Available online at https://asric.africa/natural-sciences
ASRIC Journal on Natural Sciences Vol 3 (2) (2023) 29-38

Bioactive and Cytotoxic Potential of leaf extracts of Ficus asperifolia and Phyllantus amarus on brine shrimp

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Abstract
Plants are consumed for medicinal purposes. The characterisation of these plants is required to reveal potential beneficial properties and toxicity. Therefore, this study investigated the bioactive and cytotoxicity potential of *Ficus asperifolia* and *Pyllanthus amarus*, which rural dwellers in Nigeria consume. Phytochemical studies were performed on both plants' aqueous and ethanolic leaf extracts, mineral analysis was performed using their leaf powders, and cytotoxicity was investigated using brine shrimps (artemia salina). The results showed that alkaloids, saponins, tannins, glycosides, anthocyanins, terpenoids, and triterpenes were substantially present in the aqueous extracts of *F. asperiofolia* and *P. amarus*. Significant amounts of phenolics, alkaloids, flavonoids, saponins, coumarins, glycosides, terpenoids, and triterpenes were found in the ethanolic extracts of both plant leaves. Mineral analysis of the leaf powders showed that the major elements (K, Na, Mg, and Ca) were found in high amounts. Although the trace elements (Cr, Cu, Fe, and Pb) were found in minute quantities in both plants, they were within the permissible limits set by WHO. Although the lethality rate for cells treated with the ethanolic extracts of both plants was higher than those treated with the aqueous extracts, their LC_{50} (37.33-144.89µg/ml) at 24 hours of exposure indicate potential toxicity.
Keywords: Brine shrimp, cytotoxicity assay, *Ficus asperifolia*, Leaf extracts, Phytochemical analysis, *Pyllanthus amarus*

Introduction
According to the World Health Organization (WHO), 70-80% of the world's population relies on unconventional medicine, mainly from plant sources, for primary health (WHO, 2007). Many plants are recognised for their ability to produce a wealth of secondary metabolites, and many of these natural products have been shown to present remarkable biological and pharmacological activities, which could serve as the starting point in the development of modern medicines. Although many plants have valuable properties, some also possess toxicological properties.

Ficus asperifolia (F. asperifolia) is a tree widely distributed across several African regions, including Senegal, Cameroon, Sudan, and Central and Eastern Africa (Nkafamiyaa et al., 2010). It is also found in some areas in Nigeria, especially in Isanlu, Yagba East Local Government Area of Kogi State, and Omala Local Government Area of Kogi State (Nkafamiyaa et al., 2010). *F. asperifolia,* which is often called "Ipin" (Yoruba), "Asesa" (Igbo), "Baure" (Hausa) and "Ogbaiikolo" (Igala), have been reported to be highly medicinal and have been employed as analgesics, anti-tumour and anti-cancer agents, diuretics, abortifacients, ecbolics, and menstrual cycle pain reliever (Arbonnier, 2004). Further studies have also

shown that *F. asperifolia* has been applied within local communities in Nigeria to cure many diseases, serving as antidiarrheal, anti-hyperglycemic and anti-diabetic agents and relieving people of typhoid, anaemia, and menstrual cycle pain (Emmanuel et al., 2018; Momoh et al., 2017).

Phyllanthus amarus (P. amarus) is a broad-spectrum medicinal plant recognised globally (Zheng et al., 2016). In Nigeria, it is called "Oyomokeisoamankedem" in Efik, "Iyin Olobe" in Yoruba and "Ebebenizo" in Bini. *P. amarus* is generally employed to reduce pain, expel intestinal gas, stimulate and promote digestion, perform the anti-helminth role by expelling intestinal worms, and act as a mild laxative. It has also shown antiseptic, diuretic, antiviral, anti-diabetic, hypertensive and antipyretic properties and has been utilised in treating jaundice, diarrhoea, dysentery, wound, ulcers and urogenital diseases (Abubakar et al., 2010; Calixto et al., 1998).

However, the toxicity of plants may originate from different contaminants or plant chemical compounds that are part of the plants. Various assays are used to research the potential toxicity of herbal extracts based on other biological models, such as *in vivo* assays on laboratory animals. However, recent studies employed efforts for alternative biological assays that include species of *Artemia salina, Artemia franciscana, Artemia urmiana* and *Thamnocephalus platyurus*. These toxicity tests are valuable tools for preliminary toxicity assessment (Veni and Pushpanathan, 2014; Mayorga et al., 2010; Carballo et al., 2002). One convenient way to screen bioactive natural products involves using *in vivo* lethality in a simple zoologic organism (Wakawa and Fasihuddin, 2017; McLaughlin et al., 1998). Therefore, this study aimed to screen the bioactive and mineral constituents and assess the potential toxicity of the aqueous and ethanolic leaf extracts of *Ficus asperifolia* and *Phyllantus amarus* using brine shrimp (*Artemia salina)*.

Methods: ***Chemicals and Reagents***

All the chemicals and reagents used for this study were of analar grade.

Plants and organisms collection

F. asperieolia and P. amarus were collected from the Kogi State University campus and authenticated by taxonomists in the Department of Botany, Kogi State University, Anyigba, Nigeria. The eggs of the brine shrimp (*Artemia salina)* were obtained from the Sanders Great Salt Lake (Brine Shrimp Company L.C., U.S.A.).

Preparation of sample

The plant leaves were separated from their stem, and the collected plant leaves were washed thoroughly with distilled water, air-dried and powdered with an electric blender. A hundred grams (100 g) of the pulverised plant leaves were soaked in 1000 ml of boiled water at 100 ^{o}C (aqueous) and another portion in absolute ethanol overnight in a different conical flash well covered with cotton wool. The content was then filtered, the residue was repeatedly washed with water, and the filtrate was obtained. The same process was performed on the leaf soaked in ethanol, filtered, and repeatedly washed with ethanol until the extract became colourless. The filtrate was evaporated under reduced pressure to a deep brown sticky substance in a vacuum evaporator.

Qualitative Determination of Phytochemicals of the Leaf Extracts

Phytochemical analysis of the leaf extracts for detecting saponins, tannins, phenolics, alkaloids, steroids, terpenoids, triterpenes, phlobatannins, glycosides and flavonoids was carried out using the method described by Akoh et al. (2021). Phytosterols were detected using Liberman-Burchard's test, according to Vanitha (2019). Fixed oils and fats were detected according to Kokate et al. (2009), and amino acids were determined according to Lanjwani et al. (2015).

Quantitative determination of phytochemicals of leaf extracts

Total phenolic content of the sample was estimated according to the method of Makkar *et al.* (2009). Total flavonoid content was measured by aluminium chloride colourimetric assay, while saponins were quantified using the spectrophotometric method of Adetutu et al. (2015). The quantitative determination of alkaloids was performed by distillation and titrimetric methods described by Adewolu et al. (2021), and the quantity of tannins was determined using the technique of Ajayi et al. (2010). The quantitative estimation of

coumarins, glycosides, steroids, and triterpenes was performed according to Ashidi et al. (2022) and the Association of Official Analytical Chemists, AOAC (2005). Total anthocyanin compounds of the samples were estimated using a UV-spectrophotometer by the pH differential method reported by Bakar *et al*. (2009).

Mineral analysis of leaf extracts

Mineral analysis was performed using the method described by AOAC (2005). The samples were ashed at 550°C. The ash was boiled with 10 ml of 20% hydrochloric acid in a beaker and then filtered into a 100 ml standard flask, and this was made up to the mark with deionised water. The minerals-potassium (K), sodium (Na), chromium (Cr), copper (Cu), iron (Fe), and lead (Pb)-were determined from the resulting solution using an Atomic Absorption Spectrophotometer (Accuzy 211, Bulk Scientific Inc.). Magnesium (Mg), calcium (Ca), and chromium (Cr) were determined spectrophotometrically using a UV/Vis Spectrophotometer (model 752N, Shanghai Yoke Instrument Co., Ltd).

Cytotoxicity Assay: Preparation of the brine shrimp

About 1 g of *Artemia salina* (Linnaeus) cysts (Sanders Great Salt Lake, Brine Shrimp Company L.C., U.S.A.) was aerated in 1 L capacity glass container (separating funnel) containing filtered artificial seawater (38g/1000ml NaCl solution, pH-8.2). An air pump was fitted to the water to ensure complete aeration of the cysts. After 48 hours of incubation at room temperature (25-29°C), newly hatched free-swimming nauplii (pink-coloured) were harvested from the bottom under continuous illumination of the fluorescence lamp. This collection method ensures a pure harvest of nauplii as the cyst capsules float on the surface. The freshly hatched free-swimming nauplii were used for the bioassay.

Determination of LC_{50} using brine shrimp (brine shrimp lethality assay)

The assay system was prepared with 10 ml of filtered artificial seawater containing a concentration of leaf extracts and 1% yeast extract (for feeding the *Artemia salina*) in a watch glass. In each watch glass, 20 nauplii were transferred, and the setup was allowed to remain for 24 hours under the constant illumination of a fluorescent lamp with constant aeration. The number of survived nauplii was counted with a hand lens at 3-hour intervals. Three replicates were prepared for each dose level. After 24 hours of exposure, the median lethal concentration (LC_{50}) of the test sample was obtained by plotting the percentage of the shrimps killed against the logarithm of the sample concentration (Ahmed et al., 2010; Moshi et al., 2010; Meyer et al., 1982). LC_{50} values were estimated using a probit regression analysis (Finney, 1971).

Statistical analysis

Data are expressed as mean±SEM. Significant differences were established at $P<0.05$ using One-way analysis of variance (ANOVA) (GraphPad Instat). Post-hoc Tukey-Kramer Multiple Comparison was utilised to determine the specific differences between groups of leaf extracts. LC_{50} values were estimated using probit regression analysis (SPSS 13).

Results: ***Phytochemical composition of the leaf extracts of Ficus asperifolia and Pyllanthus amarus***

Phytochemical analysis of the aqueous and ethanolic leaf extracts of *F. asperifolia and P. amarus* showed significant bioactive compounds in both plant leaves (Table 1). The result showed that alkaloids, saponins, cardiac glycosides, anthocyanins, terpenoids, and triterpenes were present in the aqueous and ethanolic extracts of *F. asperiofolia* and *P. amarus*. However, phenolics, flavonoids, coumarins, phlobatannins, and fixed oil were found only in the ethanolic extracts of both plant leaves. Meanwhile, only tannins were present in the aqueous extracts of both plant leaves.

The quantitative phytochemical evaluation of the aqueous and ethanolic extracts of *F. asperiofolia and P. amarus* showed varying amounts of bioactive compounds in both plant leaf extracts (Table 2). The result showed that the aqueous extracts of *F. asperiofolia* contained the highest percentage of tannins and steroids, while the aqueous extracts of *P. amarus* possess the highest amounts of total phenolics, anthocyanins, terpenoids, and triterpenes. However, the ethanolic extracts of *F. asperiofolia* showed the highest composition of flavonoids, coumarins, and cardiac glycosides, while alkaloids and saponins were maximally found in the ethanolic extracts of *P. amarus*.

Table 1: Phytochemical composition of the aqueous and ethanolic extracts of *F. asperifolia* and *P. amarus*

Phytochemicals	AqFa	AqPa	EtFa	EtPa
Phenolics	-	+	+	+
Alkaloids	+	+	+	+
Flavonoids	-	-	+	+
Saponin	+	+	+	+
Tannin	+	+	-	-
Coumarins	-	-	+	+
Anthocyanin	+	+	-	-
Glycosides	+	+	+	+
Steroids	-	-	+	+
Terpenoids	+	+	+	+
Triterpenes	+	+	+	+
Phlobatannin	-	-	-	-
Amino acid	-	-	-	-
Fixed oil	+	+	+	+

Keys: + = Present, - = Absent, AgFa = Aqueous extract of *F. asperiofolia*, AgPa = Aqueous extract of *P. amarus*, EtFa = Ethanolic extract of *F. asperiofolia,* EtPa = Ethanolic extract of *P. amarus*

Table 2: Quantitative composition of bioactive compounds in the aqueous and ethanolic extracts of *F. asperifolia* and *P. amarus*

Values are expressed as mean ± SEM (n=3). Means with the same superscript across the same row indicate no significant difference at p> 0.05. Keys: Nd = Not detected, AgFa = Aqueous extract of *F. asperiofolia*, AgPa = Aqueous extract of *P. amarus*, EtFa = Ethanolic extract of *F. asperiofolia*, EtPa = Ethanolic extract of *P. amarus.*

Secondary metabolites	AqFa	AqPa	EtFa	EtPa
Total phenolics (mg/100g)	10.00 ± 0.00	65.29 ± 0.01[a]	63.90 ± 0.06	63.27 ± 0.02[a]
Alkaloids (mg/100g)	10.00 ± 0.01	15.29 ± 0.07	49.42 ± 0.02	49.74 ± 0.06
Flavonoids (mg/100g)	0.00	0.00	48.39 ± 0.90	44.92 ± 0.26
Saponin (mg/kg)	52.92 ± 0.48	52.73 ± 0.09	27.04 ± 0.05	61.71 ± 5.00
Tannin (mg/100g)	1.09 ± 0.00	0.07 ± 0.01	0.00	0.00
Coumarins (µg/100g)	Nd	Nd	71.49 ± 0.06	27.08 ± 0.15
Anthocyanin (µg/100g)	0.19 ± 0.00[a]	0.27 ± 0.10[a]	Nd	Nd
Glycosides (mg/100g)	6.52 ± 0.01	7.16 ± 0.01	8.78 ± 0.02	3.05 ± 0.02
Steroids (mg/100g)	0.00	0.00	46.43 ± 0.58	51.52 ± 2.83[a]
Terpenoids (µg/100g)	60.50 ± 0.04	86.35 ± 0.20	88.13 ± 0.00	79.41 ± 0.00
Triterpenes (µg/100g)	74.60 ± 7.08[a]	78.60 ± 5.42[b]	65.60 ± 0.08[abc]	41.02 ± 5.33[c]

Mineral composition of the leaf extracts of Ficus asperifolia and Pyllanthus amarus

The mineral analysis of the leaf powders of *F. asperifolia* and *P. amarus* showed that both plant leaves have substantial amounts of all the selected major elements (K, Na, Mg, and Ca) and trace elements (Cr, Cu, Fe, and Pb) (Table 3). The result also indicates that K, Na, Ca, Cr, Cu, and Fe were found in significantly higher amounts in the leaf of *F. asperifolia*. Nevertheless, the leaf of *P. amarus* contained a significantly higher value of Mg and Pb.

Table 3: Mineral composition of *F. asperifolia and P. amarus* leaf powder

Sample	K (ppm)	Na (ppm)	Mg (ppm)	Ca (ppm)	Cr (ppm)	Cu (ppm)	Fe (ppm)	Pb (ppm)
F. asperifolia	6.4	23.1	10.1	3.7	0.005	0.41	12.6	0.1
P. amarus	5.7	22.2	14.7	3.45	0.004	0.32	9.4	0.4

RDA	3500 mg	1500 mg	280-340 mg	1000 Mg	0.025-0.035 mg	0.9 mg	9-15 mg	-
UL	3000 mg	2300 mg	350 mg	2500 Mg	1 mg	5 mg	25 mg	10 mg

RDA = Recommended daily dietary allowance for adults, UL = Tolerable upper intake level per day for adults (Dhonukshe-Rutten et al., 2013; EFSA, 2006).

Cytotoxicity profile of the leaf extracts of Ficus asperifolia and Pyllanthus amarus

The Brine shrimp lethality assay was conducted to determine the cytotoxicity of the aqueous and ethanolic extracts of *F. asperiofolia* and *P. amarus*. Table 4 shows the % lethality of the aqueous and ethanolic extracts from both plants represented by:

$$\% \text{ lethality} = \frac{\textbf{no of dead cells in the test sample}}{\textbf{\textit{no of survival in control}}} \times \mathbf{100}\%$$

As expected, the % lethality increased across all the samples and the control reference (potassium dichromate) as time progressed (6 to 24 hours) (Table 4). Similarly, the % lethality increased for all the samples and control reference as their concentration increased (31.5 to 1000 µg/ml)

Table 4: Brine shrimp lethality assay of the aqueous and ethanolic extracts of *F. asperifolia and P. amarus*

	6 hrs exposure						12 hrs exposure					18 hrs exposure					24hrs exposure				
Conc. µg/ml	**AqFa**	**AqPa**	**PDM**	**EtFa**	**EtPa**	**PDM**	**AqFa**	**AqPa**	**EtFa**	**EtPa**	**PDM**	**AqFa**	**AqPa**	**EtFa**	**EtPa**	**PDM**	**AqFa**	**AqPa**	**EtFa**	**EtPa**	**PDM**
1000	45	40	75	45	45	80	60	70	70	80	80	75	70	80	90	100	90	90	95	90	100
500	35	20	55	35	35	75	50	35	50	40	75	45	45	75	50	95	80	80	95	55	100
250	20	20	45	30	30	75	25	30	40	25	75	35	40	60	35	90	65	75	90	45	90
125	15	15	40	15	15	70	25	30	40	25	70	25	35	50	30	75	50	65	70	45	80
62.5	10	5	20	10	10	60	10	20	30	20	60	20	35	50	25	75	50	60	55	40	75
31.5	0	0	5	5	5	35	0	10	25	20	35	10	25	30	20	35	35	40	45	4	35
LC50	> 1000	> 1000	300.75	> 1000	> 1000	148.86	618.98	606.23	335.96	486.80	148.86	369.97	386.89	103.49	284.11	32.22	84.66	44.51	37.35	144.89	30.31
Ctrl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1

AqFa = Aqueous extract of *F. asperifolia*, AqPa = Aqueous extract of *P. amarus*, EtFa = Ethanolic extract of *F. asperifolia*, EtPa = Ethanolic extract of *P. amarus*. PDM = Potassium dichromate (positive control).

The derived median lethal concentration (LC_{50}) for the aqueous and ethanolic extracts of *F. asperiofolia* and *P. amarus* across the different exposures to brine shrimp (6-24 hours) are shown in Table 5. The result showed that the LC_{50} of the test samples were higher than the control reference (potassium dichromate), a known toxic compound, for all the exposure periods (Table 5).

Table 5: LC_{50} of the aqueous and ethanolic extracts of *F. asperifolia* and *P. amarus*

Sample code	6 hrs Exposure	12 hrs Exposure	18 hrs Exposure	24 hrs Exposure
AqFa	> 1000	618.90	369.92	84.66
AqPa	> 1000	606.23	386.89	44.51
EtFa	> 1000	335.96	103.49	37.35

EtPa	> 1000	486.95	284.11	144.89
PDM	300.75	148.88	32.22	30.31

AqFa = Aqueous extract of *F. asperifolia*, AqPa = Aqueous extract of *P. amarus*, EtFa = Ethanolic extract of *F. asperifolia*, EtPa = Ethanolic extract of *P. amarus*. PDM = Potassium dichromate (positive control).

Furthermore, a comparison was made between the LC_{50} of the aqueous and ethanolic extracts of *F. asperiofolia* and *P. amarus* and control reference with the toxicity index of previously established models at 24 hours of exposure (Table 6). The result showed that the LC_{50} of all the test samples (including the control reference) were lower than those indicated by Meyer's index and Clarkson's index (Table 6).

Table 6: Comparison of LC_{50} values of aqueous and ethanolic extract of *F. asperifolia* and *P. amarus* after 24 hours of exposure with toxicity index

AqFa	AqPa	EtFa	EtPa	PDM	Meyer's toxicity index (Meyer et al., 1982)	Clarkson's toxicity index (Clarkson et al., 2004)
84.66	**44.51**	**37.33**	**144.89**	***30.30***	**> 1000 (non-toxic)**	**> 1000 (non-toxic)**
					< 1000 (toxic)	**500 - 1000 (low toxic)**
						100-500 (medium toxic)
						0 - 100 (highly toxic)

AqFa = Aqueous extract of *F. asperifolia*, AqPa = Aqueous extract of *P. amarus*, EtFa = Ethanolic extract of *F. asperifolia*, EtPa = Ethanolic extract of *P. amarus*. PDM = Potassium dichromate (positive control).

Discussion

Plants have been associated with producing bioactive compounds as products of secondary metabolism, which possess biological and pharmacological properties which could revolutionise modern medicine. *F. asperifolia* and *P. amarus* are two medicinal plants utilised by local communities in Nigeria to treat anaemia, dysentery, diarrhoea, wounds, ulcers, and urogenital diseases and relieve menstrual pain (Emmanuel et al., 2018; Momoh et al., 2017; Abubakar et al., 2010). However, these plants may contain remarkable bioactive compounds and possess toxicological properties. Therefore, this study evaluated the phytochemical and mineral constituents of the aqueous and ethanolic leaf extracts of *Ficus asperifolia* and *Phyllantus amarus* and used the brine shrimp (*Artemia salina)* model to assess their potential toxicity.

Phytochemical screening reveals the chemical nature of the constituents of the plant extracts and can tell which plant would be more beneficial to man. Increased dietary intake of natural phenolics correlates with reduced coronary heart disease, hepatic-related disorders, cancer, mortality, and prolonged life expectancy (Emmanuel et al., 2022; Halliwell, 2007). Studies have also revealed that polyphenolic compounds have been closely linked with many health-based properties, including antioxidant, anticancer, antiviral, anti-hyperglycemic, and anti-inflammatory activities (Momoh et al., 2017; Amin et al., 2006). The presence of the bioactive compounds, such as alkaloids, saponins, tannins, cardiac glycosides, phenolic compounds and other beneficial compounds (Tables 1 and 2), in substantial amounts revealed by our findings are indications that both plants can inhibit the activities of various radicals. Thus, consuming these extracts may be beneficial in preventing coronary diseases and other health conditions claimed by traditional users. However, the presence of tannins in the aqueous extracts but not in the ethanol extracts could be attributed to the differential solubility of tannins in these two solvents. This is mainly because tannins have hydrophilic functional groups, allowing for easy dissolution in water, while they tend to remain in the plant material when extracted with ethanol (de Hoyos-Martínez et al., 2019; Mueller-Harvey, 2001).

Potassium (K) helps to maintain body weight and regulate the balance of water and electrolytes in the blood and tissues (Udensi and Tchounwou, 2017). Sodium (Na) is an electrolyte that regulates plasma volume, acid-base balance, and the contraction of nerves and muscles (Akpanyung, 2005). Calcium (Ca) is essential because of its role in bones, teeth, muscle system and heart functions. It also helps regulate muscle contraction required by children, infants and foetuses for bone and teeth development (Ciosek et al., 2021). Magnesium (Mg) is a cofactor of various enzymes in carbohydrate oxidation and plays a vital role in the

cell membrane's glucose transport mechanism. It is also involved in insulin secretion, binding, and activity (Velayutharaj et al., 2016). Studies have demonstrated that Mg is vital in glucose phosphorylation and metabolism, regulating insulin activity to control blood glucose levels (Chaudhary et al., 2010; Viktorínová et al., 2009). Although Mg levels in the studied plants were variable (Table 3), the levels of the studied major minerals were sufficient, as shown by previous studies (Dhonukshe-Rutten et al., 2013; EFSA, 2006). The satisfactory levels of the major minerals suggest that the plants could regulate insulin levels and nerve and muscle contractions, which could treat cardiovascular diseases.

Iron (Fe) is an essential trace element in the human body responsible for haemoglobin formation, haematopoiesis, infection control, and cell-mediated immunity (Bhaskaran, 2001). The deficiency of iron has been described as the most prevalent nutritional deficiency, and has been linked to anaemia, reduced work capacity, impairments in behaviour and intellectual performance, and decreased resistance to infection (Dioxon and Haris, 2004). Although this study showed a high range of iron (Fe) levels in the studied plants, these levels were within the permissible limit of 20 ppm set by the WHO (WHO, 1984) in edible plants, demonstrating their suitability as medicinal plants.

Chromium (Cr) is a crucial trace element with many sites of action and is required in glucose homeostasis by stimulating the insulin signalling pathway and metabolism, thus improving insulin sensitivity (Guimarães et al., 2016). The modulation of lipid metabolism by Cr in peripheral tissues also represents an additional novel mechanism of action (Qiao et al., 2009). The deficiency of Cr or its biologically active form has been implicated in insulin resistance and diabetes (Niamat et al., 2012). Cr was available in high quantity in *F. asperifolia and P. amarus* within the acceptable level for consumption (0.02 ppm), indicating their tolerance for human consumption (WHO, 1984).

Different metrics have been set for copper (Cu), another essential trace element in metabolism regulation. The permissible limits for Cu in medicinal plants in China and Singapore are 20 ppm and 150 ppm, respectively (WHO, 2011). According to Bowen (1966) and Allaway (1968), the range of Cu in agricultural produce should be between 4 and 15 ppm. Meanwhile, Reddy and Love (1999) reported that the range of Cu contents in the fifty medicinally important leaves growing in India was 17.6-57.3 ppm. However, as shown in the result (Table 3), the amount of Cu found in the studied plant leaves is within the permissible limit of 3.00 ppm set for Cu by the WHO in edible plants (WHO, 1984).

It is known that excessive concentration of lead (Pb) is toxic and can disrupt glucose uptake and regulation and induce poisoning (Khan and Awan, 2014). Despite the limit (10 ppm) set by China, Malaysia, and Thailand, the *F. asperifolia* and *P. amarus* exhibited Pb values within the range of acceptable limit concentration by the FAO/WHO (0.43 ppm) (WHO, 1984). Based on the literature review, the findings of this study (Table 3) showed that the mineral constituents of *F. asperifolia* and *P. amarus* fall within the recommended dietary allowance (RDA) and tolerable upper levels (UL) prescribed by the WHO, indicating that humans could well tolerate them.

Brine shrimp lethality is used to assess the potential toxicity of extracts using the *Artemia salina*, an in vivo model (Veni and Pushpanathan, 2014; McLaughlin et al., 1998). At six (6) hours of exposure (acute toxicity), all the plant extracts had an LC_{50} of more than 1000µg/ml, indicating that no toxicity was observed at that exposure. However, increased lethality rates were observed in the *Artemia salina*, especially after 18-24 hours of exposure to the ethanolic extracts of both plants, indicating potential toxicity. The maximum death occurred at 1000 µg/ml, while the least mortalities were recorded at 31.25 µg/ml concentration, corroborating with Omale and Omojali (2010). The potential toxicity effect was exhibited across the different timeframes when the plant leaves were extracted with ethanol and tested on the brine shrimps rather than the aqueous extract (Tables 4 and 5).

The aqueous and ethanolic extracts of *F. asperifolia,* and *P. amarus*, are toxic and highly toxic according to Meyer's toxicity index and Clarkson's toxicity index, respectively (Table 6) (Clarkson et al., 2004; Meyer et al., 1982). Although Sirajudeen *et al*. (2006) showed that the leaf extract of *P. amarus* extract had no signs of mortality in experimental rats at 5000 mg/kg, 400-1200 mg/kg of bark extracts of *F. asperifolia*

and *P. amarus* induced systemic, organ and DNA-based damages in rat models (Bakare et al., 2015; Omoniwa et al., 2014), corroborating the toxicological findings of this study. The significant lethality of the ethanolic extracts compared to the aqueous extracts using the brine shrimps might indicate that the heating reduced the effect of the toxic compounds during the aqueous extraction. So, heat-sensitive cytotoxic components might be present in the leaves of *F. asperifolia* and *P. amarus,* which calls for further investigation.

Conclusion

The phytochemical profile of the leaf extracts of *F. asperifolia* and *P. amarus* showed substantial amounts of bioactive compounds, including alkaloids, saponins, tannins, glycosides, anthocyanins, terpenoids, triterpenes, flavonoids, and saponins. The findings also revealed the characteristic level of major and trace elements in their required proportions proposed by FAO/WHO. However, the significant lethality of the extracts to the brine shrimps indicates the presence of potent cytotoxic components that are heat sensitive, which calls for further investigation. While *F. asperifolia and P. amarus* contain bioactive compounds which have the potential to ameliorate a myriad of disorders, molecular characterisation, identification, and elucidation of the active agents are required to clarify their use as potential drugs.

Acknowledgements

The authors wish to acknowledge the Department of Medical Biochemistry, Kogi State University, Anyigba, for allowing the research group to use the facilities for this research, and the Central Laboratory Unit of the University of Ilorin, who analysed some aspects of the research. Mr Olupinyo Segun of the Biochemistry Laboratory in Natural Sciences and Gideon Obaje of the Medical Biochemistry Laboratory at the College of Health Sciences of Kogi State University, Anyigba, offered technical assistance.

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