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Effect of *Phaulopsis falcisepala* (Acanthaceae) Leaves and Stems on Mitotic Arrest and Induction of Chromosomal Changes in Meristematic Cells of *Allium Cepa*

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ABSTRACT

Phaulopsis falcisepala is a herb found in the forest zone of West Africa. It is applied ethnomedicinally for treating wounds and cancer. This study investigated the chemical constituents, cytotoxicity, and antimitotic activity of *P. falcisepala* leaves and stems. Chemical constituents were evaluated using phytochemical methods. Cytotoxicity was investigated using brine shrimp lethality and *Allium cepa* root growth inhibition assays. Antimitotic activity and induction of chromosomal aberrations were assessed by cytological examination of treated *A. cepa* meristematic cells. Chemical investigation revealed the presence of diverse bioactive chemical groups, including alkaloids, terpenoids, tannins, flavonoids and saponins in various extracts of the leaves and stems of *P. falcisepala*. Aqueous and methanol extracts contained copious amounts of the phytochemicals while ethyl acetate and *n*-hexane extracts contained moderate to trace amounts. The extracts demonstrated toxicity against brine shrimps, with LC_{50} values in the range 13.1–52.2 µg/mL. The extracts significantly inhibited *A. cepa* root growth, with IC_{50} values in the range 63.91–87.4 µg/mL and 45.34– 81.15µg/mL for 24 and 48 h treatments. The extracts (IC_{50} values in the range 46.2–94.23 µg/mL and 30.79–75.81 µg/mL for 24 and 48 h treatments) produced significant antimitotic effect comparable to or greater than that of methotrexate (IC_{50} value >125 µg/mL). Cytological examinations revealed that the extracts induced mitotic arrest and chromosomal changes. This study reports for the first time the phytoconstituents and cytotoxic activity of *P. falcisepala* supporting its ethnomedicinal use and could serve as the basis for further pharmacological studies and isolation of bioactive principles

Highlights

- Various extracts of *Phaulopsis falcisepala* (PF) exhibited cytotoxicity against brine shrimps and *Allium cepa* meristematic cells
- Methanol and aqueous extracts of PF showed greater antimitotic activities than methotrexate
- The cytotoxicity of PF extracts could be due to the induction of mitotic arrest and chromosomal changes

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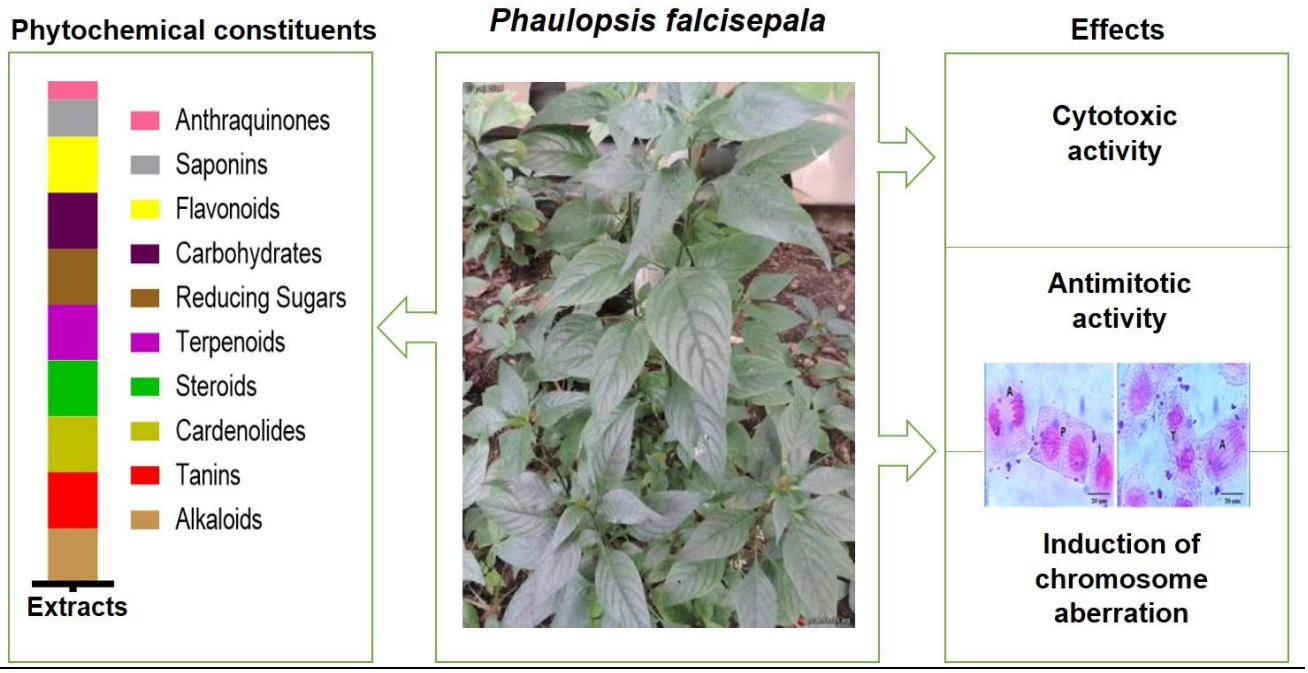
GRAPHICAL ABSTRACT



INTRODUCTION

Cancer has long been known as a dreadful disease that poses a great health burden worldwide due to its high incidence and mortality. Cancer burden continues to rise globally. Recent GLOBOCAN data indicates that cancer incidence and cancer-related mortality have increased by approximately 28% and 17%, respectively, between 2012 and 2018 [1]. This worrisome course is predicted to persist, with new cancer cases and mortality estimated to increase by additional 38% and 75%, respectively, this decade. This indicates that cancer cases would rise from 18.1 million in 2018 close to 25 million by 2030 and the number of cancer deaths from 9.6 million close to 17 million [1,2]. The factors responsible for this continuous rise in the global cancer burden are numerous and relatively well established [3]. The growing trend, however, particularly indicates the limitations of the currently existing modalities— surgery, radiotherapy and chemotherapy—for the management of cancer. There is no doubt that there is a need for novel and more effective chemotherapeutic agents. We suppose that bioactive compounds, particularly plant-derived secondary metabolites, could be explored for this purpose. In fact some secondary metabolites from plants have been used as drugs or lead compounds in chemotherapy. This is exemplified by the discovery of the first in class anticancer agents, vinca alkaloids: vincristine and vinblastine from *Catharanthus roseus*, and the development of etoposide from podophyllotoxin isolated from *Podophyllum peltatum*.

Nigeria is endowed with a rich biodiversity, with an avalanche of plants used for ethnomedicinal purposes. Medicinal plants constitute a well-known alternative for the treatment of cancer across the country. Although, the anticancer properties of some of these plants have been investigated [4,5], there are many more, including *Phaulopsis falcisepala*, which are yet to be researched.

Phaulopsis falcisepala C.B.Cl. is an upright or recumbent herb or weak undershrub that belongs to the Acanthaceae family. It is found across the forest regions of Senegal to southern

Nigeria. *P. falcisepala* is called *apa ogbe* "wound curer" or *ata-igboó* by the Yorubas and is applied ethnomedicinally for the treatment of wounds and cancer. In Ivory Coast, *P. falcisepala* is applied as baths to treat rheumatic pain and fever stiffness and topically to treat sores or blisters caused by skin infections [6]. *P. falcisepala* is also used as laxative, anti-emetic, and aphrodisiac [7]. At present there is little experimental data to evidence the medicinal and biochemical importance of *P. falcisepala*. Adesegun et al. [8] reported the *in vitro* antioxidative effect of methanolic extract of *P. falcisepala* leaves. In this study, we investigated the phytochemical constituents of the leaves and stems of *P. falcisepala* and evaluated their cytotoxicity and antimitotic activity against *Artemia salina* nauplii and *Allium cepa* meristematic cells.

EXPERIMENTAL

Plant material

Fresh material of *P. falcisepala* was collected from Forest Research Institute of Nigeria (FRIN), Ibadan with the assistance of Mr. Odewo, a plant taxonomist at FRIN. The plant material was identified, and authenticated by Mr. Adeyemo A. at FRIN and specimens were deposited at same institution (FRIN 110888). At the same period, plant material was also verified by Mr. Nodza G.I. at the Herbarium of the Department of Botany, University of Lagos (LUH 7992). The plant material was separated into leaves and stems, air dried, crushed into fine particles and then extracted.

Preparation of extracts

The pulverized leaves and stems of *P. falcisepala* (300 g each) were successively extracted with 1.5 L of *n*-hexane, ethyl acetate, methanol and water by maceration at room temperature for 9 days (per solvent). Each extract was filtered and filtrate concentrated *in vacuo* on a rotary evaporator to obtain 4 different extracts each from *P. falcisepala* leaves (*n*-hexane extract: PFLH, ethyl acetate extract: PFLE, methanol extract: PFLM and aqueous extract: PFLA) and stems (*n*-hexane extract: PFSH, ethyl acetate extract: PFSE, methanol extract: PFSM and aqueous extract: PFSA) as shown in Supplementary Fig. 1.

Chemical investigation of extracts

The extracts of the leaves (PFLH, PFLE, PFLM, and PFLA) and stems (PFSH, PFSE, PFSM, and PFSA) of *P. falcisepala* were investigated for the presence of various groups of phytochemicals, namely, alkaloids, tannins, cardenolides, steroids, terpenoids, reducing sugars, carbohydrates, flavonoids, saponins and anthraquinones using standard methods as described by Evans [9], Farnsworth [10] and Harborne [11].

Brine shrimp lethality assay

The cytotoxic effect of the extracts of the leaves (PFLH, PFLE, PFLM, and PFLA) and stems (PFSH, PFSE, PFSM, and PFSA) of *P. falcisepala* was investigated using the brine shrimp (*Artemia salina*) lethality assay as previously described [12]. Five mL of different concentrations (5, 10, 25, 50 and 100 µg/mL) of each extract was placed in a test tube and 10 brine shrimp larvae were added. Potassium dichromate ($K_2Cr_2O_7$) was used as a positive control and 1% dimethyl sulfoxide (DMSO), in seawater, served as a vehicle/negative control. The experiment was done in triplicate and maintained at room temperature for 24 h. Afterwards, the surviving shrimps were counted, the vehicle control deduction was made using the Schneider-Orelli's [13] formula and percentage mortality were calculated.

$$\text{Corrected \%mortality} = \frac{\text{\% mortality of test} - \text{\% mortality of control}}{100 - \text{\% mortality of control}} \times 100 \quad (1)$$

Allium cepa viability assay

Approximately medium-sized onion bulbs were procured, air-dried and investigated for their viability as described by Fiskesjo [14]. Dried

outer scales and roots of the onion bulbs were peeled and each bulb was grown in glass bottles containing distilled water for 48 h. The onion bulbs that sprouted properly were selected and used for the subsequent assay.

Root growth inhibition assay

The extracts were investigated for their effect on *A. cepa* root growth following 24 and 48 h treatments as described by Samuel et al. [15] and Oladipupo et al. [12]. Viable onion bulbs were grown in 30 mL of different concentrations (10, 25, 50 and 100 µg/mL) of each extract. Methotrexate (MTX) at a concentration of 125 µg/mL was used as a positive control and 1% DMSO served as a vehicle/negative control. Five replicates were maintained for each concentration and the solutions were replaced after 24 h. The root bundle length was measured for the onions at the end of each treatment period and the root growth in the treated groups was expressed as a percentage of the root growth in the vehicle control group.

Antimitotic and cytological assays

At the end of each treatment, roots from each bulb were harvested for cytological investigation. Root tips were fixed in Farmer's fluid (glacial acetic acid and ethyl alcohol 1:3, v/v) and hydrolysed in 1N HCl for 3 min. The hydrolysed root tips were developed on slides for microscopic examination by squashing, macerating and staining with aceto-orcein according to Fiskesjo [14] and Oladipupo et al. [12]. The slides were studied under a microscope using x100 objective in 5 microscopic fields and approximately 500 cells were observed for each concentration. Cells were scored for mitotic index and for chromosomal aberrations. Percentage mitotic inhibition in the treated groups was expressed as a percentage of the mitotic index in the vehicle control group. Photomicrographs of the cells were taken with a digital microscope (DB2-180M).

Statistical analyses

All statistical analyses were done on GraphPad Prism Version 6.0 (GraphPad Software, San Diego, USA). Regression analysis was used to compute the concentration required to produce 50% activity: LC_{50} (in the brine shrimp lethality assay) and IC_{50} (in root growth inhibition and antimitotic assays). Results are presented as the mean ± SEM of values obtained in replicate experiments. Statistical differences between various groups were evaluated using one-way analysis of variances (ANOVA) with post hoc Tukey's test; differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Chemical investigation

The investigation of the chemical constituents of *P. falcisepala* leaves and stems using standard methods revealed the presence of alkaloids, tannins, cardenolides, steroids, terpenoids, reducing sugars, carbohydrates, flavonoids, and saponins. Table 1 shows the relative abundance of the various groups of bioactive compounds in the different extracts.

Brine shrimp lethality assay

The results of brine shrimp lethality assay of *P. falcisepala* are as presented in Fig. 1a and b for the leaves and stems extracts, respectively. The extracts showed significant lethality against brine shrimp. This activity was not significantly different from that of potassium dichromate used as positive control. The LC_{50} of the extracts were in the range of 13.1 to 52.2 µg/mL and that of potassium dichromate was 14.4 µg/mL (Table 2).

Root growth inhibition assay

The results of root growth inhibition assay of *P. falcisepala* on *A. cepa* are as presented in Fig. 2 for the leaves and stems extracts. The figure shows the average percentage root growth from 24 and 48 h treatments. The extracts significantly inhibited *A. cepa* root growth at 100 µg/mL and

showed IC_{50} in the range of 63.91 to 87.4 µg/mL and 45.34 to 81.15 µg/mL for 24 and 48 h treatment, respectively (Table 2). This inhibitory effect was comparable to that induced by methotrexate, the positive control, which showed 33.2 and 45.6% root growths at 125 µg/mL for the same treatment periods.

Table 1. Results of chemical investigation of *P. falcisepala* leaves and stems

Chemical group	PFLH	PFLE	PFLM	PFLA	PFSH	PFSE	PFSM	PFSA
Alkaloids	+	+	++	++	–	+	+++	+++
Tannins	–	–	+++	+++	–	+	+++	+++
Cardenolides	+	+	++	++	+	+	+++	+++
Steroids	+	++	++	++	+	++	+++	+++
Terpenoids	+	++	+++	+++	+	++	+++	+++
Reducing Sugars	–	+	+++	++	–	+	+++	+++
Carbohydrates	+	+	+++	++	+	+	+++	+++
Flavonoids	–	–	+++	+++	–	+	+++	+++
Saponins	–	+	+	+++	–	–	++	++
Anthraquinones	–	–	–	–	–	–	–	+

Key: +++ = abundantly detected ++ = moderately detected, + = slightly detected, – = not detected

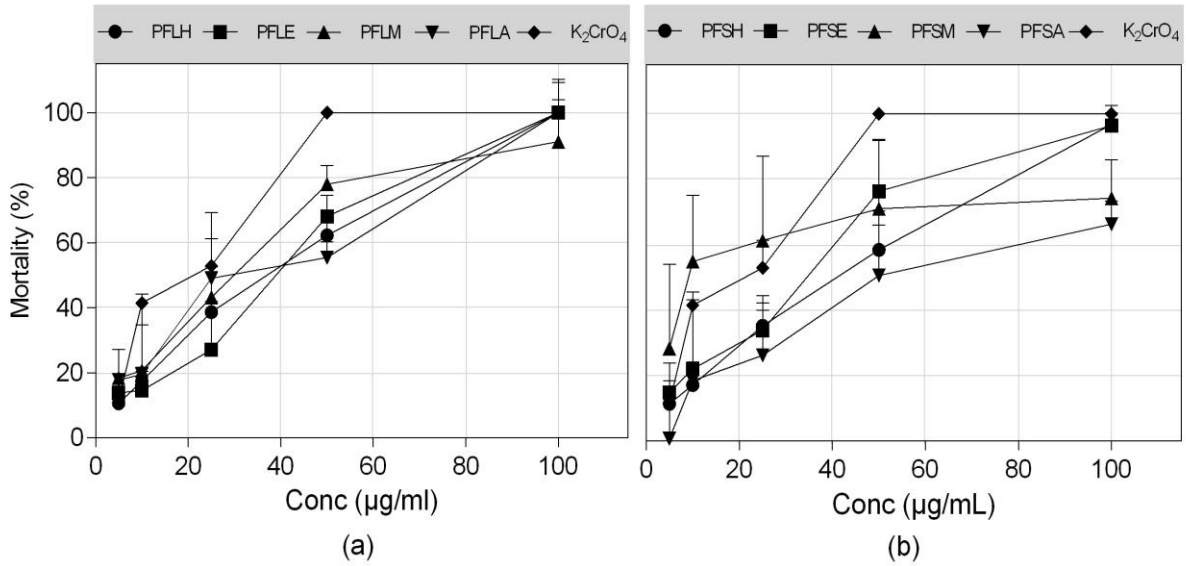


Fig. 1. Cytotoxic effect of *P. falcisepala* leaves (a) and stems (b) on brine shrimps. Values are presented as mean ± SEM; n=3. No statistically significant difference ($p > 0.05$) between all groups

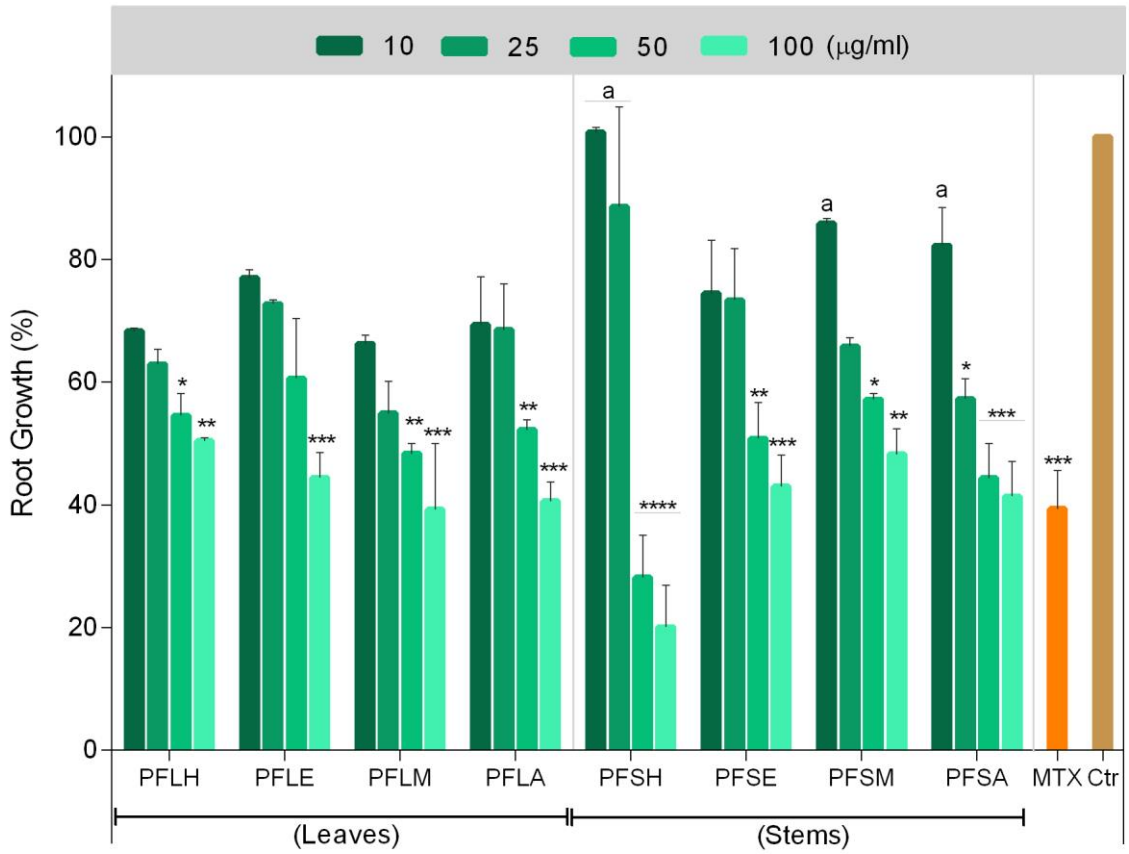


Fig. 2. Root growth (%) of *A. cepa* treated with *P. falcisepala* leaves and stems. The vehicle 1% DMSO was used as negative control (ctr) and set to 100%. Methotrexate (MTX) at 125 µg/mL act as positive control. Values are presented as mean ± SEM; n=2. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, significantly different compared to negative control; [a] significantly different compared to positive control at $p < 0.05$

Antimitotic and cytological assays

The results of antimitotic assay of *P. falcisepala* on *A. cepa* meristematic cells are as presented in Fig. 3 for the leaves and stems extracts. The figure shows the average percentage mitotic inhibition from 24 and 48 h treatments. The extracts exerted significant reduction in the mitotic indices of the cells at 100 and 50 µg/mL as indicated by the high percentage mitotic inhibition. The extracts (IC_{50} of 30.79–94.23 µg/mL) showed a higher antimitotic effect than methotrexate with an IC_{50} >125 µg/mL (Table 2). Table 3 is a summary of the chromosomal aberrations observed from the various treatments; consisting anaphase bridges (AB), laggards (L), vagrants (V), sticky or clumped chromosomes (SCC), other abnormal chromosomes (OAC) and total aberrations (TA), while Fig. 4 gives some photomicrographs showing mitotic phases and aberrations in the meristematic cells.

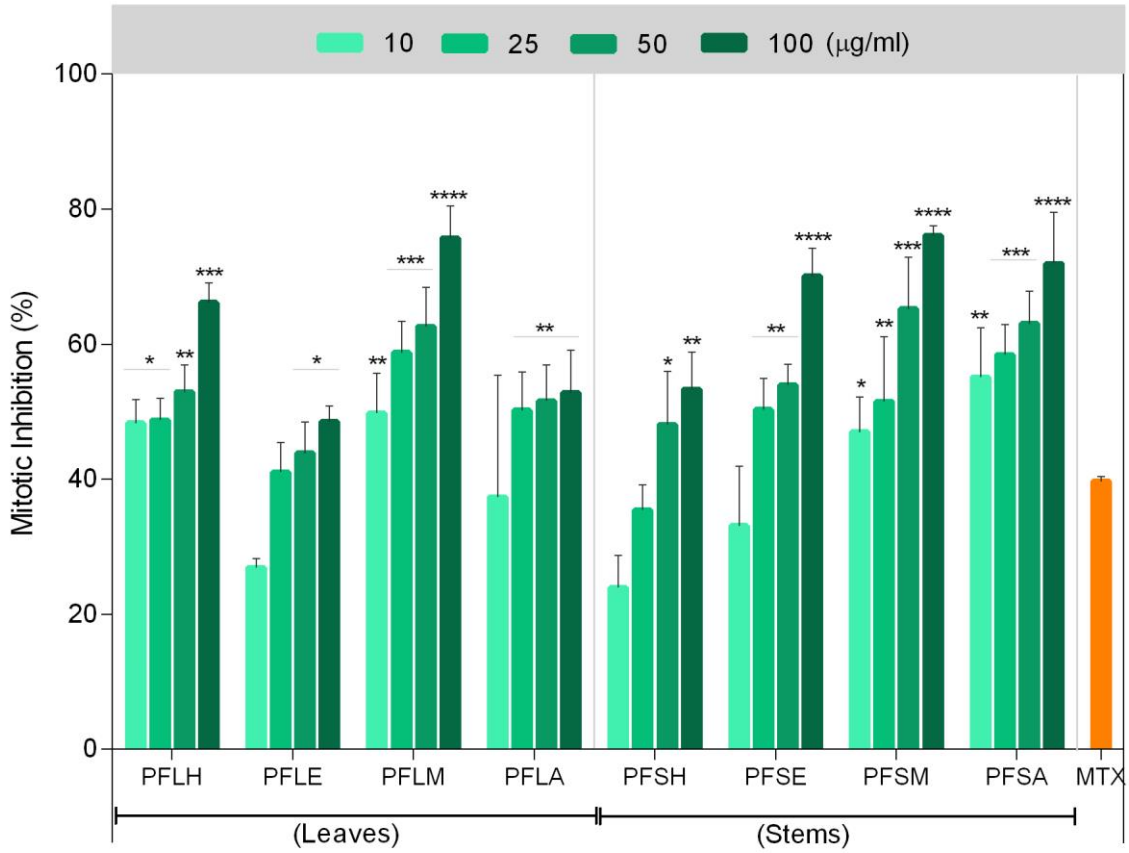


Fig. 3. Mitotic inhibition (%) of *A. cepa* meristematic cells treated with *P. falcisepala* leaves and stems. Values are presented as mean ± SEM; n=2. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, significantly different compared to negative control

Table 2. Summary of cytotoxic and antimitotic activities (LC_{50} and IC_{50} values) of *P. falcisepala* leaves and stems

Sample	Brine Shrimp Lethality (LC_{50} in µg/mL)	Root Growth Inhibition (IC_{50} in µg/mL)		Mitotic Inhibition (IC_{50} in µg/mL)	
		After 24 h	After 48 h	After 24 h	After 48 h
PFLH	26.4	85.4	79.95	58.23	45.39
PFLE	27.4	80.11	81.15	94.23	75.81
PFLM	22.6	77.32	51.67	46.2	30.79
PFLA	24.3	63.91	74.89	85.14	49.62
PFSH	28.7	66.35	45.34	90.81	63.95
PFSE	23.9	85.34	61.8	50.34	44.74
PFSM	13.1	87.4	77.93	48.38	30.79
PFSA	52.2	75.38	74.89	85.14	48.43
MTX	nt	> 125	> 125	> 125	> 125
$K_2Cr_2O_7$	14.4	nt	nt	nt	nt

nt = Not tested

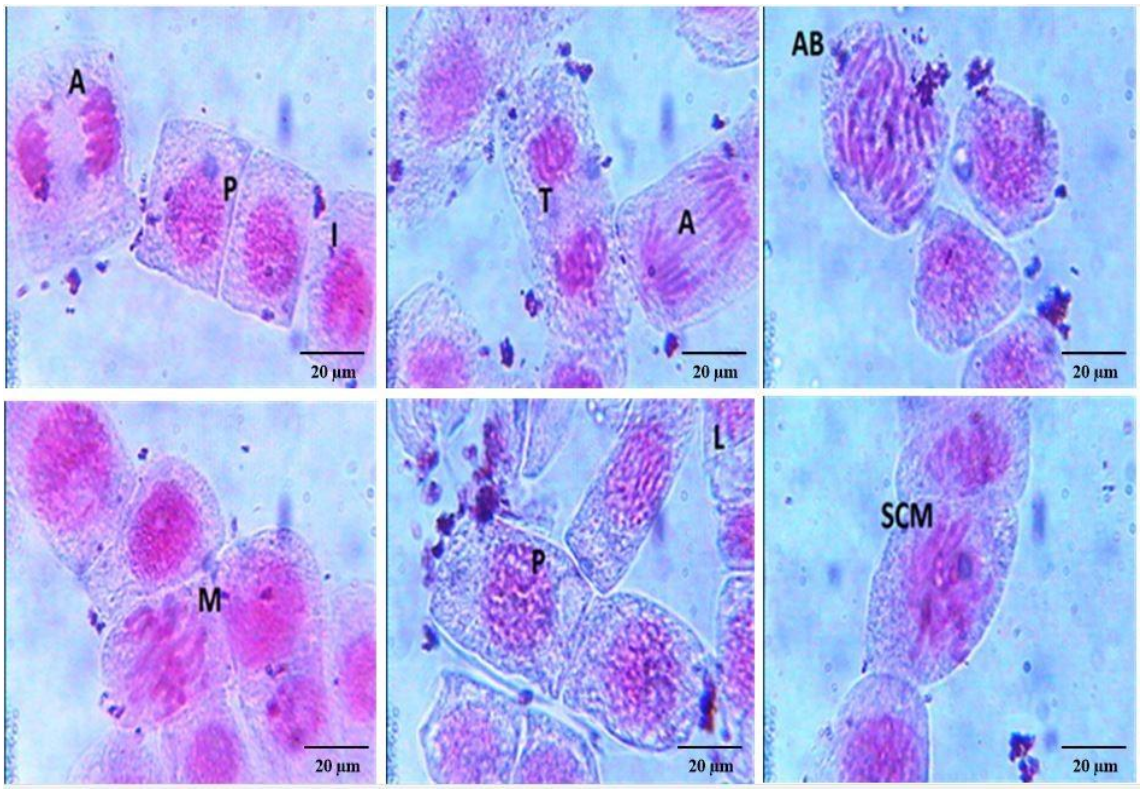


Fig. 4. Some representative photomicrographs of *A. cepa* meristematic cells showing anaphase (A), prophase (P), interphase (I), metaphase (M), telophase (T), anaphase bridges (AB), laggard (L), and sticky or clumped metaphase (SCM). Magnification x1000

Table 3. Chromosomal aberrations in *A. cepa* meristematic cells treated with *P. falcisepala* leaves and stems

Sample	Conc (µg/mL	Chromosomal aberrations					
		AB	L	V	SCC	OAC	TA

	)						
Control	–	0 ± 0	0 ± 0	5 ± 0	0 ± 0	0 ± 0	5 ± 0
MTX	125	2 ± 2	6 ± 3	14±0	9 ± 2	8 ± 2	38 ±8
PFLH	10	1 ± 1	2 ± 2	22 ± 5	6 ± 1	6 ± 2	36 ± 12
	25	4 ± 1	6 ± 1	24 ± 6	12 ± 1	9 ± 2	54 ± 12
	50	5 ± 1	8 ± 1	31 ± 11**	12 ± 6	12 ± 2	67 ± 21
	100	10 ± 2	7 ± 4	26 ± 8*	20 ± 4**	15 ± 4	77 ± 22
PFLE	10	2 ± 0	4 ± 3	21 ± 12	15 ± 4	12 ± 4	53 ± 23
	25	6 ± 1	7 ± 1	27 ± 11*	16 ± 6*	12 ± 7*	67 ± 27
	50	7 ± 1	7 ± 0	29 ± 13*	20 ± 4**	14 ± 4**	76 ± 23
	100	9 ± 2	9 ± 2	30 ± 12*	20 ± 5**	17 ± 6***	83 ± 27
PFLM	10	1 ± 1	5 ± 3	12 ± 3	7 ± 3	4 ± 2	29 ± 12
	25	3 ± 3	6 ± 1	18 ± 0	8 ± 3	9 ± 3	44 ± 10
	50	4 ± 3	8 ± 2	22 ± 5	12 ± 6	11 ± 4	56 ± 21
	100	10 ± 4	13 ± 2	23 ± 4	12 ± 1	15 ± 4**	73 ± 13
PFLA	10	1 ± 1	4 ± 2	16 ± 8	8 ± 2	9 ± 3	37 ± 16
	25	1 ± 1	6 ± 6	17 ± 5	9 ± 2	11 ± 3	43 ± 17
	50	5 ± 3	9 ± 2	19 ± 2	11 ± 4	13 ± 4*	55 ± 14
	100	6 ± 3	13 ± 4	25 ± 8	11 ± 2	14 ± 4*	67 ± 20
PFSH	10	1 ± 1	10 ± 4	24 ± 16	9 ± 1	5 ± 0	47 ± 21
	25	1 ± 0	12 ± 2	31 ± 12**	11 ± 1	7 ± 0	61 ± 15
	50	7 ± 1	15 ± 5*	32 ± 12**	13 ± 3	11 ± 2	76 ± 18
	100	8 ± 1	17 ± 6**	32 ± 14**	19 ± 3**	11 ± 2	86 ± 25*
PFSE	10	0 ± 0	4 ± 2	4 ± 4	8 ± 1	5 ± 0	21 ± 8
	25	0 ± 0	4 ± 1	9 ± 5	9 ± 2	6 ± 0	27 ± 8
	50	1 ± 1	5 ± 0	13 ± 10	11 ± 0	9 ± 2	38 ± 8
	100	2 ± 1	10 ± 4	21 ± 16	14 ± 4	11 ± 1	57 ± 20
PFSM	10	2 ± 3	6 ± 5	21 ± 6	11 ± 6	8 ± 0	47 ± 20
	25	7 ± 6	8 ± 4	28 ± 9*	13 ± 6	10 ± 0	66 ± 25
	50	9 ± 7	10 ± 6	29 ± 10*	15 ± 4	16 ± 1***	79 ± 25
	100	13 ± 11*	15 ± 8*	33 ± 10**	17 ± 5*	16 ± 1***	94 ± 36*
PFSA	10	1 ± 1	7 ± 2	19 ± 4	6 ± 3	2 ± 0	35 ± 11
	25	4 ± 3	7 ± 3	22 ± 5	8 ± 6	5 ± 4	45 ± 20
	50	5 ± 4	9 ± 2	28 ± 13	9 ± 6	8 ± 1	58 ± 26
	100	9 ± 4	10 ± 4	30 ± 14*	12 ± 5	9 ± 3	69 ± 30

*Values are mean ± S.E.M; n=2. * p < 0.05, ** p < 0.01, *** p < 0.001, significantly different compared to vehicle control. AB: anaphase bridges; L: laggards; V: vagrants; SCC: sticky or clumped chromosomes; OAC: other abnormal chromosome; TA: total aberrations*

The growing incidence of cancer and the various limitations of the conventional therapies, particularly, the high toxicity of current anticancer drugs present an imperative need for development of novel, potent and safe anticancer agents or alternative therapies. There is a renewed interest in plant-based therapeutics in both developing and developed countries due to the growing documentation attesting to their medicinal properties. This is more prominent in the search for effective drugs in the management of disease conditions, whose incidences are increasing and where the orthodox drugs are either expensive, unavailable or unsatisfactory. As part of our ongoing research into the chemical property and pharmacological importance of *P. falcisepala*, in this study, we investigated the chemical constituents, cytotoxicity, and antimitotic property of the leaves and stems of *P. falcisepala*.

The chemical investigation revealed that methanol and aqueous extracts of the leaves and stems contain copious amounts of alkaloids, tannins, cardenolides, steroids, terpenoids, reducing sugars, carbohydrates, flavonoids and saponins. The ethyl acetate and *n*-hexane extracts contained moderate to trace amounts of alkaloids, cardenolides, steroids, terpenoids and carbohydrates. The similarity of the chemical constituents of the methanol and aqueous extracts on the one hand and ethyl acetate and *n*-hexane extracts on the other hand is an indication of the presence of compounds of similar chemical groups but varying polarities. Polar and hydrophilic compounds are more amenable to methanol and aqueous extractions and are expected in the extracts of those solvents while non-polar and lipophilic molecules are expected to be present in *n*-hexane extract. The presence of these important structurally diverse chemical groups of established medicinal and biochemical importance [16,17] in *P. falcisepala* may be responsible for the supposed therapeutic effects derived from its ethnomedicinal uses.

The cytotoxicity of the leaves and stems of *P. falcisepala* was evaluated using brine shrimp lethality assay and *A. cepa* assays. All the extracts demonstrated concentration-dependent lethality. The most potent were the methanol extracts of both leaves (PFLM, LC_{50} of 22.6 µg/mL) and stems (PFSM, LC_{50} of 13.1 µg/mL). The least potent was the aqueous extract of the stems (PFLA, LC_{50} of 52.2 µg/mL). The brine shrimp lethality assay is widely employed as an indicator of general cytotoxicity. It could be extrapolated for cell line toxicity and has shown a good correlation with the detection of anticancer principles from plant extracts [18-22]. According to Meyer et al. [18], an LC_{50} value less than 1000 µg/mL is indicative of an active plant extract. Gertsch [23], however, suggests a much lower LC_{50} or IC_{50} threshold to prevent false positives. The extracts of *P. falcisepala* gave LC_{50} values < 60 µg/mL. In a similar study [24], 31 plants were investigated against brine shrimp, the most potent, *Eleusine indica*, gave an LC_{50} of 76.3 µg/mL, while cyclophosphamide, used as positive control, gave an LC_{50} of 101.3 µg/mL. Our results thus indicate that *P. falcisepala* is relatively more potent than the 31 plants as well as cyclophosphamide in this assay.

Allium cepa model is widely accepted for evaluating cytotoxicity, antimitotic activity and chromosome aberrative effect of a substance. The *A. cepa* root meristematic cells undergo rapid mitosis akin to those in cancer cells in human [25]. Hence, these cells are used for assessing the potentials of possible anticancer candidates [25-27]. As *A. cepa* cell cycle takes 24 h [28], the *A. cepa* assays were carried out for 24 and 48 h to cover 2 consecutive cell cycles. This served to assess if the meristematic cells would recover from the initial treatment-induced effects at the end of the second cycle. In the root growth inhibition assay, all the extracts of *P. falcisepala* demonstrated significant inhibitory effects. Maximum root growth was observed in the vehicle control group and was expressed as

100%. The various extracts of the leaves and stems (except PFSA) produced minimal and insignificant ($p > 0.05$) inhibitory effect at concentrations ≤ 25 µg/mL. However, at concentrations ≥ 50 µg/mL, the extracts (except PFLE) showed considerable and significant ($p < 0.05$) inhibitory effect with percentages of root growth < 60% as shown in Fig. 2. At the end of the second cycle (48 h treatment), minimal additional growth was observed in approximately 3 out of the 4 concentrations for the various extracts when compared to the first cycle (Supplementary Table 1). However, when these growths were expressed as percentages of the cumulative root growth in the vehicle control group, the extracts showed lower root growths compared to the vehicle control. This suggests that the inhibitory effect of the extracts (except PFLA and PFLE) may not be reversible, but rather cumulative, as indicated by their IC_{50} values after 24 and 48 h treatments (Table 2).

In the antimitotic assays, mitotic index was characterized by the total number of dividing cells in cell cycle and was used to determine the percentage mitotic inhibition; with high percentage mitotic inhibition indicating low mitotic index. All the extracts showed considerable antimitotic effects. At the lowest concentration (10 µg/mL), the aqueous and methanol extracts (PFLA, PFSA, PFLM, and PFSM) showed greater antimitotic activities than the positive control, methotrexate at a much higher concentration (125 µg/mL). Compared to the aqueous and methanol extracts, the ethyl acetate and *n*-hexane extracts (PFLE, PFSE, PFLH, and PFSH) exerted lower antimitotic effects. However, at 25 µg/mL, these moderately to non-polar extracts showed antimitotic activities that were either higher or comparable to that of methotrexate at 125 µg/mL. Mitotic index represents the proportion of cells in the mitotic phases of the cell cycle and is regarded as an indicator of cell proliferation. Hence, the reduction induced by *P. falcisepala* extracts on the mitotic index of *A. cepa* meristematic cells could suggest an arrest of mitotic cycle and an antiproliferative activity characteristic of clinically approved and potential antimitotic chemotherapeutic agents such as vinca alkaloids, taxanes, epothilone, hesperadin and ispinesib. The fragility of cancerous cells when they undergo mitosis served as a critical factor for the activity of antimitotic chemotherapeutics [29]. Although these agents are considered to be highly sensitive and effective for cancer treatment, they have been limited by undesirable effects including myelotoxicity and neurotoxicity [30].

At the end of the second cycle, small decrement was observed in the mitotic indices of the meristematic cells treated with the extracts in contrast to the minimal increment in those treated with the vehicle control (Supplementary Tables 2 and 3). This corroborates the results of the root growth inhibition assay, and similarly suggests that the antimitotic effect exerted by the extracts may be cumulative and not reversible, as indicated by their IC_{50} values from 24 and 48 h treatments (Table 2). A moderate negative correlation ($r = -0.54$) existed between the antimitotic effect and root growth induced by the various treatments (Supplementary Fig. 2). This indicates that the reduction of mitotic activity induced by the extracts is associated with the reduction in the root growth observed. EI-Shazly and EI-Sheikh [31] noted that plant growth consists of cell division and cell expansion, retardation of plant growth may thus result from not only arrest of mitotic cycle but also inhibition of auxin synthesis. Inhibition of plant growth could also be attributed to disturbances in natural growth regulators and chromosomal abnormalities induced by the treatment [32].

Cytological examination revealed that the extracts, as well as methotrexate, produced concentration-dependent chromosome aberrations, including bridges, laggards, vagrants, and sticky or clumped chromosomes.

The extracts caused minimal aberrations at concentrations ≤ 25 µg/mL while more aberrations were produced at concentrations ≥ 50 µg/mL. However, the total aberrations caused by the extracts at the various concentrations (except 100 µg/mL for PFSH and PFSM) were not significantly different (p > 0.05) from those produced by the vehicle control and methotrexate (Table 3). Vagrant and sticky or clumped chromosomes were the most frequently observed anomalies produced by the extracts. Stickiness is a physical aggregation that involves mostly the proteinacious matrix of chromatin [33]. A strong positive correlation (r = 0.73) was observed between the rates of occurrence of stickiness and the bridges (Supplementary Fig. 3). This substantiates the hypothesis that stickiness may be consequential of chromosome fibres misfolding; making the chromatids conjoined through subchromatid bridges [34]. Stickiness is considered as a mitotic disruption that is not likely to lead to chromosomal structural damage [35]. Vagrant chromosomes are weak c-mitotic effects and indicate disorganization and dysfunction of spindle apparatus [36]. This inactivation of spindle apparatus induced by the extracts is a typical mechanistic effect of a subclass of antimitotic chemotherapeutics known as spindle poisons (e.g. vinca alkaloids and taxanes). This suggests that the antimitotic activity of the extracts may be associated with their induction of chromosomal changes. *A. cepa* test system is a well-established in situ system with a good correlation with mammalian test systems [37]. The results obtained from this assay are indicative of the presence of cytotoxic compounds in *P. falcisepala* and could substantiate its ethnomedicinal use for cancer treatment.

CONCLUSION

This study was done to investigate the phytochemical constituents of the leaves and stems of *Phaulopsis falcisepala* and evaluate their cytotoxicity and antimitotic activity against brine shrimp nauplii and *Allium cepa* meristematic cells. The results obtained showed that the *n*-hexane, ethyl acetate, methanol and aqueous extracts of *P. falcisepala* leaves and stems contain various bioactive chemical groups of established therapeutic and pharmacological pedigree, including alkaloids, terpenoids, cardenolides and flavonoids. The extracts demonstrated cytotoxic effect and antimitotic activity on brine shrimp and *A. cepa* meristematic cells comparable to or greater than that of cyclophosphamide and methotrexate in both assays. The results further suggest that the mechanism of the observed activity could be by the induction of mitotic arrest and chromosomal changes. To the best of our knowledge, this is the first report of the chemical constituents and cytotoxic activity of *P. falcisepala.* Further studies are ongoing to isolate and characterize the active principles.

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