and 2, 2-Diphenyl-2-picrylhydrazyl (DPPH) radical activity by a model (most biologically active) of the anticancer plant was also evaluated.
**Results:** There were only twelve anticancer species that were not used in related studies: *Lannea egregia*, *Ficus exasperate*, *Croton zambesicus*, *Tetrapleurai tetraptera*, *Terminalia catappa, Zanthoxylum zanthoxyloides*, *Plumbago zelanica*, *Hilleria latifolia*, *Bryophyllum pinntu*m, *Chromolena odorata*, *Brysocarpus coccineus and Spondias mombin*. The anticancer plants contained bioactive and minerals substances like saponins, protein, lipids, magnesium, calcium, iron, zinc, and a decreased Na/K concentration. The plants had a fair amount of flavonoids and variable levels of cytotoxicity. *L. egeregia* was regarded as the prototype of the anticancer species due to its profound flavonoid concentration (85.40 g/ mL) and cytotoxicity (9.46 g/ mL) compared to other extracts. The TLB also demonstrated the presence of quercetin, with a dose-dependent antioxidant property. The anticancer model's overall antioxidant activity (34.72 g/ mL) was slightly lower than quercetin (30.44 µg/ mL) but higher than ascorbic acid (41.68 µg/ mL).
**Conclusion:** The results support the traditional use of anticancer species as nutritional and dietary supplements, whose bioactive compounds are relevant in managing cancer patients. The plants bioactive principles need to be characterized in future research.



*Keywords: Medicinal plants, Cancer, Antioxidant, Flavonoid extract, Conventional*


## 1. INTRODUCTION

Cancer is a notable deadly disease involving several other diseases. Cancers such as leukaemia, colorectal, breast, lung, and so on are responsible for over eight million annual deaths in Nigeria [1]. Risk factors for cancer include lack of physical activity, environmental, metabolic, chemical, and genetic factors [2]. Thus, the cancer burden was predicted to rise from 12·7 million new cases to 22·2 million between the years 2008 and 2030, despite the low incidence in Nigeria resulting from inadequately funded cancer recording offices and wrong diagnoses [3]. Cancer treatments range from surgical operations, radiation and immune-mediated therapies, administration of chemotherapeutics and biological compounds, and combination treatments, some of which have toxicity and side effects [4, 5]. Drugs such as fluorouracil, methotrexate and doxorubicin are commonly used in cancer chemotherapy. Scientific evidence reveals that ethnobotanical species are vital sources of therapeutics and nutritional supplements [6]. They contain secondary

metabolites like phenolics with proven biological activity resulting from their synergistic or single action [7]. Thus, ethnobotanical extracts can be administered alone or in combination with orthodox treatment in order to alleviate the toxic effects of orthodox medicine, thereby widening the therapeutic slot of anticancer agents and removing the resistance of particular anticancer therapies [8-10].
The selection of plant materials in places where traditional medicine is part of the cultural heritage of the populace is crucial in drug discovery and the preservation of traditional medical knowledge [11]. Thus, ethnobotanical species can be screened for bioactive compounds and studied for biological activity in experimental models during the development of relevant lead drugs [12]. A biological activity like cytotoxicity is a toxicity assay for the prediction of the damaging effect of a substance on organs and tissues or the anticancer potential of the substance. The use of BSLA, the 3-(4,5-demethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye conversion method, and the in-vivo hollow fiber model can all cause cytotoxicity [13-15]. The BSLA is the most commonly used method and is regarded as a convenient, easy-to-understand, and cheap method that requires a small quantity of laboratory cultured nauplii [16]. Thus, this research studied the medicinal plants used for the treatment of cancer in some parts of the south-west of Nigeria (Ogun, Oyo, Osun and Lagos states) and evaluated them for bioactive components and cytotoxicity.

## 2. METERIALS AND METHOD

### 2.1 Ethnobotanical survey of the anticancer herbs

Selection of medicinal plants used for the treatment of cancer was conducted in some locations in Ogun, Oyo, Osun and Lagos states by a physical and virtual discussion with the personnel working in the trado-medical (TM) clinics. An introductory seminar was conducted with key stakeholders. The TMP were assured of the confidentiality and use of the information for research purposes only. They were asked for the local names, and parts of the anticancer plants used for the treatment.

### 2.1.1 Anticancer herb sampling and authentication

The names of relevant anticancer herbs were subjected to internet library search via Google and Google scholar to determine the already exploited anticancer plants. The un exploited were investigated, leaving twenty-six unexploited plants specimens were verified by taxonomists and deposited within 24 hours at the Herbarium of the University of Lagos, Department of Botany and Forestry Research Institute of Nigeria, Ibadan. A portion of each plant sample was cleaned, shade dried, ground into coarse powder using an industrial blender, and preserved in clearly labeled plastic jars.

### 2. 2 Preparation of the anticancer plant's extracts

Each of the selected plant samples was subjected to ethanolic extraction according to the modified protocol of Adebayo *et al.* [17]. The plant sample (100 g) was repeatedly soaked in 80% ethanol (600 mL) and shaken at 2000 rpm on a mechanical shaker for 48 hours until a clear extract solution was obtained. The filtrates were collected by the use of muslin cloth and filtered on Whatman No. 1 filter paper. The filtrated were pooled and concentrated in a rotary evaporator. The resulting concentrated (ethanol) extract was stored at -20$^0$C until analysis. The extraction yield was calculated as shown in the equation below.

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the crude extract x 100}}{\text{Weight of sample taken for extraction}} \quad \ldots\ldots\ldots\ldots\ldots\ldots 1$$

### 2. 3 Proximate and mineral contents of the anticancer plants

A portion of each of the freshly collected plants was subjected to proximate analysis using standard protocols described in the Association of Official Analytical Chemists (AOAC) methods. The moisture, ash, crude fibre, crude protein, total carbohydrate, and lipids (fats) in each sample were determined in triplicates according to standard methods [18]. The mineral elements, which include potassium, magnesium, calcium, copper, sodium, iron, and zinc, were analyzed by atomic absorption spectrophotometry according to the method of AOAC [18].

### 2. 3 Screening for bioactive components of the anticancer plants

The solutions of the ethanolic extracts of the anticancer plants were screened for bioactive compounds by means of standard protocols [19-21]. The total flavonoid content (TFC) anticancer plants was also determined using the modified aluminium chloride colorimetric method of Sulaiman et al. [22].

### 2. 3 Cytotoxicity using the brine shrimp lethality assay (BSLA)

Cytotoxicity of the ethanolic extracts was determined by means of BSLA. A solution of sea salt (38 g/ L) was made in distilled water and adjusted to pH 8.5 with the aid of 1 M NaOH. Brine shrimp eggs (cysts) were used as the test organism. A solution of sea salt (38 g/ L) was made in distilled water and adjusted to pH 8.5 with the aid of 1 M NaOH. The sea salt solution was filtered for clarity and used for the hatching of the brine shrimp's cysts. In all, stock solutions of each ethanolic extract (10 µg/ mL to 1000 µg/ mL) were prepared in 0.1% DMSO. The control test solution was also prepared with 0.1% DMSO and used for the BSLA. The stock extract solutions and the DMSO were sterilized in an autoclave for 6 hours. The brine shrimp eggs (cysts) purchased from a pet shop in Ikeja, Nigeria were used as the test

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organism. The clean seawater was poured into a small transparent plastic tank. The cysts were added to a covered side of the tank. The tank was subjected to constant illumination and supplied with oxygen via an aerator.
After 24 hours, ten nauplii free from eggshell were selected by the use of a dropping pipette into test tubes containing freshly prepared clear sea water (2.5 mL) and inspected using a magnifying lens. The ethanolic extract (2.5 mL) was added to the test tube. Condition of the nauplii was closely monitored for 48 hours to determine the number of nauplii that survived. The control containing 0.1% DMSO was also treated as above. The percentage lethality of the nauplii was calculated for each concentration in correlation with Abott's equation [23] and expressed as a median lethal concentration ($LC_{50}$) of the test nauplii after an exposure of 48 hours. This was determined by means of probit analysis.

$$\% \text{ Lethality} = \frac{(\text{Observed mortality} - \text{Control mortality}) \times 100}{(100 - \text{Control mortality})} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

**2. 3 Extraction of flavonoids in anticancer plants**

The protocol of [24] was adopted with little modifications. The extraction of flavonoids in the anticancer plant model was done by dissolving 0.02 g/ mL ethanolic extract of the anticancer model in 80% ethanol; the extract was extracted exhaustively with concentrated n-butanol. The resulting n-butanol fraction was acidified with HCl (10%) to pH 3 and concentrated at 40ºC leaving a dry brownish residue which was re-extracted with distilled water and ethyl acetate (1:1). The ethyl acetate fraction was basified with $NaHCO_3$ to pH 9, and concentrated at 40ºC.

**2. 3 DETECTION OF QUERCETIN AND ANTIOXIDANT AGENTS**

The method of Jesionek et al. [25] was applied to the detection of quercetin. A TLC plate (5 × 4 cm2) made from alumina was activated by heating at 100°c for 10 min and allowed to cool to room temperature. Ethanolic extract, flavonoid extract, or quercetin at a concentration (10 µl) was spotted separately using a micropipette with tips onto one edge of the pencil line. The plate was placed in a development chamber with various ratios of trial solvent mixtures of methanol, n-butanol, acetic acid, ethyl acetate, chloroform, and hexane. When the solvent front travelled to the top end, the TLC plate was removed and the solvent front was marked using a soft pencil. The plate was air-dried, sprayed with a fine spray of 1% ethanolic aluminium chloride solution, left to dry, visualized on camera and the retardation factors (Rf) were calculated and recorded. The methanol/chloroform/hexane mixture (7:2:1, v/v/v) produced the best separation of the spots.

$$\mathbf{Rf} = \frac{\textbf{Distance by the sport}}{\textbf{distance by the solvent}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

In the detection of antiradical compounds in the anticancer plant, the solvent mixture containing methanol/chloroform/hexane (7:2:1, v/v/v) was used as the mobile phase for the detection of antiradical agents. The ethanolic and flavonoid extracts were spotted on separate plates in relation to quercetin and allowed to travel to the top. The dried plates were sprayed with 0.004% w/v of DPPH in 95% ethanol and observed for the development of a bright yellow colour for confirmation of antioxidant compounds. The position of the solvent front was immediately marked and the Rf values were noted.

**2.7 ANTIOXIDANT ACTIVITY**

The ability of the extracts and quercetin to scavenge free radicals was determined *in vitro* using standard protocols. The superoxide radical (SOR) scavenging activity was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a slightly modified method of Jing and Zhao [26] at 420 nm and calculated by the following equation:

$$\% \textbf{ Scavenging activity} = \frac{100\ (\text{Absorbance of the control} - \text{Absorbance of the sample})}{\text{Absorbance of the control}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots 4$$

Also, the ability of the extract to scavenge $H_2O_2$ was determined according to the method by Dehpour et al. [27] using spectrophotometric analysis at 230 nm, and the percentage of scavenged $h_2o_2$ by the anticancer plant flavonoid extracts or the standards (quercetin and ascorbic acid) was calculated as in equation four (4). The total antioxidant capacity of the extract or standards was determined by the scavenging of DPPH radical scavenging activity [28]. The rate of change of the initial purple to yellow color of the DPPH was measured at 517 nm and calculated using equation four. All analysis was done in triplicates. The median inhibitory concentrations ($IC_{50}$) of the test samples were calculated from regression equations obtained from standard curves.

**2.9 STATISTICAL ANALYSIS**

The results of the study were expressed as Mean±S.E.M. Data analysis was done using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test by means of GraphPad Prism version 5 (produced by GraphPad Software Inc., CA, USA). When $p < 0.05$, the results were considered significant.

**3. RESULTS AND DISCUSSION**

Data obtained through the survey of anticancer herbs showed that the investigated areas contain a diversity of racial groups, comprising Ijebu, Egba, Oyo, Ijesha, Awori and Egun, which are generally referred to as the Yoruba tribe. There is

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at least one traditional medical practitioner (TMP) or complementary medical clinic in each community where patients are treated for different medical challenges. During the interview, the contacted people were reluctant to divulge the information regarding the anti-cancer recipes. At the end of the interview, data concerning a total of thirty-eight anticancer herbs (plants) belonging to different botanical family, and parts used and extraction procedures was realized, out of which there was a paucity of scientific data on only twelve of the anticancer plants (Table 1). Parts such as leaves, fruit and whole are prepared by maceration, infusion and decoction.

It is interesting to note that the twelve anticancer species tested contained bioactive substances like saponins, flavonoids, and alkaloids (Table 2), as well as proximate substances like moisture, ash, crude fiber, crude protein, total carbohydrate and lipids, and mineral elements like K, Mg, Ca, Cu, Na, Fe and Zn (Table 3 and Table 4). Eleven of the chosen anticancer plants had cytotoxicity lower than 1000 ug/ mL. In contrast hand, of all the plants examined, *L. egregia* had the greatest extraction yield (7.04 %) and flavonoid concentration (79.04 ug/ mL). Thus, *L. egregia* was taken as the anticancer plant model used in the subsequent analyses.

**Table 1. Ethnobotanical studies of the selected anticancer plant samples**

| S/N | Selected Samples | | | | Voucher Number | Parts of plant used | Extraction procedure |
|---|---|---|---|---|---|---|---|
| | Family | Vernacular name | Scientific name | | | | |
| 1. | Anacardiaceae | Ekudan | *Lannea egregia* | LE | FHI112970 | Leaves | Maceration, infusion |
| 2. | Moraceae | *Ipin* | *Ficus exasperata* | FE | LUH8206 | Leaves | Decoction, maceration |
| 3. | Euphorbiaceae | Ajeobale | *Croton zambesicus* | CZ | LUH8206 | Leaves | Maceration, decoction |
| *4.* | Fabaceae | Aridan | *Tetrapleura tetraptera* | TT | LUH8516 | Fruits | Decoction |
| *5.* | Combretaceae | Furutu | *Terminalia catappa* | ZZ | LUH8572 | Leaves | Decoction |
| 6. | Rutaceae | Orin ata | *Zanthoxylum zanthoxyloides* | TC | LUH6909 | Leaves | Decoction, infusion |
| 7. | Plumbaginaceae | Inabiri | *Plumbago zelanica* | PZ | LUH6910 | Leaves | Maceration |
| 8. | Phytolaccaceae | Ewe-epa | *Hilleria latifolia* | HL | LUH6908 | Whole plant | Decoction |
| 9. | Crassulaceae | Abamoda | *Bryophyllum pinntum* | BP | LUH8577 | Leaves | Maceration |
| 10. | Asteraceae | Akintola | *Chromolena odorata* | CO | LUH8575 | Leaves | Decoction |
| 11. | Connaraceae | Amuje | *Byrsocarpus coccineus* | BC | LUH8199 | Leaves | Decoction |
| 12 | Anacardiaceae | Iyeye | *Spondias mombin* | SM | LUH8753 | Leaves | Infusion |

**Table 2: Phytochemical composition of the selected anticancer plants**

| Parameters | Tannins | Saponins | Flavonoids | Alkaloids | Cardiac glycosides | Steroids | Terpenoids |
|---|---|---|---|---|---|---|---|
| LE | + | + | + | + | - | + | + |
| FE | - | + | + | + | + | _ | + |
| CZ | + | + | + | + | + | + | + |
| TT | + | + | + | + | + | + | + |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| TC | + | + | + | + | + | + | + |
| ZZ | + | + | + | + | - | + | - |
| PZ | + | + | + | + | + | + | + |
| HL | + | + | + | + | - | + | + |
| BP | + | + | + | + | + | + | + |
| CO | + | + | + | + | + | + | - |
| BC | + | + | + | + | + | + | + |
| SM | + | + | + | + | + | + | + |

**Table 3: Proximate compositions of the selected anticancer plants**

| Parameters | Moisture (%) | Ash (%) | Crude fiber (%) | Protein (%) | Carbo-hydrate (%) | Lipid (%) |
|---|---|---|---|---|---|---|
| LE | 12.53±0.12 | 7.63±0.04 | 9.47±0.13 | 10.75±0.06 | 48.31±0.04 | 2.09±0.01 |
| FE | 9.31±0.10 | 13.08±0.03 | 7.99±0.03 | 14.12±0.02 | 45.29±0.03 | 5.67±0.03 |
| CZ | 8.15±0.04 | 6.54±0.03 | 8.51±0.04 | 8.07±0.03 | 52.50±0.10 | 1.95±0.11 |
| TT | 12.27±0.02 | 3.89±0.02 | 5.66±0.05 | 23.69±0.04 | 47.22±0.04 | 7.27±0.01 |
| TC | 17.76±0.01 | 6.89±0.03 | 9.73±0.03 | 7.42±0.02 | 53.84±0.11 | 1.83±0.01 |
| ZZ | 15.25±25 | 2.94±0.04 | 12.36±0.17 | 2.45±0.02 | 65.81±0.23 | 1.06±0.02 |
| PZ | 11.44±0.02 | 4.80±0.02 | 24.23±0.11 | 2.87±0.02 | 55.51±0.12 | 1.15±0.01 |
| HL | 20.30±0.11 | 5.22±0.03 | 22,06±0.28 | 3.88±0.02 | 54.26±0.15 | 2.05±0.01 |
| BP | 14.98±0.04 | 7.81±0.03 | 10.10±0.02 | 9.79±0.02 | 50.81±0.02 | 2.54±0.01 |
| CO | 16.25±0.02 | 5.77±0.04 | 8.84±0.02 | 11.65±0.05 | 55.15±0.05 | 2.35±0.02 |
| BC | 7.11±0.03 | 3.16±0.02 | 12.75±0.19 | 8.42±0.42 | 69.28±1.01 | 2.33±0.02 |
| SM | 14.89±0.70 | 1.98±0.02 | 10.59±0.05 | 11.01±0.04 | 65.56±1.05 | 5.01±0.01 |

*Results presented as Mean+ S.E.M.

Eleven of the chosen anticancer plants had cytotoxicity lower than 1000 ug/ mL. In contrast, of all the plants examined, *L. egregia* had the greatest extraction yield (7.04 %) and flavonoid concentration (79.04 ug/ mL). Thus, *L. egregia* was taken as the anticancer plant model used in the subsequent analyses.

**Table 4: Mineral compositions of the selected anticancer plants**

| Parameters | K (ppm) | Mg (ppm) | Ca (ppm) | Cu (ppm) | Na (ppm) | Fe (ppm) | Zn (ppm) | Na/K |
|---|---|---|---|---|---|---|---|---|
| LE | 37.14± 0.10 | 5.71± 0.01 | 156.12± 0.10 | 2.26± 0.00 | 3.11± 0.01 | 0.83± 0.00 | 0.14± 0.01 | 0.08 |
| FE | 32.10± 0.87 | 2.94± 0.04 | 240.72± 0.03 | 1.41± 0.02 | 5.21± 0.02 | 3.92± 0.10 | 0.72± 0.02 | 0.16 |
| CZ | 27.40± | 5.21± | 133.20± | 0.20± | 2.7± | 1.32± | 0.15± | 0.10 |

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|---|---|---|---|---|---|---|---|---|
| | 0.01 | 0.01 | 0.01 | 0.00 | 0.05 | 0.01 | 0.00 | |
| TT | 29.02± 0.04 | 5.25± 0.02 | 149.49± 0.05 | 1.82± 01 | 2.68± 0.01 | 1.17± 0.01 | 0.10± 0.00 | 0.10 |
| TC | 17.94± 0.20 | 14.27 ±0.07 | 108.03± 0.10 | 0.28± 0.02 | 1.4±0. 02 | 0.08± 0.01 | 0.02± 0.01 | 0.08 |
| ZZ | 31.09± 0.30 | 0.39± 00 | 109.8± 0.21 | 0.49± 02 | 6.31± 0.01 | 4.29± 0.02 | 1.72± 0.01 | 0.20 |
| PZ | 20.01± 0.04 | 9.04± 0.08 | 112.62± 0.05 | 0.18± 0.01 | 2.99± 0.01 | 2.63± 0.02 | 0.64± 0.04 | 0.15 |
| HL | 24.09± 0.07 | 1.08± 0.03 | 100.58± 0.00 | 0.33± 0.00 | 2.74± 0.01 | 0.09± 0.01 | 0.5±0. 05 | 0.11 |
| BP | 26.09± 0.60 | 5.23± 0.02 | 144.38± 0.10 | 3.23± 0.10 | 3.11± 0.01 | 1.11± 0.01 | 1.27± 0.01 | 0.12 |
| CO | 24.81± 0.20 | 6.33± 0.04 | 161.38± 0.05 | 2.10± 0.02 | 2.51± 0.02 | 1.02± 0.01 | 0.32± 0.01 | 0.10 |
| BC | 28.01± 0.20 | 2.96± 0.20 | 485.17± 0.21 | 1.71± 0.02 | 4.01± 0.01 | 1.69± 0.21 | 0.32± 0.01 | 0.14 |
| SM | 16.52± 0.04 | 7.22± 0.01 | 288.41± 0.30 | 1.62± 0.02 | 2.91± 0.03 | 1.12± 0.02 | 1.23± 0.02 | 0.18 |

*Results presented as Mean+ S.E.M.

**Table 5. Percentage extraction yields and cytotoxicity of the selected anticancer plants**

| Plant samples | Extraction yield (%) | Cytotoxicity ($LC_{50}$ ug/ mL) |
|---|---|---|
| LE | 7.04±0.02 | 09.46±1.71 |
| FE | 6.61±0.09 | 9646.62±2.87* |
| CZ | 3.36±0.09* | 160.09±5.19* |
| TT | 4.16±0.01* | 307.11±3.01* |
| TC | 6.83±0.01 | 87.38±2.01* |
| ZZ | 2.12±0.03* | 86.85±3.30* |
| PZ | 3.43±0.03* | 27.89±2.83* |
| HL | 2.29±0.08* | 63.19±0.48* |
| BP | 5.70±0.08* | 28.23±2.71* |
| CO | 5.61±0.07* | 143.23±2.17* |
| BC | 4.65±0.12* | 17.15±2.13 |
| SM | 3.92±0.45* | 153.12±3.45* |

*Values with (*) are significantly difference from others and are displayed as Mean+ S.E.M.

In the detection of quercetin using $AlCl_3$ (Plate 1), one yellow band corresponding to Rf 0.93 was observed in the quercetin (Q), ethanolic (Lu) and the flavonoid extracts (LE) of the anticancer model. Moreover, Plate 2 depicts the presence of three yellow bands (Rf 0.93, 0.13 and 0.79) in the anticancer model extracts against the purple DPPH background on the chromatogram.

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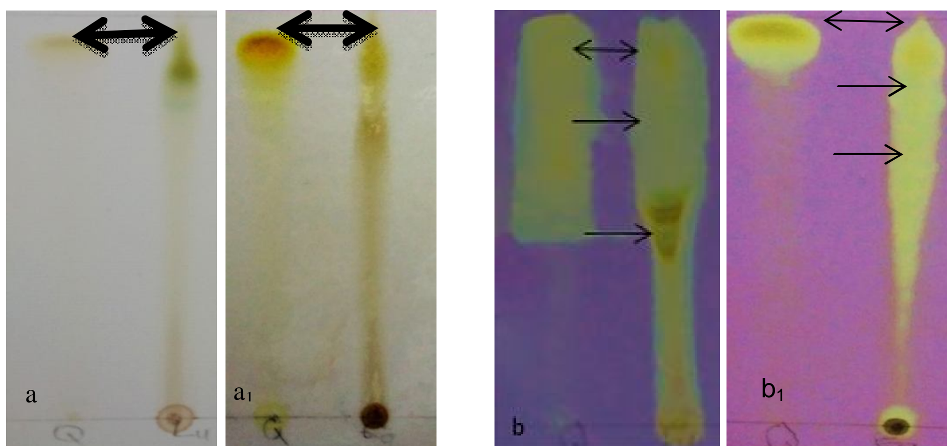
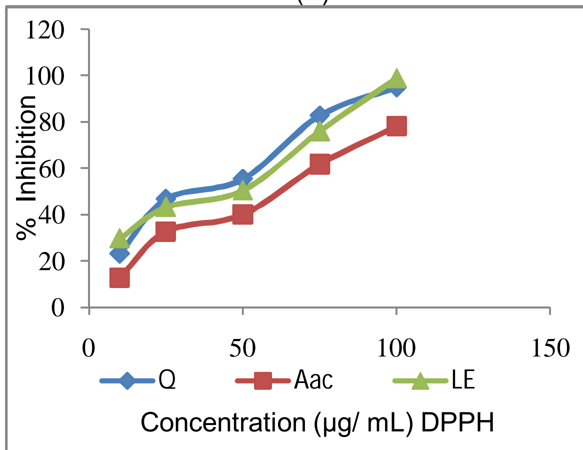


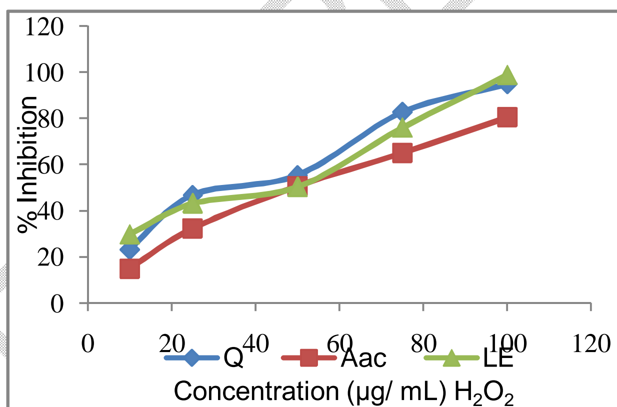

**Plate 1. Detection of quercetin** **Plate 2. Detection of antiradical agents**
*(a) Ethanolic extract of *L. egregia* (Lu), ($a_1$) Flavonoid extract of *L. egregia* (LE), Quercetin (Q),

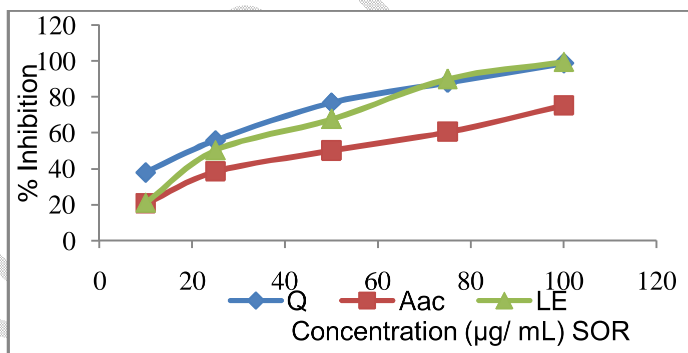
Figure 1 reveal there was a dose-dependent scavenging of SOR, $H_2O_2$ and DPPH radicals by the extract. Quercetin, Q (30.44 µg/ mL) > flavonoid extract, LE (34.72 mg/ L) > ascorbic acid, Aac (59.11µg/ L).

(A)




(B)




(c)

**Fig. 1.(A-C) Antiradical activity of anticancer plant flavonoid extract**

In developing nations like Nigeria, traditional medicine uses natural resources like plants and animals in its healthcare systems to cure ailments like cancer. A significant amount of active principles with an anticancer effect can be found in the majority of medicinal plants. Thus, phenolics like flavonoids obtained by proper selection of effective medicinal recipes can be used as templates for drug detection and investigating their mode of action [29]. An introductory seminar was conducted with key stakeholders in order to reduce the bias of information. Data from the survey on the anticancer plants

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showed that there are diverse racial groups in the investigated Yoruba communities, and this is mirrored in their dialects (Ijebu, Egba, Oyo, Ijesha, Awori and Egun). It was common unhealthy individuals to visit traditional medical (TM) or complementary medical (CM) clinics since every community visited possessed at least one TM or CM. Because the practice of TM or CM is based on inherited knowledge from ancestors, the contacted personnel were unwilling to divulge information about the anticancer recipes. It was fascinating that there was a rapid interest in the study on medicinal plants, including anticancer recipes, since only twelve of the cited anticancer plants were not exploited in related research [30]. Methods of preparation of these remedies include the maceration, infusion, and decoction of leaves, fruit, and whole parts of anticancer plants. This is in accordance with the report of Segun [31]. One of the major challenges recorded through data from the survey includes the inability of the TM and CM personnel to regulate the dosages for various administrations given to cancer patients. It is noteworthy that, aside from cancer treatment, the respondents affirmed that traditional medical knowledge could also be used to manage other diseases. Thus, this claim supports scientific investigation of the anticancer, cytotoxicity, and antimalarial properties of ethnobotanical samples such as roots, barks, leaves, or whole plants [30, 32, 33]. Just as in scientific investigations, the selected species were well identified in order to ensure miss-identification of the collected specimens by taking the samples to the herbarium, authenticating and depositing them in the herbarium while their voucher numbers were obtained for record purposes [34]. Moreover, the mostly used parts of these plants was the leaf. This corresponds with previous scientific reports, suggesting the use of easily accessible parts like leaf in herbal preparations than other parts [35].

It is commonly believed that plant extracts obtained by appropriate selection and preparation in accordance with traditional use would contain multiple molecules with antitumor activities, and are effective in killing human cancer cells and eliminating the toxic effects of synthetic chemotherapies [36]. Therefore, the information obtained through the oral seminar was correlated to the biological activities of the suggested plant extracts. Thus, the preliminary phytochemical analysis was used to determine the chemical contents of the suggested anticancer plant materials and their typical biological activities like antioxidant and cytotoxicity were investigated using standard protocols [37]. The study indicated the presence of bioactive compounds (saponins, flavonoids, and alkaloids), which justify their use in traditional medicines, characterization of the anticancer herbs, and suggest their propriety in drug development [38]. Interestingly, compounds such as saponins and flavonoids are known for their anticancer, antioxidant, anti-diabetes, antihepatotoxic, and anti-inflammatory activities and the prevention of cardiovascular dysfunction [39-41]. The nitrogenous compounds (alkaloids) are antidepressant and antitussive drugs [42, 43].

Interestingly, all the anticancer species showed biological activity to varying degrees. The quantity of the bioactive compounds in the plant extracts was greatly affected by their concentration in the selected plant rather than the solubility in extracting solvents since the same solvent was used for their extraction. This was reflected in the estimated extraction yields and the total flavonoid contents of the anticancer plants [44]. In a correlated report, Oliveira *et al*. [45] recommended the use of a mixture of ethanol (instead of methanol) and water for the solubilization of moderately polar compounds like flavonoids. This solvent mixture is appropriate because of its low cost, environmental impact, and low toxicity. This implies ethanol as an effective solvent for the extraction of flavonoids and *L. egregia* with maximum flavonoid content (79.04 ug/ mL quercetin equivalents) and cytotoxicity as the most potent anticancer plants. Therefore, the extract of *L. egregia* might contain more polar to mid-polar soluble compounds than other plant extracts. Truong *et al*. [46] reported the variation in selected extracting solvents as a factor responsible for the characterized type of natural products. The bioactive compounds in medicinal plants such as *Bulung Sangu, Coronopus didymus* and *Limnophila aromatica* were also reported in previous research [47-49]. These findings indict *L. egregia* as the most potent flavonoid anticancer plant extract.

Appreciable proximate and elemental compositions may be used to classify foods and plant materials [50]. Natural minerals (zinc, iron, calcium, and copper) are considered the most valuable phytonutrients [51]. The availability of the proximate parameters (moisture, ash, crude fiber, crude protein, total carbohydrate, and lipid) and the mineral elements in all the selected anticancer plants supports their relevance as nutritional supplements and possible immune enhancers that may prevent toxicity in cancer patients [38].

Cytotoxicity is a bioassay that detects broad-spectrum biological activity in many scientific disciplines [52]. As a result of its simplicity, rapidity, economic value, and ability to detect potent antitumor or anticancer principles, the bioassay was used to verify the scientific basis for the use of the selected plants as anticancer therapy [37]. Excitingly, the cytotoxicity of eleven of the selected anticancer plants was less than 1000 $\mu$g/ mL, and can be considered cytotoxic in accordance with the scale of Meyer [53]. The cytotoxicity of *L. egregia* ($LC_{50} \le 20$) was comparable with that of *B. coccineus* despite its relatively lower flavonoid content, and both plants can be regarded as very active cytotoxic agents. Comparatively, the other plants' extracts can be classified as active cytotoxic ($LC_{50} \le 100$ $\mu$g/ mL) with the exception of *F. exasperata* which can be said to be non-toxic ($LC_{50} \le 1000$ $\mu$g/ mL) [54]. Moreover, the toxicity of the plant extracts to the active nauplii can be seen as a programmed cell death (apoptosis), implicating the lethality as cytotoxicity on the nauplii and suggesting the action as from an anticancer drug arising from the inhibition of topoisomerases, elongation of fibers, or alteration of the cell cycle [55]. Besides, many cytotoxic plants reportedly possess anticancer, cytotoxic or antitumor properties, presumably caused by the free radical scavenging activity of extractable phytochemicals such as polyphenols (flavonoids)

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and alkaloids [56, 57]. Also, a significant correlation of BSLA cytotoxicity with the anticancer properties of medicinal plants has also been documented [58]. In related studies, the anticancer activity of botanical species such as *Mentha piperita* (L), *Urtica massaica*, and *Tragia involucrata* (L) was demonstrated [59].

TLC analysis is usually thought of as a vital separation process that precedes column chromatography in order to reduce the cost and wastage of reagents or chemicals [60]. It is also applied as TLC bioautography (TLB) to detect compounds with antioxidant, antidiabetic, or antimicrobial activity. In the TLB profiling of the anticancer model extracts (*L. aegregia* leaf) using diverse solvent mixtures, the presence of quercetin was obvious as a yellow colored band amidst the brown to green bands during the TLC fingerprint involving combinations of hexane, chloroform: methanol (1:2:7). This indicts the solvent mixture as an effective solvent for the isolation of the plant's quercetin and some other active compounds in the plant extracts. In a recent report, separation of flavonoids was achieved in the extracts of *Grewia bicolar* , *Carissa bispinosa* and *Ficus sycomorus* by this same solvent combination [61]. Other solvent mixtures have been applied for the separation of flavonoids. These include the combination of toluene: ethyl acetate: formic acid in a 58: 33: 9 [62] and ethylacetate: acetone: acetic acid: water in a ratio of 6:2:1:1 (Birk *et al.,* 2005). Furthermore, the presence of a mixture of bioactive components illustrates the ability of some of these compounds to work in synergy during biological actions like cytotoxicity, anticancer, antimicrobial, and antioxidant properties [63, 64]. Flavonoids including quercetin have been unearthed by the use of TLB to analyse *Leea indica* and *Ageratum conyoides* [62].

Besides, Afolabi *et al.* [65] demonstrated that flavonoids are effective therapeutic mediators in the treatment of oxidative stress-related assaults. Moreover, the characterization of flavonoids' antioxidant activity and their antioxidative effects has been of immense interest [66]. In this study, TLB-DPPH profiling validated the antiradical potential of quercetin and at least two other compounds in the flavonoid extract of the anticancer plant. Some of these compounds appeared similar to the flavonoids in the $AlCl_3$ stained bioautography TLC bioautography is a screening test through which biological activity like antiradical, antimicrobial, or antidiabetic activity of a natural product can be qualitatively investigated [67]. In this study, the purple-colored DPPH was neutralized by the quercetin or antiradical agents in the extract, which donated hydrogen and formed yellow-colored diphenyl-picrylhydrazine [68]. Thus, the plant extracts contained antioxidant compounds like flavonoids (quercetin) [69]. The findings in this experiment are also in accordance with those of scientists [25, 70]. Evaluation of antioxidant activity is a way of determining biological activity. The inclusion of antioxidants into the diet or medicinal remedy protects the body against the damaging effects of free radicals, which may harm the genes, alter proteins and the immune system, and affect lipid peroxidation in order to cause cancer [71]. The antioxidant activity of anticancer plants like *L. egregria* was previously reported by Idowu *et al.* [72]. Thus, the antioxidant property might have donated electrons to free radicals produced by DPPH, SOR, or $H_2O_2$ and neutralized their radicals. Thus, the administration of the selected anticancer plants by the TMP might be preventing oxidative stress-associated diseases like cancer [73].

**4.1 CONCLUSION**

The cytotoxic bioactive compounds such as flavonoids in the selected anticancer plants' extracts provide medical support to the exploitation of the understudy plants in traditional medical practice for cancer treatment. Besides, the presence of proximate compounds, mineral elements, and antioxidant characterisers like quercetin in the plant extract further justified the traditional use of the anticancer plants as a traditional remedy for deficient nutritional and medical supplements and as a possible chemotherapy-adjuvant for patients receiving treatment from TMP. Moreover, the compounds may serve as novel anticancer agents.

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