

## ANTICANCER ACTIVITIES OF EXTRACTS OF *Peristrophe bicalyculata* (RETZ) NEES

Received for publication, September 20, 2012

Accepted, November 20, 2012

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### Abstract

The anticancer activity of leaf extracts of *Peristrophe bicalyculata* was evaluated. Results showed that the methanolic ethylacetate fraction with an  $IC_{50}$  of  $15.60 \pm 0.52 \mu\text{g/ml}$  was potentially very toxic against human mouth epidermal carcinoma (KB) cells. After partial purification of the methanolic ethylacetate fraction, seven fractions were obtained; five of which were inactive ( $IC_{50}$  values above  $500 \mu\text{g/ml}$ ) and one moderately toxic ( $IC_{50} = 235 \pm 33.05 \mu\text{g/ml}$ ), hence not considered. The  $IC_{50}$  value of the most cytotoxic fraction (fraction 5) ( $3.50 \pm 0.21 \mu\text{g/ml}$ ) was not different from that of cisplatin ( $3.32 \pm 0.09 \mu\text{g/ml}$ ), but was significantly ( $P < 0.05$ ) higher than the value obtained for doxorubicin ( $0.43 \pm 0.02 \mu\text{g/ml}$ ) and vincristine ( $0.05 \pm 0.01 \mu\text{g/ml}$ ). The partially-purified fraction induced apoptosis in KB cells after 24 and 48 hours in a dose-dependent manner. The percentage of apoptotic cells in cultures treated with this fraction was significantly ( $P < 0.05$ ) higher than that in control cultures, but lower than cultures treated with cisplatin. The GC-MS analysis of the fraction indicated an abundance of andrographolide 2(3H)-furanone and aromadendrene oxide, both of which possess anticancer properties. The results provide scientific evidence supporting the anticancer activity of the methanolic ethylacetate fraction of the plant. In conclusion, the methanolic ethylacetate fraction of *Peristrophe bicalyculata* is a potential source of chemotherapeutic agent.

**Keywords:** *Peristrophe bicalyculata*, Sulphorhodamine B assay, anticancer, apoptosis, necrosis, andrographolide

### Introduction

*Peristrophe bicalyculata* (Retz) Nees belongs to the family Acanthaceae. It is native to warm tropical regions of Africa, including Mauritania, Niger, Nigeria, India, Burma and Thailand. The Hausas in Northern Nigeria call it 'tubanin dawaki' meaning 'flower of the horse'. In Berer and Wolof languages of Senegal, it is called 'buben' or 'moto' (1). In the Indore district of India, the local name is 'chotiharjori' (2). The leaves of the plant have analgesic, antipyretic, anti-inflammatory activity (3), antibacterial and fungistatic properties (1,4). In South-west Nigeria, the leaves are used for the treatment of hypertension and cardiovascular-related diseases (5). Studies have demonstrated the anticancer activity of oils from *Peristrophe*

*bicalyculata* using MCF-7 (human breast tumour) and MDA-MB-468 (human breast tumour) cells (6). The anticancer activities of crude extracts of the plant against EAC cell lines have also been reported (7,8). Cancer, a disease characterized by the uncontrolled growth and spread of abnormal cells, is a major public health burden in both developed and developing countries (9). It is second only to cardiovascular disease as the main cause of death in developed countries. Although cancer is reported more in the developed world, about two-thirds of all cancers occur in developing countries with many of them either not reported or wrongly diagnosed (10). Current treatment methods for cancer have yielded good results, but there is need for further improvement of both current therapeutic strategies and the search for novel agents. Natural products, especially plants, are useful sources of treatment of various diseases, including cancer (11). Thus, current research efforts are aimed at discovering new anticancer agents from plants, especially based on ethnomedical data, including the selection of plants on prior knowledge of their use in folk medicine. However in the case of cancer, which is a complicated and heterogeneous disease, the use of such data alone may be inadequate. Hence, there is the need to determine the cytotoxic effects of plants on different types of carcinoma (12). In this study, the anticancer effects of extracts of *Peristrophe bicalyculata* on human cervical cancer (HeLa), human colon adenocarcinoma (HT-29), human hepatocellular liver carcinoma (HepG2) and human mouth epidermal carcinoma (KB) cells, were determined, with the aim of identifying the anticancer component(s) present in the plant, and to possibly identify potential source of anticancer agents from the extracts.

## Materials and methods

### *Plant Material*

The leaves of *Peristrophe bicalyculata* were harvested at maturity in the month of June, 2010 at Ibadan (7° 26' N and 3° 54' E), Oyo State, Nigeria. It was identified and authenticated by the botanist, Mal Abubakar Adamu, in the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, with a voucher number 2863.

The powdered leaves of *Peristrophe bicalyculata* (500 g) were extracted in n-hexane (defatting) using Soxhlet apparatus, before extracting with methanol (70%). The extract was then suction-filtered, concentrated under reduced pressure using with a rotary evaporator (Buchi, Switzerland) and lyophilized. The methanol extract was dissolved in distilled water (1000 ml) and partitioned in ethylacetate and n-butanol, using separating funnel to obtain methanol, methanolic ethylacetate, methanolic butanol and methanolic water fractions, which were concentrated and lyophilized.

*Peristrophe bicalyculata* (50g) was also extracted in distilled water (500 ml) by stirring (Harmony Hot Plate Stirrer, Japan) for 30 minutes to obtain cold water extract. Another 50g was boiled for thirty (30) minutes in hot water to obtain hot water extract. The extracts were sieved using a muslin cloth and then filtered under suction pressure with a Whatman's filter paper. They were then concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland), lyophilized (Christ Alpha 1-2 LD, Germany) and stored at 4°C until needed.

### *Anticancer Activity of Peristrophe bicalyculata*

#### *Cell lines*

Human cervical cancer (HeLa), human colon adenocarcinoma (HT-29), human hepatocellular liver carcinoma (HepG2) and human mouth epidermal carcinoma (KB) were obtained from the American Type Culture Collection (Rockville, MD) and stored in nitrogen tanks.

#### *Experimental Design*

**Cell Culture:** A stock of each cell line from the nitrogen tank was quickly thawed in 70°C water by swirling gently. This was transferred into a 25 cm<sup>3</sup> plastic culture flask, containing 5 ml complete DMEM supplemented with 10% (v/v) foetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 mg/ml). Cells were incubated in a temperature-controlled and humidified incubator (Shel Lab, model 2123TC, USA) with 5% CO<sub>2</sub> at 37°C.

At confluence, approximately 10,000 cells were seeded per well into a 96-well plate. The total volume was adjusted to 200 µL with the complete DMEM and incubated in a temperature-controlled and humidified incubator (Shel Lab, model 2123TC, USA) with 5% CO<sub>2</sub> at 37°C for 24 hours.

**Antiproliferative Assay:** Anti-proliferation activity on cancer cell lines was determined by the modified SRB assay (17). Briefly, the culture plates were divided into 3 groups: the reference plate, the treated plate and control plate. For the reference plate, after 24-h incubation, the cells were fixed with ice-cold 50% trichloroacetic acid (TCA) solution at 4°C for 2 h. The plates were then washed properly with distilled water and air-dried overnight. The fixed plate was dyed by adding 50 µL 0.4% SRB solution to each well to allow staining at room temperature for 30 minutes, before washing with 1% acetic acid to remove unbound dye. The plates were then air-dried overnight again. The bound SRB was solubilised by adding 100 µL Tris-solution to each well and shaken for 30 minutes on a shaker platform. The absorbance of all plates was taken at 540 nm with a microplate reader (Bio-Rad, Model 680, Philadelphia, PA19102-1737, USA). All experiments were done in triplicate.

For the treated plates, after the first 24-h incubation, the cells were treated with 20 µl of the polar extracts (in case of non-polar extracts, 2µL was used) at the different concentrations. To the control plate (C) of the polar extracts, 20 µL complete DMEM was added, while 2 µl DMSO was added to control plates of non-polar extracts. The plates were then re-incubated for 24 h, fixed and dyed using the same steps described above for reference plate. The percentage cell growth (%G) was calculated, thus:

$$\%G = (T_{\text{treat}} - T_0/C - T_0) \times 100$$

Where  $T_{\text{treat}}$  is the absorbance of the extract-treated plates,

C is the absorbance of the control plate, and

$T_0$  is the absorbance of the reference plate.

The concentration of extract providing 50% inhibition (IC<sub>50</sub> mg/mL) was calculated from the graph of correlation of percentage growth against concentrations of the extracts.

**Partial Purification of Most Active Extract.** The most active extract was obtained after repeating the experiment three times. Thin layer and column chromatographic separation of the extract was carried out, and the anticancer activity of all fractions determined.

**Acridine orange/ethidium bromide (AO/EB) staining.** Morphological assessment of apoptotic cells was performed using the 96-well-based-AO/EB staining method (18). Briefly, 10,000 cells were seeded per well in 96-well microtitre plates divided into different groups based on sample concentration and number of incubation days. After 24-h incubation in appropriate medium, they were treated with the standard drug, cisplatin at 1000, 100 and 10 µg/mL; the extract at 1000, 500 and 250 µg/mL. The cells were incubated for another 24 and 48 h at 37°C in a 5% CO<sub>2</sub> atmosphere. Thereafter, 8 µL of AO/EB dye mix was added to each well. The cells were then viewed under fluorescent light microscope (Olympus CK 40, Postfach 10 49 08, 20034, Hamburg, Germany). Morphological characteristics such as chromatin condensation, nuclear pyknosis, number of nuclear body fragments and irregular edges around the nucleus; round, clear edged, uniformly stained cell nuclei were observed and counted in untreated and treated cells.

### ***Gas Chromatography and Mass Spectrometry Analysis of the Partially Purified Anticancer Fraction of *Peristrophe bicalyculata****

The gas chromatography and mass spectrometry (GC-MS) analysis was conducted using Shimadzu machine with chromatographic system (model GC-2010). The mass analysis machine (GC-MS QP2010) was connected to the column RTX-5MS.30m.0.25mm internal diameter.

Spectra were generated using the in-built softwares (GC-MS solution version 2.5 SUI). GC-MS real time and GC-MS post-run analyses were evaluated using three libraries: NIST, Wiley intergrated and a domestic library using a CAS number of EMBRAPA Genetic Resources and Biotechnology. Each spectrum was confirmed by two replicated readings to ensure reproducibility. All peaks were further analysed for compound identification, based on their similarities with structures available in the libraries.

#### ***Statistical Analysis***

Data obtained were expressed as mean  $\pm$  standard error of mean (mean  $\pm$  SEM) and analysed using SPSS 17. The significance among groups was determined by one-way analysis of variance and LSD post-hoc test was applied for multiple comparisons. Values of  $P < 0.05$  were regarded as statistically significant.

## **Results**

### ***Anticancer Activity of Extracts of *Peristrophe bicalyculata****

From the results obtained (Table 1), the methanolic ethylacetate fraction with an  $IC_{50}$  of  $15.60 \pm 0.52 \mu\text{g/mL}$  inhibited 50% of the KB cancer cells at concentrations significantly lower ( $P < 0.05$ ) than the hexane extract, with an  $IC_{50}$  of  $328 \pm 52.1 \mu\text{g/mL}$ . The  $IC_{50}$  values of the methanol extract, methanolic water and methanolic butanol fraction were  $>500 \mu\text{g/mL}$ . However, the standard drugs, cisplatin ( $3.32 \pm 0.09 \mu\text{g/mL}$ ), doxorubicin ( $0.43 \pm 0.02 \mu\text{g/mL}$ ) and vincristine ( $0.05 \pm 0.01 \mu\text{g/mL}$ ) inhibited 50% of the cancer cells at concentrations significantly lower ( $P < 0.05$ ) than that of the methanolic ethyl acetate ( $15.6 \pm 0.52 \mu\text{g/mL}$ ) and hexane ( $328 \pm 52.1 \mu\text{g/mL}$ ) extract.

The  $IC_{50}$  value of vincristine ( $0.02 \pm 0.001 \mu\text{g/mL}$ ) against HeLa cells was significantly lower than the values obtained for cisplatin ( $3.42 \pm 1.95 \mu\text{g/L}$ ), doxorubicin ( $2.74 \pm 0.15 \mu\text{g/mL}$ ) and all the extracts. With respect to HT-29, the  $IC_{50}$  value of vincristine ( $0.07 \pm 0.03 \mu\text{g/mL}$ ) was significantly lower than those of cisplatin ( $1.12 \pm 0.56 \mu\text{g/mL}$ ), doxorubicin ( $1.13 \pm 0.24 \mu\text{g/mL}$ ) and all the extracts tested. The  $IC_{50}$  value of the methanolic butanol fraction ( $298 \pm 20.50 \mu\text{g/mL}$ ) was significantly ( $P < 0.05$ ) lower than the value obtained for the methanolic water fraction ( $490 \pm 42.6 \mu\text{g/mL}$ ), while the hexane extract, methanol extract and methanolic ethylacetate fractions had  $IC_{50}$  values above  $500 \mu\text{g/mL}$ . Also, the  $IC_{50}$  values of cisplatin ( $5.52 \pm 0.76 \mu\text{g/mL}$ ), doxorubicin ( $0.42 \pm 0.25 \mu\text{g/mL}$ ) and vincristine ( $2.87 \pm 0.71 \mu\text{g/mL}$ ) against HepG2 were significantly ( $P < 0.05$ ) lower than those of the methanolic ethylacetate fraction, hexane extract and methanolic butanol fraction (Table 1).

The methanolic ethylacetate fraction of *Peristrophe bicalyculata* was partially purified by column chromatography after thin layer chromatographic separation. The thin layer chromatography was carried out using ethyl acetate and hexane (5:5) which gave six separate constituents, visible under UV-spectrum. Column chromatography using silica gel was then run with hexane (100%), hexane:ethylacetate (8:2), hexane:ethylacetate (5:5), hexane:ethylacetate (3:7), ethylacetate:chloroform (9:10), ethyl acetate:methanol (9:1) and ethylacetate: methanol (1:1).

Seven fractions (Figure 1) obtained after partial purification were tested for their anticancer activity against KB cancer cells by the sulphorhodamine B (SRB) assay. From the results (Table 2), fractions 1, 2, 4, 6 and 7 inhibited 50% of the cancer cells at concentrations

above 500 µg/mL, which was significantly ( $P < 0.05$ ) higher than the concentration at which fraction 3 ( $235 \pm 33.05$  µg/mL) inhibited the cells. Fraction 5 inhibited the cancer cells at a concentration ( $3.50 \pm 0.21$  µg/mL) significantly ( $P < 0.05$ ) lower than fraction 3 ( $235 \pm 33.05$  µg/mL), fractions 1, 2, 4, 6 and 7 ( $>500$  µg/mL), but not different from that of the standard drug, cisplatin ( $3.32 \pm 0.09$  µg/mL). The  $IC_{50}$  values of the standard drugs, doxorubicin ( $0.43 \pm 0.02$  µg/mL) and vincristine ( $0.05 \pm 0.01$  µg/mL) were significantly lower than that of fraction 5 ( $3.5 \pm 0.21$  µg/mL).

#### *Apoptotic Effect of Partially Purified Extract of Peristrophe bicalyculata*

There was a significant ( $P < 0.05$ ) increase in percentage apoptotic cells 24 h and 48 h after treatment with the partially-purified anticancer fraction of *Peristrophe bicalyculata* (fraction 5) and cisplatin compared to control cells (Figure 2). The percentage of apoptotic cells in cultures treated with 10 µg/mL ( $15.06 \pm 2.31$  %) cisplatin was not significantly different from those obtained in cultures treated with fraction 5 at 250 ( $12.49 \pm 1.96$  %) and 500 µg/mL ( $16.25 \pm 1.87$  %); while the percentage of apoptotic cells in cultures treated with 100 µg/mL cisplatin ( $22.74 \pm 2.44$  %) was not significantly different from the value obtained in cells treated with 1000 µg/mL of fraction 5 ( $21.20 \pm 2.17$  %). Forty-eight (48) hours after treatment, the percentage of apoptotic cells increased significantly ( $P < 0.05$ ) compared to that recorded after 24 h treatment. There was a significant increase ( $P < 0.05$ ) in the percentage of apoptotic cells in cultures treated with 1000 µg/mL cisplatin ( $81.22 \pm 3.62$  %), compared to cultures treated with 100 µg/mL ( $63.35 \pm 3.84$  %), 10 µg/mL ( $49.73 \pm 5.23$  %) and those treated with fraction 5 (Figure 2).

#### *Necrotic Effect of Partially-purified Extract of Peristrophe bicalyculata*

Treatment of cells with cisplatin and partially-purified fraction (fraction 5) after 24 h resulted in a significant ( $P < 0.05$ ) increase in necrotic cells, compared to control culture (Figure 3). The percentage of necrotic cells in cultures treated with the partially-purified fraction (fraction 5) at 1000 µg/mL ( $33.62 \pm 2.94$  %) was significantly higher than that of cells treated at either 500 µg/mL ( $24.77 \pm 3.68$  %) or 250 µg/mL ( $18.28 \pm 2.12$  %), but was not different from the percentage recorded in cultures treated with cisplatin at 1000 µg/mL ( $37.33 \pm 3.22$  %). After 48 h, the percentage of necrotic cells rose significantly ( $P < 0.05$ ) in cisplatin and extract-treated cultures at all concentrations, compared to the corresponding treatments after 24 h. Cell cultures treated with 10 µg/mL ( $48.30 \pm 7.10$  %) cisplatin had significantly higher percentage of necrotic cells than those treated with 250 µg/mL fraction 5 ( $37.84 \pm 5.68$  %) (Figure 3).

**Table 1:** The  $IC_{50}$  (µg/ml) values for anticancer activity of extracts of *Peristrophe bicalyculata*

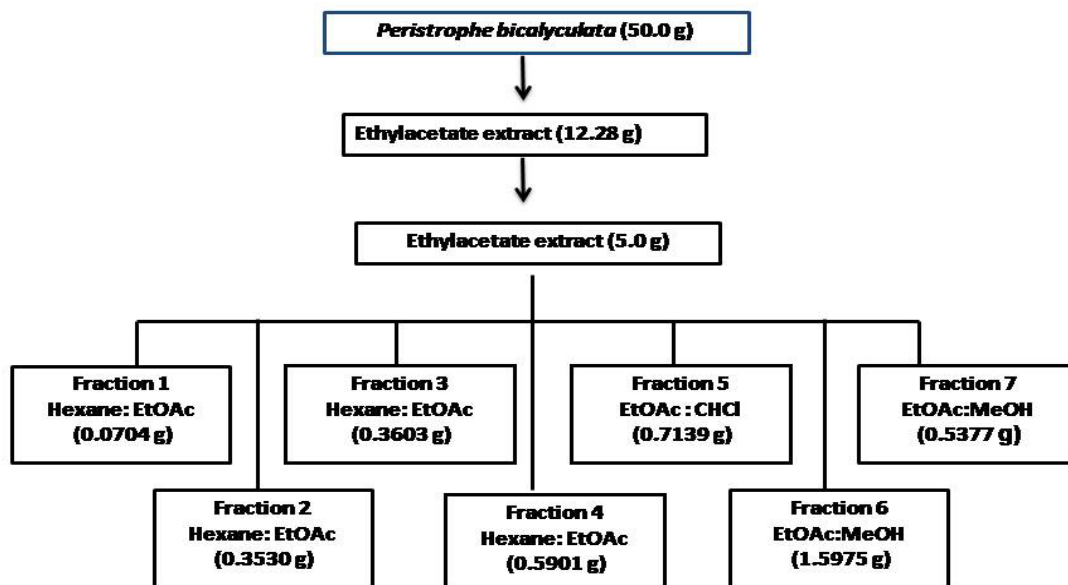
| Sample                      | IC <sub>50</sub> values (µg/ml ± SD) |                           |                          |                          |
|-----------------------------|--------------------------------------|---------------------------|--------------------------|--------------------------|
|                             | KB                                   | HeLa                      | HT-29                    | Hep G2                   |
| Hexane extract              | 328 ± 52.1 <sup>a</sup>              | >500                      | >500                     | >500                     |
| Methanol extract            | >500                                 | 430 ± 34.0 <sup>g</sup>   | >500                     | ND                       |
| Methanolic water fraction   | >500                                 | >500                      | 490 ± 42.6 <sup>f</sup>  | ND                       |
| Methanolic butanol fraction | >500                                 | >500                      | 298 ± 20.50 <sup>k</sup> | >500                     |
| Methanolic ethylacetate     | 15.60 ± 0.52 <sup>b</sup>            | 450 ± 25.60 <sup>g</sup>  | >500                     | 395 ± 37.0 <sup>r</sup>  |
| Cisplatin                   | 3.32 ± 0.09 <sup>c</sup>             | 3.42 ± 1.95 <sup>h</sup>  | 1.12 ± 0.56 <sup>y</sup> | 5.52 ± 0.76 <sup>p</sup> |
| Doxorubicin                 | 0.43 ± 0.02 <sup>d</sup>             | 2.74 ± 0.15 <sup>h</sup>  | 1.13 ± 0.24 <sup>y</sup> | 0.42 ± 0.25 <sup>m</sup> |
| Vincristine                 | 0.05 ± 0.01 <sup>e</sup>             | 0.02 ± 0.001 <sup>j</sup> | 0.07 ± 0.03 <sup>s</sup> | 2.87 ± 0.71 <sup>n</sup> |

Values are mean ± SEM

Values with different superscripts in the same column are significantly different ( $P < 0.05$ )

> 500 =  $IC_{50}$  values higher than 500 µg/ml (inactive)

ND = Not determined



**Fig. 1. Yield and fractions obtained from ethylacetate extract of *Peristrophe bicalyculata***

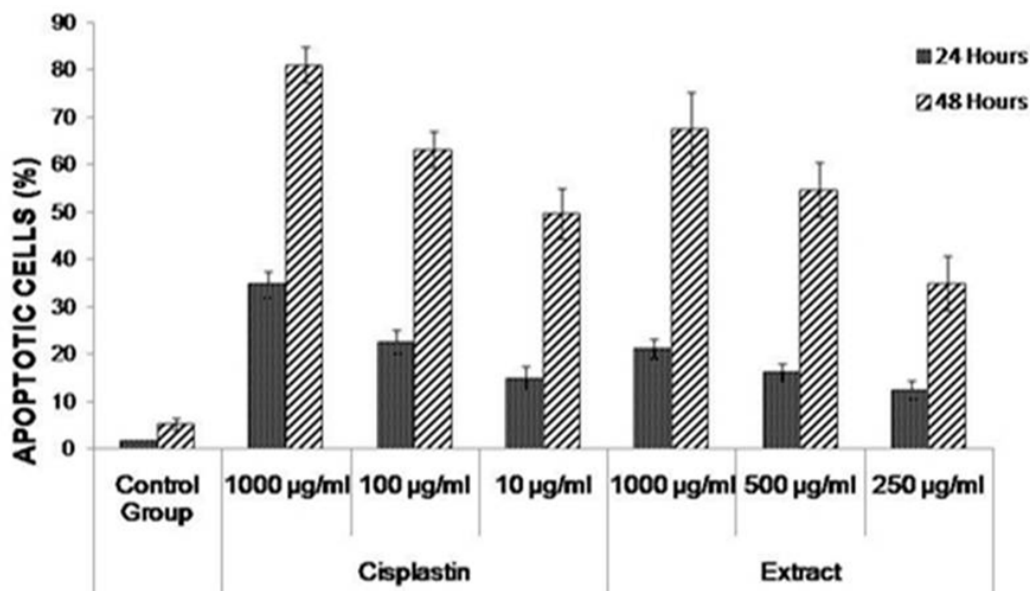
**Table 2: Anticancer activity of fractions of *Peristrophe bicalyculata***

| Fractions   | IC <sub>50</sub> values (µg/ml) |
|-------------|---------------------------------|
| Fraction 1  | >500                            |
| Fraction 2  | >500                            |
| Fraction 3  | 235 ± 33.05 <sup>a</sup>        |
| Fraction 4  | >500                            |
| Fraction 5  | 3.50 ± 0.21 <sup>b</sup>        |
| Fraction 6  | >500                            |
| Fraction 7  | >500                            |
| Cisplatin   | 3.32 ± 0.09 <sup>b</sup>        |
| Doxorubicin | 0.43 ± 0.02 <sup>d</sup>        |
| Vincristine | 0.05 ± 0.01 <sup>e</sup>        |

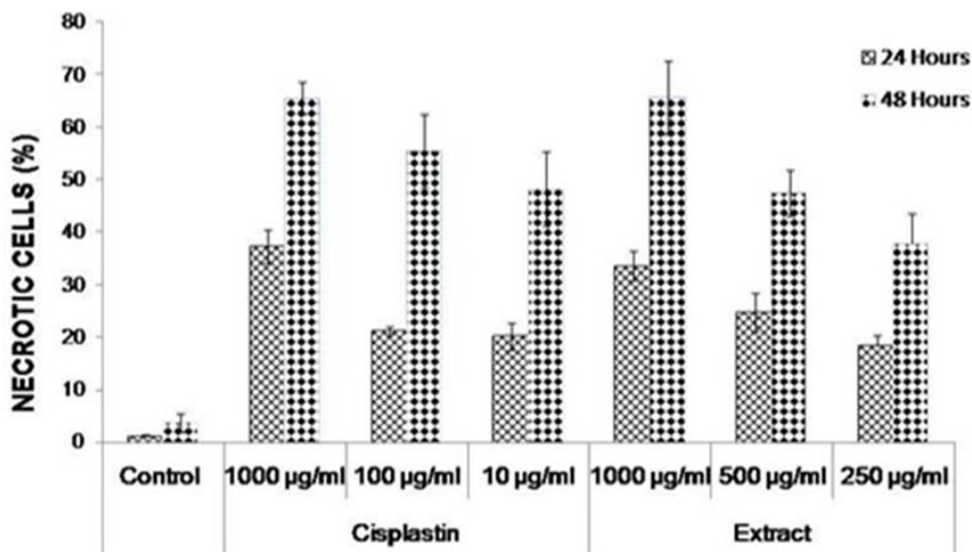
Values are mean ± SEM

<sup>a,b,c,d,e</sup> = Values with different superscript letters are significantly different (P < 0.05)

> 500 = IC<sub>50</sub> values is higher than 500 µg/ml (inactive)



**Fig. 2. Percentage of apoptotic human mouth epidermal carcinoma cells after 24 and 48 hours treatment with *Peristrophe bicalyculata***



**Fig. 3. Percentage of necrotic human mouth epidermal carcinoma cells after 24 and 48 hours treatment with *Peristrophe bicalyculata***

*GC-MS Analysis of the Partially Purified Anticancer Fraction of Peristrophe bicalyculata*

The gas chromatogram of the partially-purified antihypertensive fraction of *Peristrophe bicalyculata* is shown in Figure 4. Six (6) distinct peaks were obtained and the compounds identified were andrographolide 2(3H)-furanone (RT: 58.63 minutes; molecular weight: 350 g), 2,4,5-Trimethoxybenzaldehyde (RT: 47.34 minutes; molecular weight: 196 g), Diazoprogerone (RT: 50.22 minutes; molecular weight: 338 g), cis-Z-alpha-bisabolene epoxide (RT: 52.04 minutes; molecular weight: 220 g), aromadendrene oxide (RT: 57.58 minutes; molecular weight 220 g) and 3,11-Bis[(trimethylsilyl)oxy]androstan-17-one o-benzyloxime (RT: 56.33 minutes; molecular weight: 555 g). Andrographolide 2 (3H)-furanone was the most abundant of all the compounds identified.

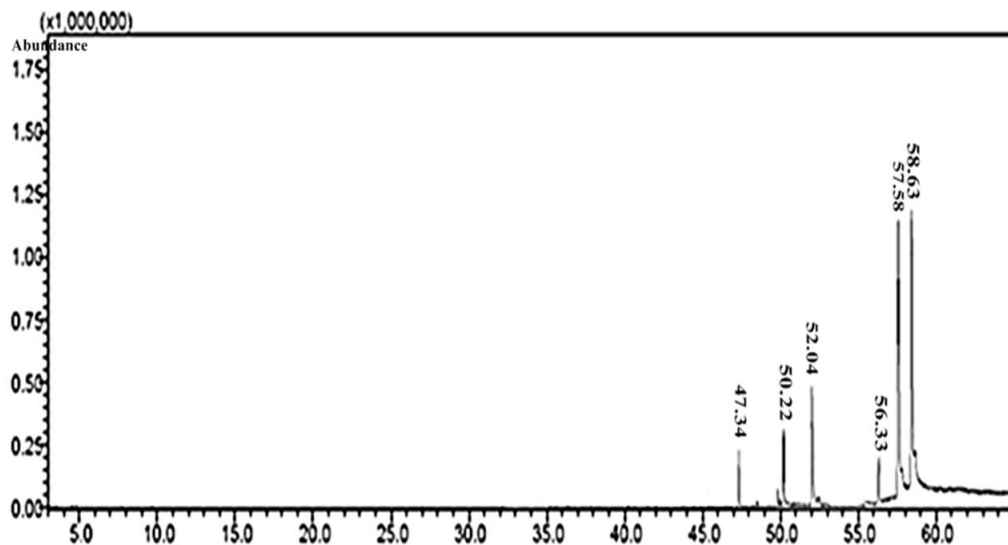


Fig. 4. Gas chromatogram of partially purified anticancer fraction of *Peristrophe bicalyculata*

## Discussion

In this study, the anticancer activity of *Peristrophe bicalyculata* on four cancer cell lines: HeLa, HT-29, HepG2 and KB was investigated using the sulphorhodamine B assay (SRB). The assay was most preferred for cytotoxicity testing because it relies on the uptake of the negatively charged pink aminoxanthine dye, sulphorhodamine B (SRB) by basic amino acids in the cells, unlike the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) or 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide sodium salt (XTT) that relies on the activity of the mitochondria. Therefore, if the function of the mitochondria is inhibited by variations in cellular levels of NADH, glucose and other factors, the results will show the cells are not alive or not proliferating (33). The anticancer activity of *Peristrophe bicalyculata* (Table 1) shows that the hexane, methanol and methanolic butanol fractions were active against some of the cell lines, but they may only be classified as potentially harmful or moderately toxic (34). However, the methanolic ethylacetate fraction, with an  $IC_{50}$  of  $15.60 \pm 0.52$   $\mu\text{g/mL}$  may be classified to be potentially very cytotoxic against KB cells. Further purification of this fraction gave seven fractions; five (fractions 1,2,4,6 and 7) of which were inactive, with  $IC_{50}$  values above 500  $\mu\text{g/mL}$ , and one (fraction 3) moderately toxic. Fraction 5 was most active with an  $IC_{50}$  value of  $3.5 \pm 0.21$   $\mu\text{g/mL}$ . This result supports the findings, demonstrating the anticancer activity of extracts and essential oils of *Peristrophe*



*bicalyculata* in different cancer cell lines (6,7,8). Several studies have also reported potent anticancer properties of extracts of plants, belonging to the same family as *Peristrophe bicalyculata*, Acanthaceae (35,36,37).

It is a conceivable fact that one essential strategy for cancer therapy is to target the lesions that suppress apoptosis in the tumour cells, as can be found in most cancer chemotherapy drugs which exert cytotoxic effects on malignant cells by inducing apoptosis (38,39,40). Thus, it is suggestive that the partially-purified anticancer fraction (TLC spot 5) of *Peristrophe bicalyculata* may be a possible anticancer drug candidate. This is because the morphological changes observed on treating KB cells with this fraction demonstrated its ability to induce apoptosis. Although its mechanism of action has not been elucidated, the anticancer activity of *Peristrophe bicalyculata* may be attributed to its high antioxidant activity and its high phenolic content. Phenols are known to inhibit cancer cells by metabolizing xenobiotic enzymes that alter metabolic activation of potential carcinogens, alteration of hormone production, inhibition of aromatase to prevent the development of cancer cells and by disturbing the cellular division during mitosis at the telophase stage (41). It has also been reported that phenols reduce the amount of cellular protein and mitotic index, and the colony formation during cell proliferation of cancer cells (42).

Apoptosis or programmed cell death is a normal physiological process that eliminates unwanted cells and maintains homeostasis in healthy tissues. Studies have demonstrated that tumour growth is regulated by a balance between cell proliferation and apoptosis (40). It has also been suggested that the loss of apoptotic control in favour of cell proliferation is responsible for cancer initiation and progression (38). Hence, one essential strategy for cancer therapy adopted in the present study was to target the lesions that suppress apoptosis in the tumour cells (39). The presence of compounds known to be cytotoxic against cancer cells in the partially purified anticancer fraction, obtained in the present study may play major roles in the anticancer activity of the plant. Andrographolide 2(3H)-furanone, the most abundant compound present in the partially-purified anticancer fraction of *Peristrophe bicalyculata* is a derivative of andrographolide, the principal bioactive chemical constituent isolated from *Andrographis paniculata* Nees. Andrographolide has shown credible anticancer potential in various investigations (43, 44). *In vitro* studies demonstrated its ability to induce cell-cycle arrest and apoptosis in a variety of cancer cells at different concentrations (45). It has also shown potent immunomodulatory and anti-angiogenic activities in tumorous tissues. Synthetic analogues of the compound have also exhibited similar activities (44). Both *Peristrophe bicalyculata* and *Andrographis paniculata* Nees belong to the same family and, hence, the finding of the present study is in agreement with the reports demonstrating the presence of similar compound and action in plants belonging to the same family (43, 46).

Aromadendrene oxide has been isolated and characterized from the stems of *Sandorium koetjape*, and shown to be significantly cytotoxic against many cultured human cancer cells, especially P-388 cells (47). 2,4,5-Trimethoxybenzaldehyde has been isolated from *Antrodia camphorata* and shown to be chemoprotective against colon and breast cancer (48). It manifests anti-inflammatory properties by inhibiting cyclooxygenase 2 in MDA-MB-231 cells (49).

The present results are in agreement with the findings that extracts and essential oils of the plant were cytotoxic against some cancer cells (6,7,8). The present study did not only show that extracts of the plant were cytotoxic against KB cells, but also demonstrated that they inhibited cancer cells by inducing apoptosis. The proximate mechanism by which apoptosis was induced, which was not investigated in the present work, requires further studies.

## Conclusions

In conclusion, this study has shown that *Peristrophe bicalyculata* may be a potential source of valuable anticancer therapy.

## Acknowledgements

A part of this study was supported by Tertiary Education Trust Fund (TETFUND) of Nigeria. The authors wish to express their gratitude to Prof. Dr. Aranya Manosroi and Prof. Dr. Jiradej Manosroi for giving us the opportunity to carry out some parts of this work at the Science and Technology Research Institute, Chiang Mai University, Thailand.

## Authors' Note

The corresponding author was previously at the Natural Product Research and Development Centre, Science and Technology Research Institute, Chiang Mai University, Chiang Mai, Thailand.

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