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

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Authors' contributions

This work was carried out in collaboration among all authors. Each author had a separate role in the project's design, data collection, writing of the initial and amended drafts of the article, and revision the draft paper. This completed manuscript was given their approval for publishing. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Medicinal plants used by traditional medical practitioners (TMP) to treat cancers are considered safe when used alone or combined 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 for physiochemical composition and a possible antioxidant and cytotoxic potential to validate the basis for the use of the selected anticancer plants.
Methodology: Online academic literature searches were done on the cited plants to identify the already-exploited anticancer plants. The ethanolic extracts of the plant were examined for the presence of bioactive components and their total flavonoid content, with focusing 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 mineral 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 µg/mL) and cytotoxicity (9.46 µg/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 µg/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 plant's 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 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].

Many current cancer treatment methods are expensive and not readily accessible. 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 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]. Consumption of anticancer medicinal plants results in a low incidence of cancer among the populace, and 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]. Some of the first plant-derived anticancer agents were vinca alkaloids, vincristine, vinblastine, and cytotoxic agents (podophyllotoxins) [12]. Likewise, the under studied anticancer plants (*C. zambesicus*, *T. tetraptera*, *T. catappa, Z. zanthoxyloides*, *P. zelanica*, *H. latifolia*, *B. pinntu*m, *C. odorata*, *B. coccineus and S. mombin*) are available locally. They are used for the treatment of ailments including cancer, epilepsy, gonorrhoea and diabetes mellitus. The plants' biological activities, such as antioxidant, antibacterial, anticancer anti-inflammatory and anticonvulsant, were previously documented [13-15]. The physiochemical composition and putative pharmacological activities of understudied plants can be explored in order to justify the rationale for their usage and demonstrate potential as lead drugs.

Biological activity is a method of investigating a chemical substance's mechanism of action. Plant compounds can be probed for possible biological activity and documented as antioxidant, anticancer, anti-inflammatory and others. 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 cause cytotoxicity [16-18]. 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 [19]. The ethnobotanical species can be screened for bioactive compounds and studied for biological activity in experimental models while developing relevant lead drugs [20]. Thus, this research studied the medicinal plants used for cancer treatment in some parts of the south-west of Nigeria (Ogun, Oyo, Osun and Lagos states) and evaluated them for bioactive components and cytotoxicity in other to establish their potential use as anticancer remedies.

2. EXPERIMENTAL DETAILS

2.1 Ethnobotanical Survey of the Anticancer Herbs

Selection of medicinal plants used for cancer treatment 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 an internet library search via Google and Google scholar to determine the already exploited anticancer plants. The unexploited were investigated, leaving twenty-six exploited 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 a coarse powder using an industrial blender, and preserved in clearly labelled plastic jars.

2.2 Preparation of the Anticancer Plant's Extracts

Each of the selected herb samples was subjected to ethanolic extraction according to the modified protocol of Adebayo *et al*. [21]. 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 using a muslin cloth and filtered on Whatman No. 1 filter paper. The filtrates from each plant were pooled and concentrated in a rotary evaporator. The resulting concentrated extracts were regarded as ethanol extract of each plant. The extracts were stored at -200C until analysis. The extraction yield was calculated as shown in the equation below.

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

2.3 Proximate and Mineral Contents of the Anticancer Herbs

A portion 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. 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 [22].

2.4 Screening for Bioactive Components of the Anticancer Herbs

The solutions of the ethanolic extracts of the anticancer plants were screened for bioactive compounds using standard protocols [23-25]. The total flavonoid content (TFC) of anticancer plants was also determined using the modified aluminium chloride colourimetric method of Sulaiman *et al.* [26].

2.5 Cytotoxicity Using the Brine Shrimp Lethality Assay (BSLA)

Cytotoxicity of the ethanolic extracts was determined using BSLA. A solution of sea salt (38 g/L) was made in distilled water, adjusted to pH 8.5 with 1 M NaOH, and used for the culture. The sea salt solution was filtered for clarity and used for the culture by hatching the brine shrimp's eggs (cysts) at room temperature. 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 organism. The clean seawater was poured into a small transparent plastic tank. The cysts were added to a covered side of the tank, subjected to constant illumination and supplied oxygen via an aerator.

After 24 hours, ten nauplii free from eggshells were selected using 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. The 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 Abbott's equation [27] and expressed as a median lethal concentration (LC50) of the test nauplii after an exposure of 48 hours. The LC_{50} was determined through probit analysis.

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

2.6 Extraction of Flavonoids in Anticancer Plants

The protocol of Lee and his group [28] 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.

2.7 Detection of Quercetin and Antioxidant Agents

The method of Jesionek *et al.* [29] was applied to detect quercetin. A TLC plate (5 × 4 cm2) made from alumina was activated by heating at 100°c for 10 min and cooled 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, and visualized on camera. 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.

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

In detecting 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 detecting antiradical agents. The ethanolic and flavonoid extracts were spotted on separate plates concerning quercetin detection 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 bright yellow colour for confirmation of antioxidant compounds. The position of the solvent front was immediately marked, and the Rf values were noted.

2.8 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 [30] at 420 nm and calculated by the following equation:

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

Also, the ability of the extract to scavenge H_2O_2 was determined according t o the method by Dehpour et al. [31] 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 [32]. The rate of change of the initial purple to the yellow colour of the DPPH was measured at 517 nm and calculated using equation four. All analysis was done in triplicates. The test samples' median inhibitory concentrations (IC_{50}) were calculated using 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 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.

Ethanol, a low-toxic polar solvent able to solubilize polar or intermediate antioxidant compounds, was used for this investigation. 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 µg/mL. In contrast hand, of all the plants examined, *L. egregia* had the greatest extraction yield (7.04%) and flavonoid concentration (79.04 µg/mL). Thus, *L. egregia* was taken as the anticancer plant model used in the subsequent analyses.

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

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.

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	+	+	+	+	+	+	+
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.*

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±0.01	5.21±0.01	133.20±0.01	0.20±0.00	2.7±0.05	1.32±0.01	0.15±0.00	0.10
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} µg/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*

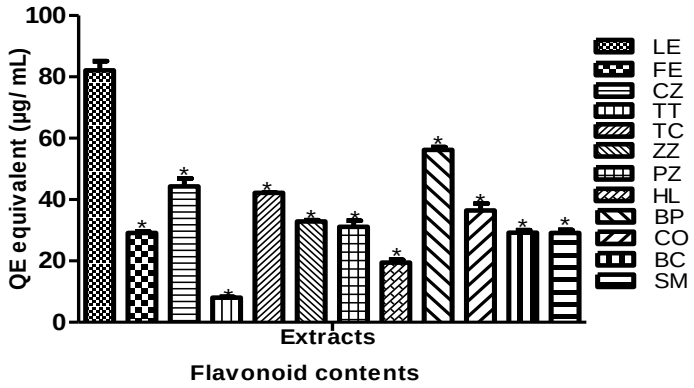

Fig. 1. Flavonoid contents measured in quercetin equivalent and presented as mean ± S.E.M. values with (*) are significantly difference from others at $P < 0.05$

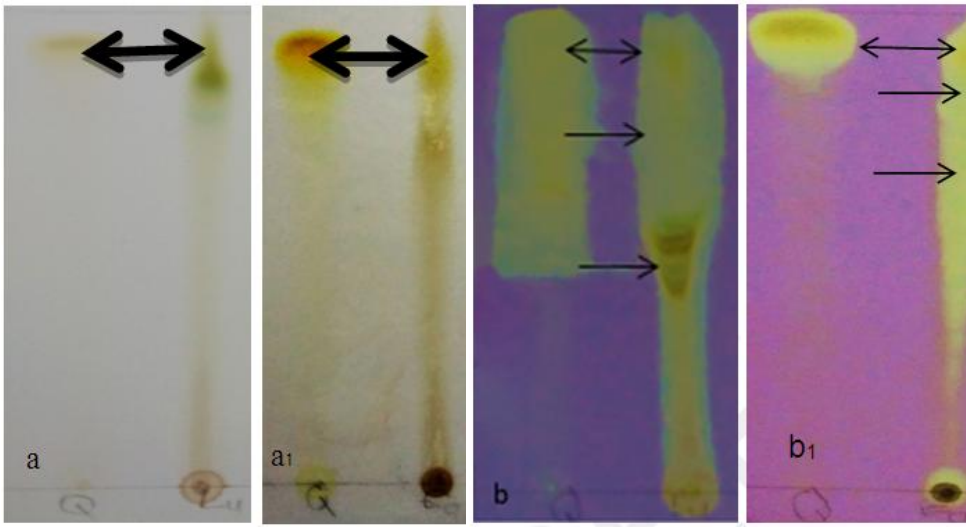

Plate 1. Detection of quercetin **Plate 2. Detection of antiradical agents**

**(a) Ethanolic extract of L. egregia (Lu), (a_1) Flavonoid extract of L. egregia (LE), Quercetin (Q),*

Fig. 2. reveals a dose-dependent scavenging of SOR, H2O2 and DPPH radicals by the extract. Quercetin (Q) flavonoid extract (LE) and ascorbic acid (Aac)

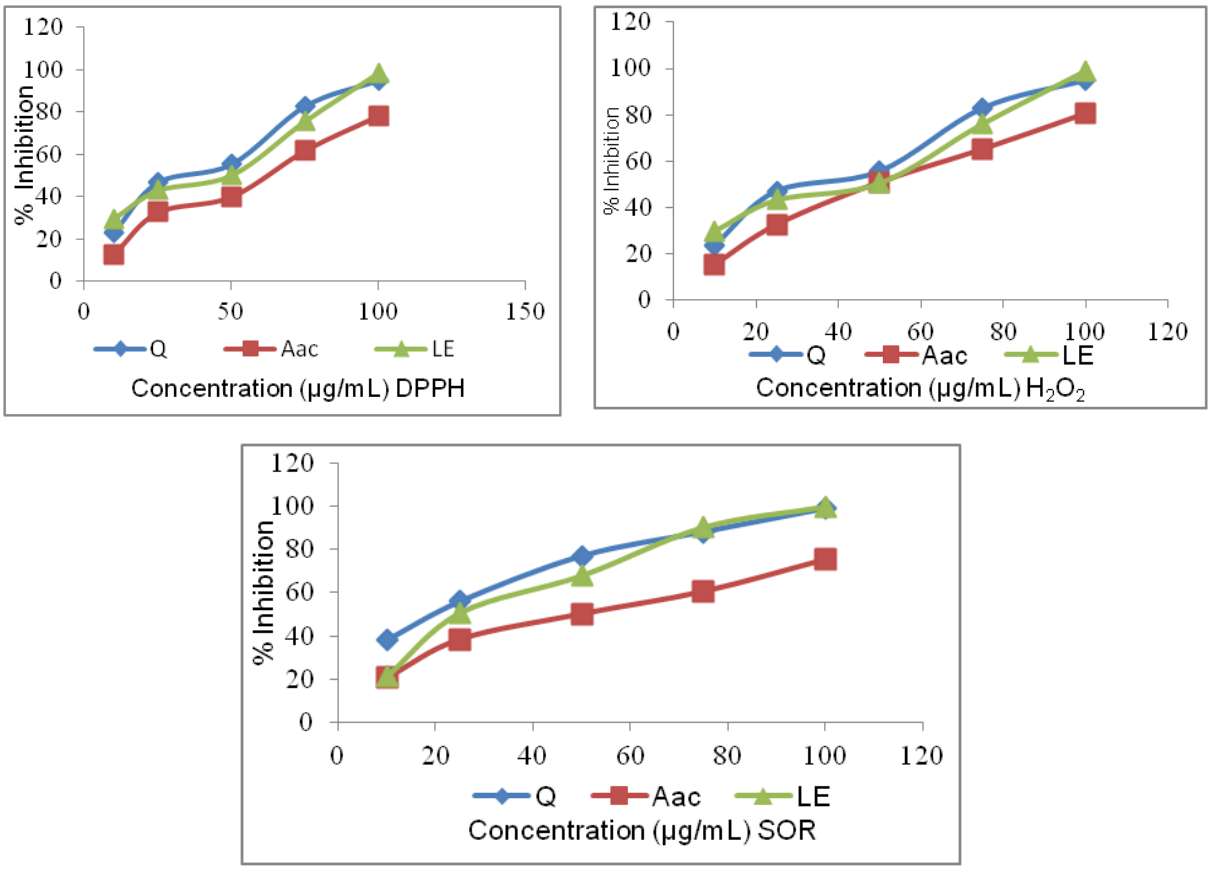

Fig. 2. Antiradical activity of anticancer plant flavonoid extract

Table 6. Median inhibitory concentration (IC50) of the flavonoid extract

Samples	Quercetin	Ascorbic acid (Aac)	*L. egregia*
	IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)
Superoxide radical	18.31 ± 0.52	53.67 ± 0.96	29.40± 0.10
H_2O_2	38.22± 0.25	61.33 ± 0.81	34.53± 0.91
DPPH	30.44 ±0.80	59.11 ± 0.21	34.72 ± 0.01

**Results are presented as Mean ± S.E.M.*

Table 6 displays the median inhibitory concentrations for the test samples, which indicate the overall antioxidant activity (IC50). The anticancer model's (L. egregia), LE activity (34.72 µg/mL) was higher than ascorbic acid, Aac (41.68 µg/mL) but slightly less effective than quercetin, Q (30.44 µg/mL). In this instance, the sequence of the IC50 was Q (30.44 µg/mL)> LE (34.72 µg/mL)> Aac (59.11 µg/mL).

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 [33]. An introductory seminar was conducted with key stakeholders to reduce information bias. Data from the survey on the anticancer plants showed that there are diverse racial groups in the investigated Yoruba communities, mirrored in their dialects (Ijebu, Egba, Oyo, Ijesha, Awori and Egun). It was common for 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 of medicinal plants, including anticancer recipes since only twelve of the cited anticancer plants were not exploited in related research [34]. Methods of preparation of these remedies include the maceration, infusion, and decoction of leaves, fruit, and whole parts of anticancer plants. This finding is per the report of Segun [35]. One of the significant 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 [34,36,37]. As in scientific investigations, the selected species were well identified to ensure the 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 [38]. Moreover, the primarily used part of these plants was the leaf, which corresponds with previous scientific reports, suggesting the use of easily accessible parts like the leaf in herbal preparations than other parts [39].

It is commonly believed that anticancer plant extracts obtained by appropriate selection and preparation following traditional use would contain multiple molecules with antitumor activities. They effectively kill human cancer cells and eliminate synthetic chemotherapies' toxic effects [40]. 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. Their typical biological activities, like antioxidant and cytotoxicity, were investigated using standard protocols [41]. 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 [42]. Interestingly, compounds such as saponins and flavonoids are known for their anticancer, antioxidant, anti-diabetes, anti-hepatotoxic, and anti-inflammatory activities and the prevention of cardiovascular dysfunction [43-45]. Nitrogenous compounds (alkaloids) are antidepressant and antitussive drugs [46,47].

Excitingly, 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 [48]. In a correlated report, Oliveira et al. [49] 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 is an effective solvent for extracting flavonoids and L. egregia with maximum flavonoid content (79.04 µg/mL quercetin equivalents) and cytotoxicity as the most potent anticancer plants. Therefore, L. egregia might contain polar to mid-polar soluble compounds than other plant extracts. Truong et al. [50] 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 [51-53]. 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 [54]. Natural minerals (zinc, iron, calcium, and copper) are considered the most valuable phytonutrients [55]. The availability of the proximate parameters (moisture, ash, crude fibre, 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 [42].

Cytotoxicity is a bioassay that detects broad-spectrum biological activity in many scientific disciplines [56]. 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 [41]. Excitingly, the cytotoxicity of eleven of the selected anticancer plants was less than 1000 µg/mL and can be considered cytotoxic following the scale of Meyer [57]. The cytotoxicity of L. egregia (LC50 ≤ 20 µg/mL) 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 (LC50 ≤ 100 µg/mL) except for F. exasperata which can be said to be non-toxic (LC50 ≤ 1000 µg/mL) [58].

Moreover, the toxicity of the plant extracts to the active nauplii can be regarded as 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 fibres, or alteration of the cell cycle [59]. 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) and alkaloids [60,61]. Also, a significant correlation between BSLA cytotoxicity with the antitumor or anticancer properties of medicinal plants has been documented [62]. In related studies, the anticancer activity of botanical species such as Mentha piperita (L), Urtica massaica, and Tragia involucrata (L) was demonstrated [63].

TLC analysis is considered a vital separation process that precedes column chromatography to reduce the cost and wastage of reagents or chemicals [64]. It is 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 evident as a yellow-coloured 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 antioxidant compound like quercetin and some other active compounds in the plant extracts. In a recent report, the separation of flavonoids was achieved in the extracts of Grewia bicolar , Carissa bispinosa and Ficus sycomorus by this same solvent combination [65]. 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 [66] and ethyl acetate: 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 [67,68]. Flavonoids, including

quercetin, have been unearthed using TLB to analyze Leea indica and Ageratum conyoides [66].

Besides, Afolabi et al. [69] 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 [70]. 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. These compounds appeared similar to the flavonoids in the aluminium chloride3-stained bioautography. TLC bioautography is a screening test through which a natural product's biological activity, like antiradical, antimicrobial, or antidiabetic, can be investigated qualitatively [71]. In this study, the purple-coloured DPPH was neutralized by the quercetin or antiradical agents in the extract, which donated hydrogen and formed yellow-coloured diphenyl-picrylhydrazine [72]. Thus, the plant extracts contained antioxidant compounds like flavonoids (quercetin) [73]. The findings in this experiment are also in line with reports from scientists [29,74]. 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 to cause cancer [75]. The antioxidant activity of anticancer plants like L. egregria was previously reported by Idowu et al. [76]. Thus, the antioxidant property might have donated electrons to free radicals produced by DPPH, SOR, or H2O2 and neutralized their radicals. Thus, the administration of the selected anticancer plants by the TMP might prevent oxidative stress-associated diseases like cancer [77].

4. 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 characters 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. Thus, it is recommended for the plants bioactive principles be characterized and investigated for propriety in anticancer drug development.

CONSENT

The consent of each participant involved in the oral survey was obtained before the study commenced.

ETHICAL CONSEDRATIONS

The authors declare that there is no ethical concerns.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Uchendu OJ. Cancer incidence in nigeria: A tertiary hospital experience. Asian Pacific Journal of Cancer Care. 2020;5(1): 27-32.
2. Jedy-Agba E, Curado MP, Ogunbiyi O, Oga E, Fabowale T, Igbinoba F, et al. Cancer incidence in nigeria: A report from population-based cancer registries. Cancer epidemiology. 2012;36(5): e271-e8.
3. Bray F, Jemal A, Grey N, Ferlay J, Forman D. Global cancer transitions according to the human development index (2008–2030): A population-based study. The lancet oncology. 2012;13(8):790-801.
4. Abbas Z, Rehman S. An overview of cancer treatment modalities. Neoplasm. 2018;1:139-57.
5. Aslam MS, Naveed S, Ahmed A, Abbas Z, Gull I, Athar MA. Side effects of chemotherapy in cancer patients and evaluation of patients opinion about starvation based differential chemotherapy. Journal of Cancer Therapy. 2014;2014.

6. Adedosu OT, Imodoye SO, Adeleke GE, Ajiboye A. Aqueous extract of Hibiscus sabdariffa calyx showed antioxidative effects and ameliorate acetaminophen (paracetamol)-induced hepatotoxicity and nephrotoxicity in rats.
7. Shukla S, Mehta A. Anticancer potential of medicinal plants and their phytochemicals: A review. Brazilian Journal of Botany. 2015;38(2):199-210.
8. De Melo JG, Santos AG, de Amorim ELC, Nascimento SCd, de Albuquerque UP. Medicinal plants used as antitumor agents in brazil: An ethnobotanical approach. Evidence-based complementary and alternative medicine. 2011;2011.
9. Lin SR, Chang CH, Hsu CF, Tsai MJ, Cheng H, Leong MK, et al. Natural compounds as potential adjuvants to cancer therapy: Preclinical evidence. British journal of pharmacology. 2020;177(6):1409-23.
10. Mohan L. Plant-based drugs as an adjuvant to cancer chemotherapy. Alternative Medicine—Update. 2021.
11. Onimhawo J, Ebhomienlen T. Esan traditional medicine: Problems and prospects. International Journal of Advancements in Research & Technology. 2014;3(2).
12. Raina H, Soni G, Jauhari N, Sharma N, Bharadvaja N. Phytochemical importance of medicinal plants as potential sources of anticancer agents. Turkish Journal of Botany. 2014;38(6):1027-35.
13. Ahmed F, Ahmed KM, Abedin M, Karim A. Traditional uses and pharmacological potential of ficus exasperata vahl. Systematic Reviews in Pharmacy. 2012;3(1):15.
14. Fernandes JM, Cunha LM, Azevedo EP, Lourenço EM, Fernandes-Pedrosa MF, Zucolotto SM. Kalanchoe laciniata and bryophyllum pinnatum: An updated review about ethnopharmacology, phytochemistry, pharmacology and toxicology. Revista Brasileira de Farmacognosia. 2019;29:529-58.
15. Okokon JE, Dar A, Choudhary MI. Immunomodulatory, cytotoxic and antileishmanial activity of phytoconstituents of Croton zambesicus. Phytopharmacology Journal. 2013;4(1):31-40.
16. Bahuguna A, Khan I, Bajpai VK, Kang SC. Mtt assay to evaluate the cytotoxic potential of a drug. ||| Bangladesh Journal of Pharmacology. 2017;12(2):115-8.
17. Bijnsdorp IV, Peters GJ, Temmink OH, Fukushima M, Kruyt FA. Differential activation of cell death and autophagy results in an increased cytotoxic potential for trifluorothymidine compared to 5-fluorouracil in colon cancer cells. International journal of cancer. 2010;126(10):2457-68.
18. Tolosa L, Donato MT, Gómez-Lechón MJ. General cytotoxicity assessment by means of the mtt assay. Protocols in in vitro hepatocyte research: Springer; 2015. p. 333-48.
19. Sarah QS, Anny FC, Misbahuddin M. Brine shrimp lethality assay. Bangladesh J Pharmacol. 2017;12(2):186-9.
20. Popović Z, Matić R, Bojović S, Stefanović M, Vidaković V. Ethnobotany and herbal medicine in modern complementary and alternative medicine: An overview of publications in the field of i&c medicine 2001–2013. Journal of ethnopharmacology. 2016;181:182-92.
21. Adebayo IA, Arsad H, Samian MR. Antiproliferative effect on breast cancer (mcf7) of moringa oleifera seed extracts. African Journal of Traditional, Complementary and Alternative Medicines. 2017;14(2):282-7.
22. Jude CI, Catherine CI, Ngozi MI. Chemical profile of tridax procumbens linn. Pakistan Journal of Nutrition. 2009;8(5):548-50.
23. Harborne A. Phytochemical methods a guide to modern techniques of plant analysis: springer science & business media; 1998.
24. Kokate C. Practical pharmacognosy-4th reprinted edition. New Delhi, Vallabh Prakasan. 1999;107:124.
25. Sofowora A. Recent trends in research into african medicinal plants. Journal of ethnopharmacology. 1993;38(2-3):197-208.
26. John B, Sulaiman C, George S, Reddy V. Total phenolics and flavonoids in selected medicinal plants from kerala. International journal of pharmacy and pharmaceutical sciences. 2014;6(1):406-8.
27. Abbott WS. A method of computing the effectiveness of an insecticide. J econ Entomol. 1925;18(2):265-7.
28. Lee Y, Howard L, Villalon B. Flavonoids and antioxidant activity of fresh pepper (capsicum annuum) cultivars. Journal of Food Science. 1995;60(3):473-6.
29. Jesionek W, Majer-Dziedzic B, Choma IM. Separation, identification, and investigation

of antioxidant ability of plant extract components using TLC, LC–MS, AND TLC–DPPH•. Journal of liquid chromatography & related technologies. 2015;38(11):1147-53.
30. Jing T, Zhao X. The improved pyrogallol method by using terminating agent for superoxide dismutase measurement. Prog Biochem Biophys. 1995;22(1):84-6.
31. Dehpour AA, Ebrahimzadeh MA, Fazel NS, Mohammad NS. Antioxidant activity of the methanol extract of Ferula assafoetida and its essential oil composition. Grasas y aceites. 2009;60(4):405-12.
32. Ruch RJ, Cheng S-j, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989;10(6): 1003-8.
33. Adedosu OT, Oyedeji AT, Iwaku T, Ehigie AF, Olorunsogo OO. Hepatoprotective activity and inhibitory effect of flavonoid–rich extract of Brysocarpus coccineus leaves on mitochondrial membrane permeability transition pore. Asian Journal of Natural & Applied Sciences. 2014;3(3):92-100.
34. Fitsiou E, Pappa A. Anticancer activity of essential oils and other extracts from aromatic plants grown in greece. Antioxidants. 2019;8(8):290.
35. Segun PA, Ogbole OO, Ajaiyeoba EO. Medicinal plants used in the management of cancer among the ijebus of South-western Nigeria. Journal of herbal medicine. 2018;14:68-75.
36. Kuete V, Tchinda CF, Mambe FT, Beng VP, Efferth T. Cytotoxicity of methanol extracts of 10 Cameroonian medicinal plants towards multi-factorial drug-resistant cancer cell lines. BMC complementary and alternative medicine. 2016;16(1):1-12.
37. Maroyi A. Evaluation of pharmacological properties, phytochemistry and medicinal uses of Cissampelos owariensis. Research Journal of Pharmacy and Technology. 2021;14(6):3416-22.
38. Rivera D, Allkin R, Obón C, Alcaraz F, Verpoorte R, Heinrich M. What is in a name? The need for accurate scientific nomenclature for plants. Journal of Ethnopharmacology. 2014;152(3):393-402.
39. Farooq A, Amjad MS, Ahmad K, Altaf M, Umair M, Abbasi AM. Ethnomedicinal knowledge of the rural communities of dhirkot, azad jammu and kashmir, pakistan. Journal of ethnobiology and ethnomedicine. 2019;15(1):1-30.
40. Majolo F, Delwing LKdOB, Marmitt DJ, Bustamante-Filho IC, Goettert MI. Medicinal plants and bioactive natural compounds for cancer treatment: Important advances for drug discovery. Phytochemistry Letters. 2019;31:196-207.
41. Hossain MS, Uddin MS, Moniruzzaman M, Asaduzzaman M. Comparative study of cytotoxic potential and phytochemical screening of Xanthosoma sagittifolium rhizome and Syngonium podophylam leaf. J Med Plant Res. 2015;3(3):43-6.
42. Usunomena U, Paulinus ON. Phytochemical analysis and mineral composition of Annona muricata leaves. Magnesium. 2016;9619:801.
43. Akinpelu B, Igbeneghu O, Awotunde A, Iwalewa E, Oyedapo O. Antioxidant and antibacterial activities of saponin fractions of erythropheleum suaveolens (guill. And perri.) stem bark extract. Scientific Research and Essays. 2014;9(18):826-33.
44. Imran M, Rauf A, Abu-Izneid T, Nadeem M, Shariati MA, Khan IA, et al. Luteolin, a flavonoid, as an anticancer agent: A review. Biomedicine & Pharmacotherapy. 2019;112:108612.
45. Xu L, Zhang P-P, Fang X-Q, Liu Y, Wang J-Q, Zhou H-Z, et al. A ruthenium (ii) complex containing a p-cresol group induces apoptosis in human cervical carcinoma cells through endoplasmic reticulum stress and reactive oxygen species production. Journal of Inorganic Biochemistry. 2019;191:126-34.
46. Hamid HA, Ramli AN, Yusoff MM. Indole alkaloids from plants as potential leads for antidepressant drugs: A mini review. Frontiers in pharmacology. 2017;8:96.
47. Shoaib M, Shah SWA, Ali N, Shah I, Ullah S, Ghias M, et al. Scientific investigation of crude alkaloids from medicinal plants for the management of pain. BMC Complementary and Alternative Medicine. 2016;16(1):1-8.
48. Dhar G, Akther S, Sultana A, May U, Islam MM, Dhali M, et al. Effect of extraction solvents on phenolic contents and antioxidant capacities of Artocarpus chaplasha and Carissa carandas fruits from bangladesh. Journal of Applied Biology and Biotechnology. 2017;5(3):0-4.
49. Oliveira Chaves J, Corrêa de Souza M, Capelasso da Silva L, Lachos-Perez D, Torres-Mayanga PC, da Fonseca

Machado AP, et al. Extraction of flavonoids from natural sources using modern techniques. 2020.
50. Truong D-H, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of Severinia buxifolia. Journal of food quality. 2019;2019.
51. Noreen H, Semmar N, Farman M, McCullagh JS. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant Coronopus didymus. Asian Pacific journal of tropical medicine. 2017;10(8):792-801.
52. Sasadara M, Wirawan I, editors. Effect of extraction solvent on total phenolic content, total flavonoid content, and antioxidant activity of bulung sangu (gracilaria sp.) seaweed. IOP Conference Series: Earth and Environmental Science; 2021: IOP Publishing.
53. Vieito C, Fernandes É, Velho MV, Pires P. The effect of different solvents on extraction yield, total phenolic content and antioxidant activity of extracts from pine bark (Pinus pinaster subsp. Atlantica). Chemical Engineering Transactions. 2018;64:127-32.
54. Ganogpichayagrai A, Suksaard C. Proximate composition, vitamin and mineral composition, antioxidant capacity, and anticancer activity of Acanthopanax trifoliatus. Journal of advanced pharmaceutical technology & research. 2020;11(4):179.
55. Bongoni R, Steenbekkers L, Verkerk R, Van Boekel M, Dekker M. Studying consumer behaviour related to the quality of food: A case on vegetable preparation affecting sensory and health attributes. Trends in Food Science & Technology. 2013;33(2):139-45.
56. Pandit AP, Joshi SR, Dalal PS, Patole VC. Curcumin as a permeability enhancer enhanced the antihyperlipidemic activity of dietary green tea extract. BMC complementary and alternative medicine. 2019;19(1):1-10.
57. Meyer B, Ferrigni N, Putnam J, Jacobsen L, Nichols D, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta medica. 1982;45(05):31-4.
58. Sowemimo A, Fakoya F, Awopetu I, Omobuwajo O, Adesanya S. Toxicity and mutagenic activity of some selected nigerian plants. Journal of ethnopharmacology. 2007;113(3):427-32.
59. Reddy U. Cytotoxicity assay of ethanolic extracts of amalakyadi churna and its ingredients using artemia salina.
60. Del Socorro MML, Bendoy CP, Dacayana CML. Cytotoxic effects of betel vine, piper betle linn. Leaf extracts using Artemia salina leach (brine shrimp lethality assay). Journal of Multidisciplinary Studies. 2014;3(1).
61. Nabavi SM, Šamec D, Tomczyk M, Milella L, Russo D, Habtemariam S, et al. Flavonoid biosynthetic pathways in plants: Versatile targets for metabolic engineering. Biotechnology advances. 2020;38:107316.
62. Ogbole OO, Segun PA, Adeniji AJ. *In vitro* cytotoxic activity of medicinal plants from nigeria ethnomedicine on rhabdomyosarcoma cancer cell line and hplc analysis of active extracts. BMC complementary and alternative medicine. 2017;17(1):1-10.
63. Ndossi BA, Kilonzo M. Brine shrimp lethality assay of selected medicinal plants in tanzania. J Complement Altern Med Res. 2018;5(3):1-6.
64. Ekalu A, Habila JD. Flavonoids: Isolation, characterization, and health benefits. Beni-Suef University Journal of Basic and Applied Sciences. 2020;9(1):1-14.
65. Gwatidzo L, Dzomba P, Mangena M. Tlc separation and antioxidant activity of flavonoids from carissa bispinosa, Ficus sycomorus, and Grewia bicolar fruits. Nutrire. 2018;43(1):1-7.
66. Tambunan AP, Bahtiar A, Tjandrawinata RR. Influence of extraction parameters on the yield, phytochemical, tlc-densitometric quantification of quercetin, and lc-ms profile, and how to standardize different batches for long term from Ageratum conyoides l. Leaves. Pharmacognosy journal. 2017;9(6).
67. Pezzani R, Salehi B, Vitalini S, Iriti M, Zuñiga FA, Sharifi-Rad J, et al. Synergistic effects of plant derivatives and conventional chemotherapeutic agents: An update on the cancer perspective. Medicina. 2019;55(4):110.
68. Zhang L, McClements DJ, Wei Z, Wang G, Liu X, Liu F. Delivery of synergistic polyphenol combinations using biopolymer-based systems: Advances in physicochemical properties, stability and bioavailability. Critical Reviews in Food

Science and Nutrition. 2020;60(12): 2083-97.
69. Afolabi OK, Wusu AD, Ugbaja R, Fatoki JO. Aluminium phosphide-induced testicular toxicity through oxidative stress in wistar rats: Ameliorative role of hesperidin. Toxicology Research and Application. 2018;2:2397847318812794.
70. Selamoglu Z, Ustuntas H, Ozgen S. Traditional and complementary alternative medicine practices of some aromatic plants in the human health. Research Journal of Biology. 2016;4(2): 52-4.
71. Ramírez-Salcedo HE, Barrientos-Ramírez L, Vargas-Radillo JJ, Rodríguez-Macías R, Ruíz-López MA, Virgen-Calleros G. Inhibición de colletotrichum gloeosporioides y botrytis cinerea con extractos de Guazuma ulmifolia lam. Revista mexicana de fitopatología. 2019;37(2):330-44.
72. Choma IM, Olszowy M, Studziński M, Gnat S. Determination of chlorogenic acid, polyphenols and antioxidants in green coffee by thin-layer chromatography, effect-directed analysis and dot blot–comparison to hplc and spectrophotometry methods. Journal of separation science. 2019;42(8):1542-9.
73. Fathoni A, Ilyas M, Maehara S, Gusta A, editors. Evaluation of the potency of endophytic fungi associated with artemisia annua as antibacterial and antioxidant. IOP Conference Series: Earth and Environmental Science; 2020: IOP Publishing.
74. Victoria DT, Samrot AV. Tlc bio-autography guided identification of antioxidant fraction from aegle marmelos rind. Research Journal of Pharmaceutical Biological and Chemical Sciences. 2015;6(2):179-83.
75. Gupta RK, Patel AK, Shah N, Choudhary AK, Jha UK, Yadav UC, et al. Oxidative stress and antioxidants in disease and cancer: A review. Asian Pacific journal of cancer prevention. 2014;15(11):4405-9.
76. Idowu PA, Ekemezie LC, Olaiya CO. Phytochemical, antioxidant and antimicrobial studies of lannea egregia engl. & k. Krause (anacardiaceae) extracts and chromatographic fractions. Journal of Phytomedicine and Therapeutics. 2020; 19(1):348-63.
77. Barku VY. Wound healing: Contributions from plant secondary metabolite antioxidants. Wound healing-current perspectives: IntechOpen Rijeka, Croatia. 2019;13.

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