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Anti-proliferative and cytotoxic activities of *Heliconia psittacorum* L. f. (Heliconiaceae) and *Ficus coronata* Spin. (Moraceae) leaves

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Abstract
The cytotoxicity, anti-proliferative and antioxidant effects of leaves of *Heliconia psittacorum* and *Ficus coronata* were studied using standard models. Concentration-dependent anti-proliferative activity ($P \geq 0.5$) which became maximal at 5mg/ml was observed for the methanol extract, chloroform and aqueous fractions of both plants at every incubation period. Growth inhibition of guinea corn seeds (anti-proliferative activity) at 48 and 72h. was: methanol extract > aqueous fraction > chloroform fraction for *H. psittacorum,* and chloroform fraction > methanol extract > aqueous fraction for *F. coronata.* Their chloroform fraction: *H. psittacorum* (LC_{50}, 5.0 mg/ml) and *F. coronata* ($LC_{50,}$ 6.5 mg/ml) were more cytotoxic against tadpoles than their aqueous fractions. Ferric reducing power assay at 40-100 µg/ml gave concentration-dependent antioxidant activity, which was higher for *H. psittacorum.* This was also comparable to that of ascorbic acid. These findings hereby suggest potential of *H. psittacorum* and *F. coronata* in the treatment of oxidative stress-induced diseases.

Keywords: *Heliconia psittacorum, Ficus coronata,* anti-proliferative activity, cytotoxicity, antioxidant activity

INTRODUCTION

Cancer is considered as a major cause of death worldwide with increasing number of cases yearly due to changing lifestyle of the global population [1]. This has consequently generated an increasing amount of cancer research directed towards the investigation of plant-derived anticancer compounds, many of which have been used in traditional herbal treatments for centuries [2].

The ornamental plant, *Heliconia psittacorum* L. f. (Heliconiaceae) is a perennial herb native to the Caribbean and South America where it is used for ulcers of the scalp. *H. psittacorum* appears to have no known medicinal properties, unlike its related species, *H. bihai* (L.) L. that is employed to ease foetus expulsion after childbirth and as diuretic [3]. To date, there is no publication on medicinal properties of *H. psittacorum. Ficus coronata* Spin (Moraceae), commonly known as sandpaper fig, is native to Australia but is now widespread and found in other parts of the world including Kenya and Papua New Guinea [4]. It is a shrub up to 15m cultivated for its edible fruit and its leaves are used as sandpaper for polishing wood. The only known research on *F. coronata* is the

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antibacterial investigation by HPLC reported by Smyth et al [5].

In the absence of any literature information on the cytotoxicity and antiproliferative activities of these two plants, and the growing concern for cancer therapy by exploiting the potential of African biodiversity, we therefore investigated the activities and report our findings herein.

EXPERIMENTAL

Collection and preparation of plant materials. Leaves of *H. psittacorum* were harvested from trees growing along Medical Laboratory premises, Igbinedion University teaching hospital. *F. coronata* leaves were obtained from the back of Stallion hostel, Crown estate, Igbinedion University, Okada (IUO) in October 2017. They were authenticated (voucher nos. *H. psittacorum*: IUO/16/114 and *F. coronata:* IUO/12/045A) at the Department of Phamacognosy herbarium, College of Pharmacy, IUO, Edo State, Nigeria.

Extraction of plant material and fractionation. Plant samples were cut into pieces, dried at room temperature (25^{o}C) for 3 weeks, and ground into a coarse state using the locally fabricated laboratory milling machine. 500g of each sample were extracted to exhaustion with methanol in a Soxhlet apparatus. The crude extract was concentrated *in vacuo*, weighed (Table 1) and refrigerated until needed. An appropriate amount of the crude methanol extract was fractionated with chloroform and ethyl acetate in a separatory funnel to yield chloroform, ethyl acetate and aqueous fractions. All fractions were reduced *in vacuo* and yields recorded (Table 1).

Phytochemical screening. Basic phytochemical screening was carried out on the crude methanol extract of each plant according to Evans [6] and suspected secondary metabolites recorded.

Determination of Anti-proliferative effect of crude extract and solvent fractions on guinea corn (*Sorghum bicolor*). Guinea corn (*Sorghum bicolor*) seeds were obtained from a local market in Okada Town, Ovia North East Local Government Area, Edo state. Viability test was performed according to Ikpefan et al [7]. The seeds were put in a beaker of distilled water, stirred and viable seeds that sank were decanted and used. They were surface-sterilized with 95% ethanol for 1 minute, rinsed again with distilled water to make ready for use. According to Ikpefan et al [7], twenty sterilized seeds were introduced into each petri-dish (10cm diameter) lined with absorbent cotton wool and overlaid with Whatman no.1 filter paper. Concentrations of 5mg/ml, 2.5mg/ml, 1mg/ml, 0.5mg/ml and 0.25mg/ml of the methanol crude extract and each of the aqueous, chloroform, and ethyl acetate fractions were separately applied. A negative control (water) and positive control (chloramphenicol, 0.1mg/ml) were similarly set up. All experiments were replicated thrice, and petri-dishes incubated in dark cupboard at room temperature for 72h. The length (mm) of the radicles emerging from the seeds were measured at 24h, 48h and 72h, average determined and recorded (±SEM). Results were subjected to standard statistical analyses. Percentage inhibition of growth of seeds at 72h with 5mg/ml of all test agents was determined using the formula:

% inhibition =
{(mean radicle length of negative control - mean radicle length of test agent) x 100} / (mean radicle length of negative control)

Determination of cytotoxic effects of extract and partitioned fractions on tadpoles. Tadpoles (3-4 day old) were scooped from stagnant water in Crown estate, IUO. Using the method of Obuotor and Onajobi [8], ten viable tadpoles were selected with the aid of pipette into different beakers containing 30ml natural water from tadpole habitat. The volume was made up to 49ml

with distilled water and 1ml of stock solution containing 1mg/ml, 2mg/ml, 5mg/ml, 10mg/ml and 20mg/ml of extract and various fractions. The experiment was replicated thrice and assay beakers incubated at room temperature for 24h. A negative control (water) and positive control (chloramphenicol, 0.1mg/ml) were similarly set up. Mortality was determined and mean ±SEM recorded.

Estimation of total phenolic content. Total phenolic content was measured using the modified Folin-Ciocalteau method [9]. 1 ml of extract or fraction was mixed with 2ml of 7.5% Sodium carbonate (Na_2CO_3) and 2ml of Folin-Ciocalteau reagents. After incubation using water bath at 40^0C for 45min, the absorbance of the reaction mixture was measured at 765nm in a UV-Visible Spectrophotometer (model SM23A; Micro field® England). The calculation of total phenol content was based on the calibration curve of the gallic acid standard (0.2- 1.0 mg/ml) and the data was expressed as milligram gallic acid equivalents (GAE) per gram) plant extract.

Estimation of flavonoid content. The total flavonoid content was determined using the aluminum chloride colorimetric method [10]. 1ml of the plant sample was mixed with 0.1ml of 10% aluminium chloride hexahydrate ($AlCl_3.6H_2O$), 0.1ml of 1M potassium acetate (CH_3COOK) and 2.8ml of deionized water. After incubation at room temperature for 40min, the absorbance of the reaction mixture was measured at 415nm on a UV-visible Spectrophotometer (model SM23A; Microfield®, England). Flavonoid contents were calculated on the basis of the calibration curve of quercetin standard [2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-1-benzopyran-4-one, 98% purity,) at 0.1- 0.5 mg/ml, and expressed as milligram quercetin equivalents per gram plant extract.

Ferric Reducing Power Assay. Ferric reducing power was determined as described by Yen and Chen [11] by mixing various concentrations of plant extract (or solvent fraction) and standard ascorbic acid solution at 10, 20, 40, 60, 80 and 100µg/ml in 1ml of methanol with phosphate buffer (2.5ml, 0.2M at pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$) (2.5ml, 1%). The mixture was incubated at 50^{o}C for 20min. Trichloracetic acid (2.5ml, 10%) was added to the mixture, centrifuged at 3000g for 10min at room temperature. 2.5ml of supernatant were mixed with 2.5ml distilled water and ferric chloride (0.5ml, 0.1%), and the absorbance of the reaction mixture measured at 700nm as indicative of increased reducing power. All the tests were performed in triplicate and a graph was plotted for the average of the observation.

Statistical analysis. Results of triplicate determinations were expressed as mean± SEM and subjected to statistical analysis using one-way analysis of variance (ANOVA) and difference at $P \geq 0.5$ were considered significant.

RESULTS AND DISCUSSION

Saponin, flavonoid, alkaloid and glycosides were detected in *H. psittacorum* (Table 1). The profile of *H. psittacorum* and *F. coronata* leaves is presented in Table 1. From Table 2, mean radicle length of guinea corn seeds treated with methanol extract and fractions of the two plants decreased with concentration, being maximal at 5mg/ml. Anti-proliferative activity was found to be concentration-dependent attaining a maximum at 5mg/ml at every incubation period for all tested agents of both plants. Growth inhibition generally increased with time for both plants (Fig. 1). Order of growth inhibition (anti-proliferative activity) at 48 and 72h. was methanol extract > aqueous fraction > chloroform fraction for *H. psittacorum* leaf (Fig. 1). For *F. coronata*

leaf, chloroform fraction > methanol extract > aqueous fraction also at 48 and 72h. From this investigation, aqueous fraction (79.8% inhibition) of *H. psittacorum* and chloroform fraction (96.3% inhibition) of *F. coronata*, could serve as promising anti-proliferative agents in the development of cytotoxic drugs. According to this present study, *F. coronata* (76.9 - 96.3% inhibition at 72h) was a more potent anti-proliferative agent than *H. psittacorum* (35.8 - 81.6%). *Ficus* species are an important group of antiproliferative agents and previous studies included those of *F. rumphii* [12], *F. pseudopalma* [13] and *F. pumila* [14] on human cancer cell lines. This present investigation is a further contribution to the compendium of potential anticancer agents. Aside from *Ficus* species, similar research works have also been published [7,15,16]. Phenols [14] and other phytochemicals [2] have been linked with growth inhibitory activity.

All the tested agents of *H. psittacorum* and *F. coronata* leaf, at concentration range of 1.0- 20mg/ml, displayed a dose-dependent mortality profile on tadpoles (Table 3). Order of cytotoxic potency was: chloroform fraction > methanol extract > aqueous fraction for *H. psittacorum,* and methanol extract > chloroform fraction> aqueous fraction for *Ficus coronata.* From the study, chloroform fraction of both plants (LC_{50} values of 5.0 mg/ml and 6.5 mg/ml, respectively) of *H. psittacorum* and *F. coronata* were more active than their aqueous fractions (Table 3). Methanol extract and solvent fractions of both plants were less active than the positive cytotoxic agent, chloramphenicol tested at 0.1mg/ml. Judging from the LC_{50} values of these plants, *H. psittacorum* seems a more potent cytotoxic agent. Abbood [17] reported cytotoxic activity of a related species, *F. religiosa* leaves on cancer cell lines linked to the phenolic compounds, serotonin and tannic acid.

Table 4 showed the result of antioxidant activity using the ferric reducing power assay model. The standard antioxidant agent, ascorbic acid and all tested samples of *H. psittacorum* and *F. coronata* gave concentration-dependent ferric reducing power activity at tested concentration (20-100 µg/ml). Data of activity relative to ascorbic acid indicated that methanol extract and the two fractions of *H. psittacorum* gave higher antioxidant effects at 40-100 µg/ml than *F. coronata*. Antioxidant activity at 100 µg/ml was ranked: aqueous fraction > methanol extract > chloroform fraction for *H. psittacorum,* and chloroform fraction > methanol extract > aqueous fraction for *F. coronata.*

Table 1: Profile of *Heliconia psittacorum* and *Ficus coronata* and yield of extract and fractions

Plant	Common name	Voucher number	Morphological part	Aspect	Location
Heliconia psittacorum L. f. Heliconiaceae	heliconia	IUO/16/114	Leaves	Herb	Igbinedion University Teaching Hospital (Medical Laboratory premises), Okada
Ficus coronata Spin. Moraceae	Sandpaper fig	IUO/12/045A	Leaves	Shrub	Behind Stallion 3 hostel, Crown estate, Okada

Plant	Yield			
	Crude methanol extract	Aqueous fraction[+]	Chloroform fraction[+]	Ethyl acetate fraction[+]
H. psittacorum	10%	17%	16%	7%
F. coronata	9.45%	42.62%	52.45%	3.31%

[+]Relative to crude extract

Table 2: Mean radicle length (cm) of guinea corn seeds treated with *Heliconia psittacorum* and *Ficus coronata*

Concentration (mg/ml)	24h		48h		72h	
	Crude methanol extract					
	H. psittacorum	*F. coronata*	*H. psittacorum*	*F. coronata*	*H. psittacorum*	*F. coronata*
0.25	1.79 ± 0.08^{a}	1.41 ± 0.23^{b}	2.75 ± 0.19^{l}	2.76 ± 0.35^{l}	3.34 ± 0.27^{u}	5.39 ± 1.17^{v}
0.5	1.18 ± 0.04^{c}	1.45 ± 0.17^{d}	1.95 ± 0.14^{m}	2.76 ± 0.35^{n}	2.31 ± 0.04^{x}	5.39 ± 1.17^{w}
1.0	1.35 ± 0.08^{e}	1.52 ± 0.69^{f}	1.56 ± 0.09^{o}	2.63 ± 0.20^{p}	2.07 ± 0.08^{y}	4.47 ± 0.28^{z}
2.5	1.13 ± 0.08^{g}	0.55 ± 0.13^{h}	1.41 ± 0.07^{q}	1.01 ± 0.11^{q}	1.65 ± 0.14^{a}	1.68 ± 0.13^{a}
5.0	0.71 ± 0.02^{i}	0.28 ± 0.20^{j}	0.57 ± 0.08^{t}	0.65 ± 0.19^{t}	1.14 ± 0.07^{b}	1.22 ± 0.21^{b}
Positive control (chloramphenicol, 0.1mg/ml)	0.24 ± 0.11^{j}		0.63 ± 0.06^{t}		$1.07\pm0.12^{a,b}$	
Negative control (distilled water)	1.85 ± 0.22^{r}		$2.66\pm0.07^{l,n,p}$		6.20 ± 0.09^{c}	
Concentration (mg/ml)	Aqueous fraction					
	H. psittacorum	*F. coronata*	*H. psittacorum*	*F. coronata*	*H. psittacorum*	*F. coronata*
0.25	1.35 ± 0.09^{a}	1.83 ± 0.28^{a}	2.81 ± 0.05^{f}	3.14 ± 0.39^{g}	3.55 ± 0.15^{s}	4.84 ± 0.72^{t}
0.5	1.24 ± 0.08^{b}	1.72 ± 0.35^{b}	1.39 ± 0.09^{h}	2.76 ± 0.25^{i}	2.43 ± 0.08^{u}	3.94 ± 0.18^{v}
1.0	0.88 ± 0.09^{c}	1.35 ± 0.08^{c}	1.45 ± 0.17^{j}	2.20 ± 0.17^{k}	2.21 ± 0.16^{w}	3.04 ± 0.17^{x}
2.5	0.74 ± 0.03^{d}	0.64 ± 0.08^{d}	1.38 ± 0.10^{l}	1.38 ± 0.14^{l}	2.31 ± 0.21^{y}	2.21 ± 0.02^{y}
5.0	0.53 ± 0.05^{e}	0.21 ± 0.13^{e}	0.61 ± 0.09^{m}	1.28 ± 0.16^{n}	1.25 ± 0.10^{z}	1.43 ± 0.28^{z}
Positive control (chloramphenicol, 0.1mg/ml)	0.24 ± 0.11^{e}		0.63 ± 0.06^{m}		1.07 ± 0.12^{z}	
Negative control (distilled water)	$1.85\pm0.22^{a,b,c}$		$2.66\pm0.07^{f,i,k}$		6.20 ± 0.09^{c}	
Concentration (mg/ml)	Chloroform fraction					
	H. psittacorum	*F. coronata*	*H. psittacorum*	*F. coronata*	*H. psittacorum*	*F. coronata*
0.25	3.35 ± 0.05^{a}	1.63 ± 0.50^{b}	4.00 ± 0.09^{k}	2.87 ± 0.18^{l}	4.41 ± 0.10^{x}	4.87 ± 0.42^{x}
0.5	3.63 ± 0.19^{c}	1.08 ± 0.27^{d}	4.07 ± 0.04^{o}	3.26 ± 0.22^{m}	4.10 ± 0.14^{y}	4.87 ± 0.42^{y}
1.0	3.60 ± 0.07^{e}	1.37 ± 0.11^{f}	3.82 ± 0.06^{p}	2.64 ± 0.04^{q}	4.65 ± 0.68^{z}	3.55 ± 0.05^{a}
2.5	3.81 ± 0.23^{g}	0.45 ± 0.05^{h}	4.07 ± 0.29^{t}	1.04 ± 0.06^{r}	4.32 ± 0.27^{b}	1.86 ± 0.09^{c}
5.0	1.48 ± 0.08^{i}	0.00 ± 0.00^{j}	2.38 ± 0.01^{u}	0.11 ± 0.04^{v}	3.98 ± 0.23^{d}	0.23 ± 0.15^{e}
Positive control (chloramphenicol, 0.1mg/ml)	$0.24\pm0.11^{h,j}$		0.63 ± 0.06^{v}		1.07 ± 0.12^{c}	
Negative control (distilled water)	$1.85\pm0.22^{b,d,f,i}$		$2.66\pm0.07^{q,u}$		6.20 ± 0.09^{d}	

The values above are mean of three replicates. n=3. Mean ± SEM. Similar alphabet across the row per each concentration (and relative to the controls) indicates no significant difference at $P \geq 0.5$

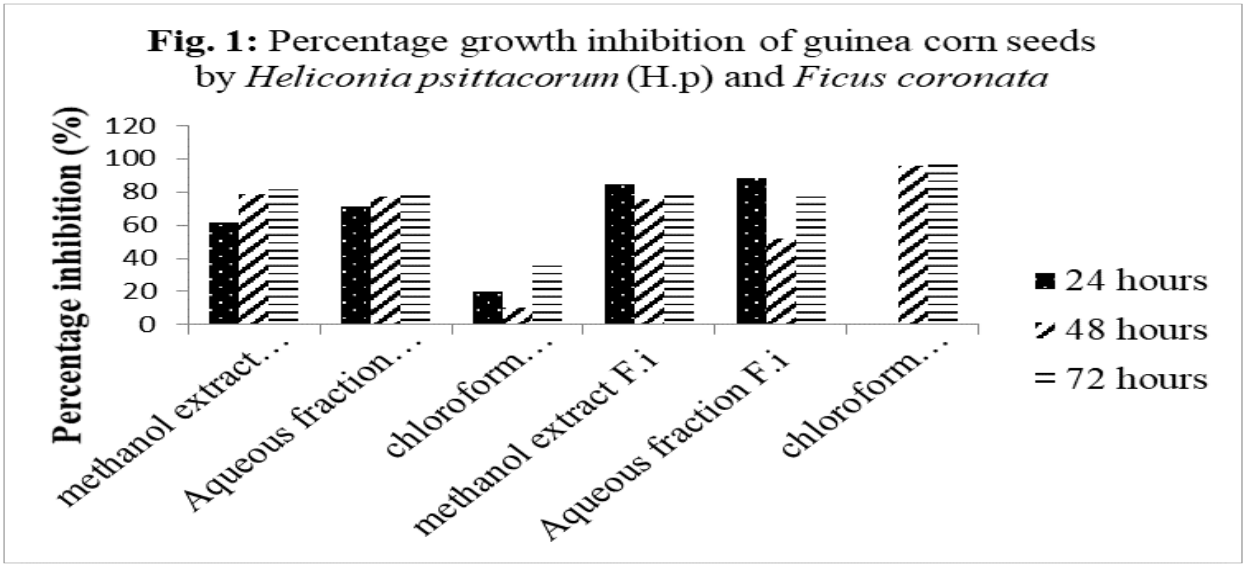
Table 3: Mean mortality (%) of crude extract and fractions of *Heliconia psittacorum* and *Ficus coronata* on tadpoles

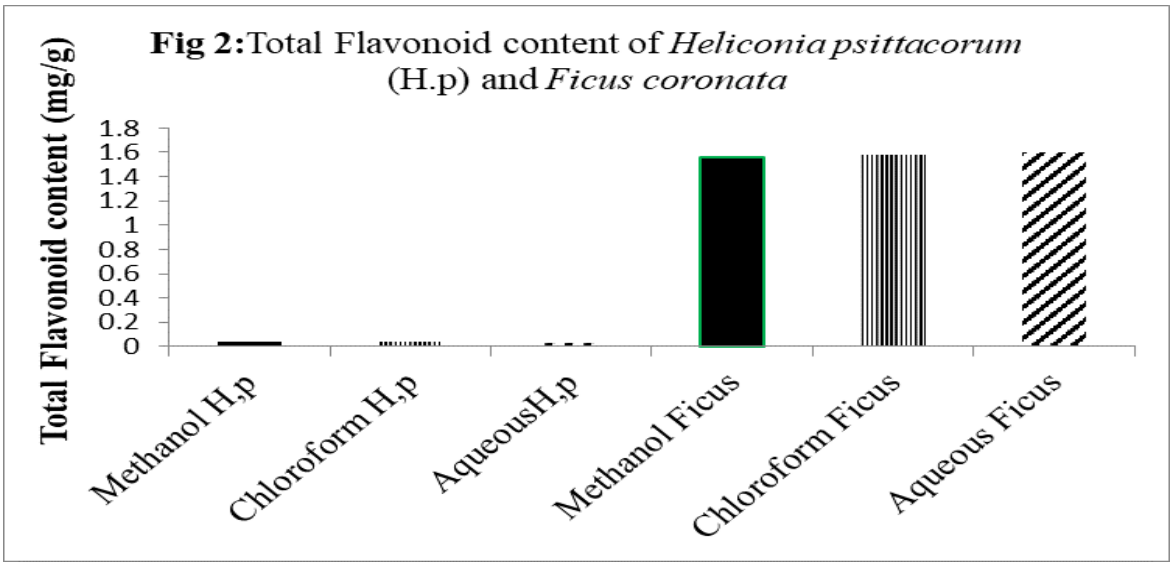
Test agent/ concentration	1mg/ml	2mg/ml	5mg/ml	10mg/ml	20mg/ml	LC_{50} (mg/ml)
			H. psittacorum			
Methanol	10.00±4.7	30.00±4.7	46.66±2.7	86.66±7.2	93.33±5.4	5.2
Chloroform	6.66±2.7	16.66±2.7	50.00±4.7	80.00±4.7	93.33±2.7	5.0
Aqueous	6.66±2.7	23.33±4.7	30.00±4.7	56.66±6.4	96.66±2.7	5.7
Negative control (water)			0			
Positive control (Chloramphenicol, 0.1 mg/ml)			100			
			F. coronata			
Methanol	16.66±4.7	36.66±4.7	56.66±4.7	80.00±8.3	96.66±4.7	3.8
Chloroform	3.33±3.8	16.66±4.5	36.66±4.7	76.66±12.5	100	6.5
Aqueous	3.33±3.8	13.33±4.7	26.66±2.1	40.00±8.3	80.00±8.3	12.7
Negative control (water)			0			
Positive control (Chloramphenicol, 0.1 mg/ml)			100			

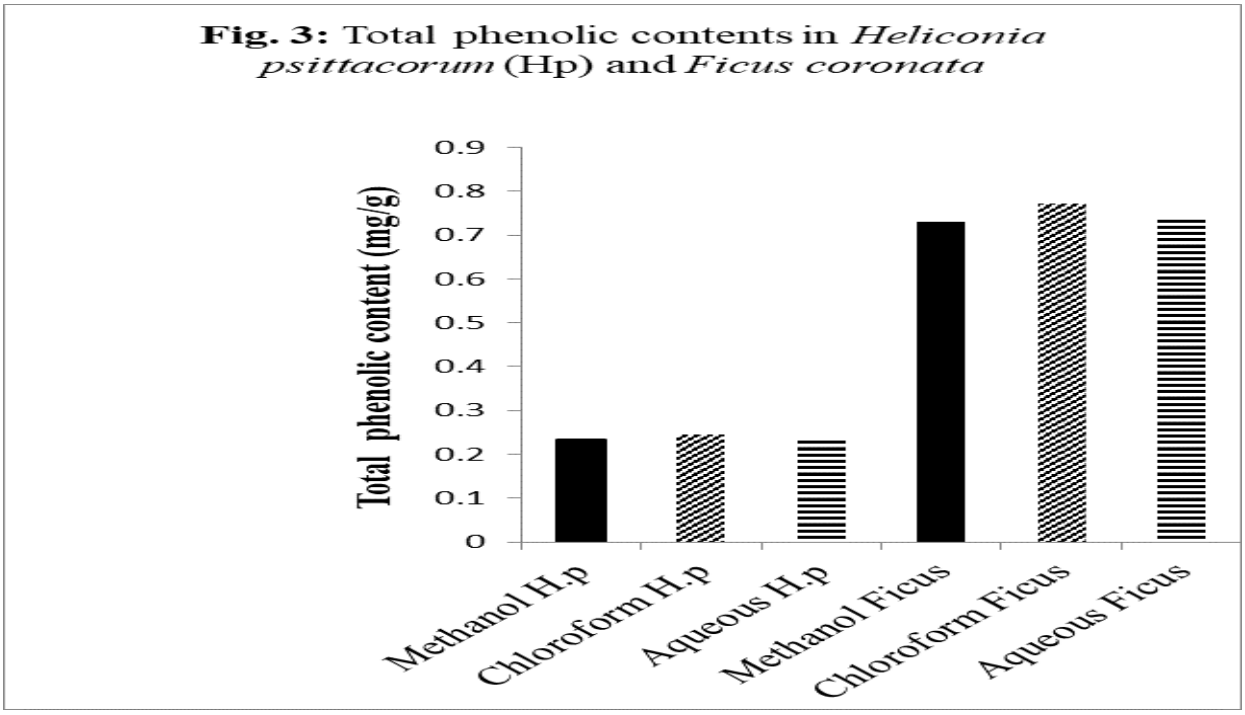
Table 4: Ferric reducing power activity of extracts and fractions of *Heliconia psittacorum* and *Ficus coronata*

Test agent	Absorbance measurements									
	20 µg/ml		40 µg/ml		60 µg/ml		80 µg/ml		100 µg/ml	
	H.p.	*F.c.*	*H.p.*	*F.c.*	*H.p.*	*F.c.*	*H.p.*	*F.c.*	*H.p.*	*F.c.*
Methanol extract	0.540	0.647	1.105	0.710	1.492	0.798	1.301	0.977	1.702	1.312
Chloroform fraction	0.592	0.746	1.462	0.781	1.662	0.934	1.180	1.304	1.191	1.369
Aqueous fraction	0.596	0.511	1.084	0.619	1.498	0.888	1.753	0.947	1.777	1.015
Ascorbic acid	0.920		1.320		1.420		1.500		1.520	

H.p. = H. psittacorum; *F.c. = F. coronata*







In addition, *H. psittacorum* exhibited comparable effects to the standard antioxidant agent, ascorbic acid. Antioxidant properties of related species, *H. rostata* rhizomes [18], *F. benjamina* [19] and *F. carica* [20] leaves among others, have been published. This investigation therefore serves to expand the database of antioxidants in the *Ficus* and *Heliconia* genera. Mechanism of action of antioxidants has been suggested to involve preventing metals from participation in initiation of lipid peroxidation and oxidative stress through metal-catalyzed reactions [21]. Our finding complements those of Sofidiya et al [22] and Adedokun et al. [21] on the importance of antioxidants in the management of tumor-related diseases. Antioxidants serve the purpose of improving health and reducing the risk of some metabolic diseases like diabetes and cancer.

F. coronata leaf gave higher flavonoid (0.869-0.898 mg/g quercetin equivalent) (Fig. 2) and phenolic (0.731- 0.772 mg/g gallic acid equivalent) (Fig. 3) contents in its methanol extract, aqueous and chloroform fractions than *H. psittacorum* leaf. While phenol content in *F. coronata* were thrice that of *H. psittacorum*, the flavonoid content in *F. coronata* was exceedingly greater than that of *H. psittacorum.* Apart from the chloroform fraction of both plants, which was richer in phenols, other tested agents had comparable amounts of these polyphenols. Plant flavonoids have been linked to the treatment of heart diseases and cancer [23], and this corroborates this current investigation on *F. coronata, which* is an abundant source of this phytoconstituent. The genus, *Ficus* has been described as being a very rich source of polyphenolic compounds especially flavonoids, which are relevant in the prevention of oxidative stress-related diseases [24]. There appears to be no correlation between the phenol contents of the plants and ferric reducing power effect. This data possibly suggests *F. coronata* as a better antioxidant plant.

Conclusion. The anti-proliferative and antioxidant activities of *H. psittacorum* and *F. coronata* are being reported for the first time. These indices, as well as the significant amount of phenols and flavonoids suggest better potential for *F. coronata* in the development of anti-tumor drugs. The active aqueous and chloroform fractions of these plants will require further bioactivity-guided chromatography to isolate the bioactive constituents.

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