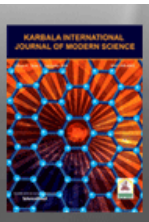

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Anticancer And Antiangiogenic Activities Of Alkaloids Isolated From Lantana Camara By Adsorption On The Magnetic Nanoparticles

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Abstract

Lantana camara L. (*L. camara*) is a perennial shrub that contains low amounts of alkaloids. In the present study, the magnetic nanoparticles (MNPs) were used to isolate positively charged alkaloids from the methanolic extract of *L. camara* leaves. The crude alkaloid was fractionated using HPLC to separate the highest peak of the alkaloid fraction (HPAF). The crude alkaloids (CA) and HPAF were tested for their antiproliferative effect against cancer cell lines (MCF-7, HCT-116, and HeLa) and endothelial cells line (EA.hy926) as a standard cell line. Antiangiogenic properties were examined using rat aortic ring assay. HPAF exhibited a profound anticancer effect against MCF-7 and HeLa cell lines (with IC_{50} = 0.027 µg / mL and 5.90 µg / mL, respectively, while it displayed reasonably mild cytotoxicity against the HCT-116 cell line (IC_{50} = 8.38 µg / mL). The CA also demonstrated a significant anticancer effect against MCF-7 and HeLa and a weak cytotoxic effect against colon cancer HCT-116 cells. Cationic alkaloids displayed selective antiproliferative activity against HeLa while it was utterly safe on the normal test cell line. HPAF demonstrated remarkable antiangiogenic activity in non-toxic doses. Also, cationic alkaloids showed significant antiangiogenic effects. The use of magnetic nanoparticles (MNPs) to separate small quantities of precious compounds is handy and cost-effective.

Keywords

Anticancer, alkaloids, antiangiogenic, Lantana camara L, and magnetic nanoparticles

Cover Page Footnote

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1. Introduction

Cancer is a serious hazard to human health, and it causes death to millions of people worldwide annually. In Iraq, the Cancer Registry reported more than twenty thousand newly diagnosed cancer cases in 2012 [1]. The crude incidence of all cancer types was 61.69 per 100,000 (70.59 in women and 53.31 in men). Breast cancer morbidity is reported as the highest compared to other cancer types in Iraq [1]. The search for cheap, efficient, and easy-to-manufacture therapeutics for cancer has always been a hot area of research. Cancer treatment represents a significant challenge as there is no specific therapeutics regime for all kinds of cancers, besides conventional chemotherapy, which employs cytotoxic drugs associated with severe side effects and sometimes chemoresistance [2]. Hence, there is a need for new therapeutic compounds to reduce adverse effects and combat resistance. Scientific communities worldwide placed tremendous efforts to discover and develop new drugs directly from natural sources, especially from plants used in traditional medicine [3,4] or from synthetic derivatives [5,6]. Medicinal plants serve as an abundant reservoir for bioactive agents that improve human health by treating various diseases. It has been used to treat cancer and continued to be used as a home remedy by some traditional healers in developing countries. Several natural compounds obtained from medicinal plants, including alkaloids, triterpenoids, and flavonoids, proved to have anticancer properties [7−12].

Alkaloids are classified as a phytochemicals group that contains a nitrogen atom in their structure, mostly in a heterocyclic ring. It has potent antineoplastic effects against various cancers [13]. The majority of cancer drugs approved by the FDA are alkaloids of natural origins such as camptothecin and vinblastine. Chemotherapy medications are formulated to interact with fast-growing cancer cells. Several studies showed that alkaloids isolated from plants such as berberine, sanguinarine, and matrine are capable of triggering apoptosis and inhibiting cancer cell proliferation [14−16].

Angiogenesis is a process of developing new blood vessels from pre-existing vasculature, is a major pathological component of some diseases such as cancer, rheumatoid arthritis, obesity, and coronary heart disease [17]. Tumour angiogenesis is crucial for solid tumors to grow and metastasis. It facilitates the supply of oxygen nutrients to the tumor. Besides, it acts as an avenue for the dissemination of malignant cells to a distant organ. Therefore, tumor angiogenesis is a good target for cancer therapy and prevention [18].

Lantana camara L. (*L. camara*) is a tropical plant that exists in 60 countries. Few studies reported the isolation of some bioactive compounds from *L. camara,* such as lantadene, which is a common pentacyclic triterpenoid in the plants. Lantadene showed antitumor effects against human promyelocytic leukemia cells, cervical, colon, and lung cancers [19,20]. *L. camara* contains a relatively small percentage of alkaloids that are difficult to detect or extract [21,22]. Alkaloids in the leaves of *L. camara* have been detected qualitatively, in a tiny percentage, using conventional protocols of phytochemical screening [22,23]. Some phytochemical studies failed to detect alkaloids in *L. camara* due to their minute concentration in the plant tissues, or adequately it could be masked by other substances in the extract [24]. In the present work, a new method was invented to separate specific alkaloids by adsorption on the iron oxide magnetic nanoparticles (MNPs), which are negatively charged [25,26] that preferably and selectively adsorbed alkaloids. To the best of our knowledge, this the first research that reports the isolation of alkaloids from *L. camara* using novel techniques that recruit the MNPs as a separating medium. The crude alkaloids and the isolated highest peak of the alkaloid fraction (HPAF) were evaluated for their anticancer and anti-angiogenic properties.

2. Materials and methods

2.1. Materials

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was supplied by Thermo-Fisher Scientific (Massachusetts, USA). L-glutamine, aminocaproic acid, aprotonine B, gentamicin, fibrinogen, aprotinin, bovine serum albumin, DMSO, potassium bromide, deuterated chloroform, anhydrous $MgSO_4$ were supplied by Sigma−Aldrich (Taufkirchen, Germany). Petroleum ether from GCC (London, UK), while chloroform, $FeCl_3.6H_2O$, $FeSO_4.7H_2O$, HCl, NaOH from BDH Chemicals Ltd. (Poole, England). Acetic acid and ammonia solution were supplied by Fluka Chemicals Ltd. (Neu-Ulm, Germany), while n-hexane was purchased from

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Thomas–Baker (Mumbai, India). Toluene, NaCl, and methanol were supplied by SDFCL (Mumbai, India).

2.2. Plant material

L. camara leaves were hand-picked during the flowering stage (Spring-Summer 2016) from the Agricultural Unit of the University of Kufa, Najaf, Iraq. The plant samples were identified by a senior taxonomist (Dr. Aboothar Hatem) in the herbarium. The specimen was given voucher No. IQ Mena-1. The irrelevant materials were removed and the collected leaves were dried in the shadow at room temperature. The powdered leaves were oven-dried at 40 °C for 3 h to ensure complete dryness prior extraction process to obtain the correct weight of the dried *L. camara* leaves.

2.3. Extraction and fractionation

One kilogram of the powdered leaves was extracted by petroleum ether (5 L) for 4 h by using Soxhlet extractor to remove the essential oils and fatty materials. The extraction was repeated with petroleum ether in the Soxhlet extractor until further extraction gave a colorless solution. The petroleum ether extract was separated, and the residues assumed to contain alkaloids were then exhaustively extracted with methanol by Soxhlet for 3 h. The extract was concentrated to dryness using a rotary evaporator. The crude methanolic extract was thoroughly mixed with acetic acid (10%, 1 L), diluted with 500 mL of water, and let to stand overnight. After filtration of the mixture to remove insoluble materials, the pH of the aqueous filtrate was increased to 9.5 by adding drops of aqueous ammonia solution. It was then shaken three times with chloroform in a large separating funnel, and the chloroform extracts were dried over anhydrous magnesium sulfate, filtered, and evaporated to obtain alkaloid residue [27].

2.4. Synthesis of the new Fe_3O_4 MNPs

In the present study, Fe_3O_4 MNPs were synthesized by a coprecipitation process recently improved in our lab [28]. In brief, in 100 mL of 0.5 M HCl solution, 0.08 mol of 0.04 mol of FeSO4.7H2O and FeCl3•6H2O is dissolved. Then one liter of 1,5 M NaOH solution was applied dropwise to the mixture under intense stirring at 80 °C. The resulting black Fe_3O_4 precipitate was separated using a magnet. The MNPs were continually washed with deionized H_2O until the supernatant's pH becomes neutral. Then, the suspensions were dried overnight at 40 °C in the oven. The separated MNPs were dried at 50 °C for 4 h and stored closed until use. Transmission electron microscopy (TEM) was used to measure the size and visualize the morphology of the prepared MNPs.

2.5. Extraction of cationic alkaloids using MNPs

To separate the cationic alkaloids fractions (abbreviated as AF) from the *L. camara* crude alkaloid mixture, one milliliter of the crude alkaloid mixture was mixed with 250 mg MNPs in a large (5 mL) Eppendorf tube. The above ratio of the MNPs/alkaloids solution was obtained empirically (by previous experiments) to optimize the best ratio that assures complete adsorption of cationic alkaloids on the MNPs surface. After incubation for 2 h at room temperature, MNPs coated with alkaloids were separated magnetically and washed twice to remove any soluble materials. To recover the adsorbed cationic alkaloids, two milliliters of 0.1 M HCl were added to the MNPs and shaken for 1 h to assure the release of most adsorbed cationic alkaloids to the solution [29]. After centrifugation at 4000 rpm for 20 min, the mixture was exposed to a magnetic field to remove any colloidal MNPs, and the supernatant containing cationic alkaloids were separated and examined for alkaloids. A strongly positive test (reddish-brown precipitate) was formed after adding a few drops of Mayer's reagent to the supernatant, indicating a relatively high concentration of cationic alkaloids separated by MNPs [30].

2.6. Separation of cationic alkaloids using HPLC

The HPLC system (Agilent Technologies 1200 infinity, USA) was equipped with a UV–visible photodiode array detector, auto-sampler, quaternary pump, column incubator, and degasser. The HPLC analysis was performed on an XDB C18 analytical column (particle size = 5 μm, dimensions of the column = 4.6 × 250 mm) with isocratic elution. The acetic acid-methanol–water mobile phase was used at a ratio (82:18:0.3, v/v/v). The reverse-phase HPLC assay was performed using an isocratic system at a flow rate of 1.6 mL/minute using a constant temperature maintained at 25 °C. The detection was carried out by ultraviolet (UV) detector at λ_{max} 280 nm. Total runtime was less than 20 min for each injection, and data acquisition was carried out by Agilent ChemStation software. Twenty microliters of the alkaloid's solution were injected into the column through the injection valve. According to the chromatogram data,

peak fractions were manually recorded. Each fraction was evaporated under decreased pressure into dryness and dissolved in methanol. The major fraction (with highest peak area) was further subjected to purification using the preparative HPLC method. Three peaks, one major high peak, and two secondary low peaks were obtained with retention times 2.27, 2.57, and 4.51 (minute. second). The third peak (RT = 4.51) with the highest peak area was collected as a single fraction. This peak showed the most decisive positive test for alcohol, indicating the high alcohol contents in this fraction and called the highest peak of the alkaloid fraction (HPAF).

2.7. Antiproliferative assay

The antiproliferative effect of the alkaloids extracted from *L. camara* was performed by MTT assay according to the Mosmann (1983) method [31] with minor modifications [32]. Briefly, 1.5×10^4 cells in one hundred microliters of fresh culture medium were seeded in each well of the 96-well plate and incubated overnight. The stock solutions of HPAF, AF, and CA were diluted with the media of the cell culture medium to obtain six serial dilutions (2.84, 5.67, 11.36, 22.72, 45.44, 90.88 μg/mL). One hundred microliters were applied to each well from each concentration. The medium was aspirated from wells after two days of the treatment, and 10% v/v of MTT solution (5 mg/mL in sterile PBS) were added to all wells and incubated for 3 h at 37 °C in 5% CO_2 incubator. The formed formazan salts were dissolved in 200 μl DSMO added to each well. Absorbance was measured at a primary wavelength of 570 nm and a reference wavelength of 620 nm by using i-control™-Microplate reader equipped with a software (TECAN Group Ltd., Switzerland) for calculating the concentration form absorbance. The negative control (Blank) was 0.1% v/v DMSO solution. The results are expressed as a mean % inhibition to the negative control ± standard deviation. The assay was performed in quadriceps and the mean of results were calculated.

2.8. Antiangiogenic effect of L. camara alkaloids using "rat aortic ring assay"

All the animal studies were officially approved by the "Animal Ethics Committee of Malaysia Division of the EMAN Research Office" (Approval No. AEA-2020-2092-EA) in April 11th, 2020. The anti-angiogenic property of HPAF, AF, and CA was assessed on *ex vivo* rat aorta ring assay as described previously with slight modifications [33].

2.8.1. Preparation of aortic rings

In the CO_2 chamber, the rats were humanely euthanized. A midline was developed with the sternum splitting into the abdominal and thoracic cavities. Then, excision of the thoracic aortas, rinsing with serum-free urine, and separation of the fibroadipose tissue were performed and the aortas were transformed into small circles roughly 1 mm thickness.

2.8.2. Preparation of tissue culture plates

The lower layer medium was prepared by adding fibrinogen (3 mg/mL) and aprotinin (5 μg/mL) to the M199 basic medium. Then, in a 48-well plate, 300 μL of this mixture was added to each well. The sections of the aortic ring are then positioned in each well. Ten microliters of thrombin solution (NIH U/mL of thrombin in 0.5 M NaCl and bovine serum albumin) were added to each well to coagulate at 37 °C in the 5% CO_2 incubator for 1.5 h. The top layer medium was primed by adding the aminocaproic acid at 0.1%, aprotonine B at 1%, FBS at 20% v/v, gentamicin at 0.6%, and L-glutamine at 1% to the basic medium M199. For evaluation, the anti-angiogenic effect of tested samples various concentrations of each sample was added, at 100 μg/mL, to the top layer medium and were incubated in 5% CO_2 incubator at 37 °C. After two days, the top layer medium was replaced with a freshly prepared medium. DMSO was used as a negative control.

2.8.3. Quantification of outgrowth of the blood vessels

The method of Nicosia et al. (1997) was used to quantify the degree of blood vessel outgrowth [34]. Briefly, at the 5th day of growth, the width of the blood vessels from the primary tissue was determined by an inverted light microscope supplied with 4X magnification power. "Lecia Quin computerized imaging system". The growth gap was estimated to be at least 20 blood vessels per ring. The blood vessels were picked to decrease the bias at regular intervals across the rings. The experiment was repeated three times, each replicate containing six rings, and the experiments were repeated three times, and the results were expressed as a mean % inhibition ± standard deviation (SD).

2.9. Statistical analysis

The results were expressed as a mean ± SD. The mean of the results was used in all experiments. The differences between means were analyzed using the

pooled t-test. Pearson correlation coefficient (r) was used to express the correlation between HPAF and the inhibition percentage of microvessels formation. The differences are considered significant when $p < 0.05$. The figures were plotted using Microsoft Excel software (2016). All statistical analysis was conducted using the SPSS Statistics Program (IBM-USA) version 25 (2017).

3. Results and discussion

3.1. Anticancer effect of HPAF and crude alkaloids of L. camara

The antiproliferative effect of extracted crude alkaloids and purified alkaloid (HPAF) was assessed against three cancer cell lines in addition to one standard cell line using MTT assay. Table 1 shows the values of median inhibitory concentration (IC_{50}) of each cell line. The highest peak HPAF exhibited selective cytotoxicity against the tested three cancer cell lines. It showed profound anticancer effect against breast cancer cell lines (MCF-7) in shallow doses (with $IC_{50} = 0.027$ μg/mL), which is much better than Tamoxifen (standard drug) with $IC_{50} = 6.70$ μg/mL, followed by cervical cancer cell line (HeLa) and colon cancer cell line (HCT-116) ($IC_{50} = 5.9$ μg/mL and 8.38 μg/mL respectively), the isolated highest peak also exhibited a mild cytotoxicity against the human endothelial cells (EA.hy926).

The crude alkaloid (CA) demonstrated significant anticancer effect against MCF-7 with $IC_{50} = 1.60$ μg/mL (more than fourfold better than tamoxifen), also has a significant activity against HeLa ($IC_{50} = 6.60$ μg/mL), whereas it showed weak or moderate cytotoxic effect against colon cancer cells and standard endothelial EA. hy926 cell line with IC_{50}s = 449.85 and 31.77 μg/mL, respectively. The shapes of the cell lines after treatment with the three samples compared to the negative controls are presented as images in Fig. 1. The figure shows the top two of the cells most affected by the three extracts. The dose-dependent cytotoxic effect of HPAF on the four cell lines, HCT-116, MCF-7, HeLa, and EA. hy926 are expressed as means in Fig. 2. Crude alkaloids (CA) displayed selective antiproliferative activity against cervical cancer cells (HeLa) with $IC_{50} = 2.10$ μg/mL; interestingly, it was utterly safe on standard endothelial cell lines (with $IC_{50} > 1000$ μg/mL).

Table 1
IC_{50} Values of HPAF, ordinary crude alkaloids (CA), and alkaloid fraction extracted by MNPs (AF) on three human cancer cell lines and one standard cell line.

Tested Samples	Cell lines (IC_{50} in μg/mL[a])			
	MCF-7	HeLa	HCT-116	EA.hy926
HPAF	0.027	5.90	8.38	0.29
CA	9.20	2.10	15.57	212.13
AF	1.60	6.60	449.85	31.77
Tamoxifen	6.70	–	–	12.64
5-fluorouracil	–	–	5.30	–
Betulinic Acid	–	18.50	–	25.70

[a] The median inhibitory concentrations (IC_{50}) were determined by analyzing linear regression equations obtained from log-concentration-response curves of three different tests (n = 3).

A couple of decades ago, studies claimed that stem barks and roots of *L. camara* contain a lantanine (a quinine-like alkaloid) with robust antispasmodic and antipyretic properties [35]. However, it was tough to detect alkaloids in the plant using conventional phytochemical methods [36]. Recently few studies reported the presence of alkaloids in various *L. camara* extracts [37,38]. However, there is no record for the chemical structure of alkaloids from *L. camara*. To this end, a new technique was employed to isolate alkaloids from *L. camara* by using MNPs. MNPs are used as a surface to adsorb and positively-charged alkaloids from the crude extract in an attempt to tackle the issue of the difficulty of isolating alkaloids via conventional chromatographic techniques from *L. camara* leaves [22]. Before applying MNPs for separation, the crude extract was defeated using petroleum ether until alkaloids can be natural to detect by Mayer's qualitative test [26]. As such, CA and positively charged AF can be extracted and analyzed by HPLC using a successive separation method. Three peaks were identified; the major peak with the highest area was collected as a single fraction, which was subjected to spectroscopic techniques as an alkaloid fraction with a high alcoholic positive result, which is called HPAF.

In the current work, nanotechnology was utilized, in a cost-effective approach, to separate minute amounts of alkaloids depending on their opposite charges and the magnetic properties of the MNPs. Besides, MNPs have a functional adsorption capacity for the cationic species more than neutral or anionic species [39,40]. The extraction technique is cost-effective because it is feasible to free MNPs from the substances that adhere to its surface to be reused for isolation of bioactive compounds several times. Recently, different plant extracts were used in the green synthesis of MNPs [41,42]. However, the use of MNPs in extracting a precious compound from plant extract was not widely used. In the present study, MNPs are particularly advantageous to extracting alkaloids from crude extracts

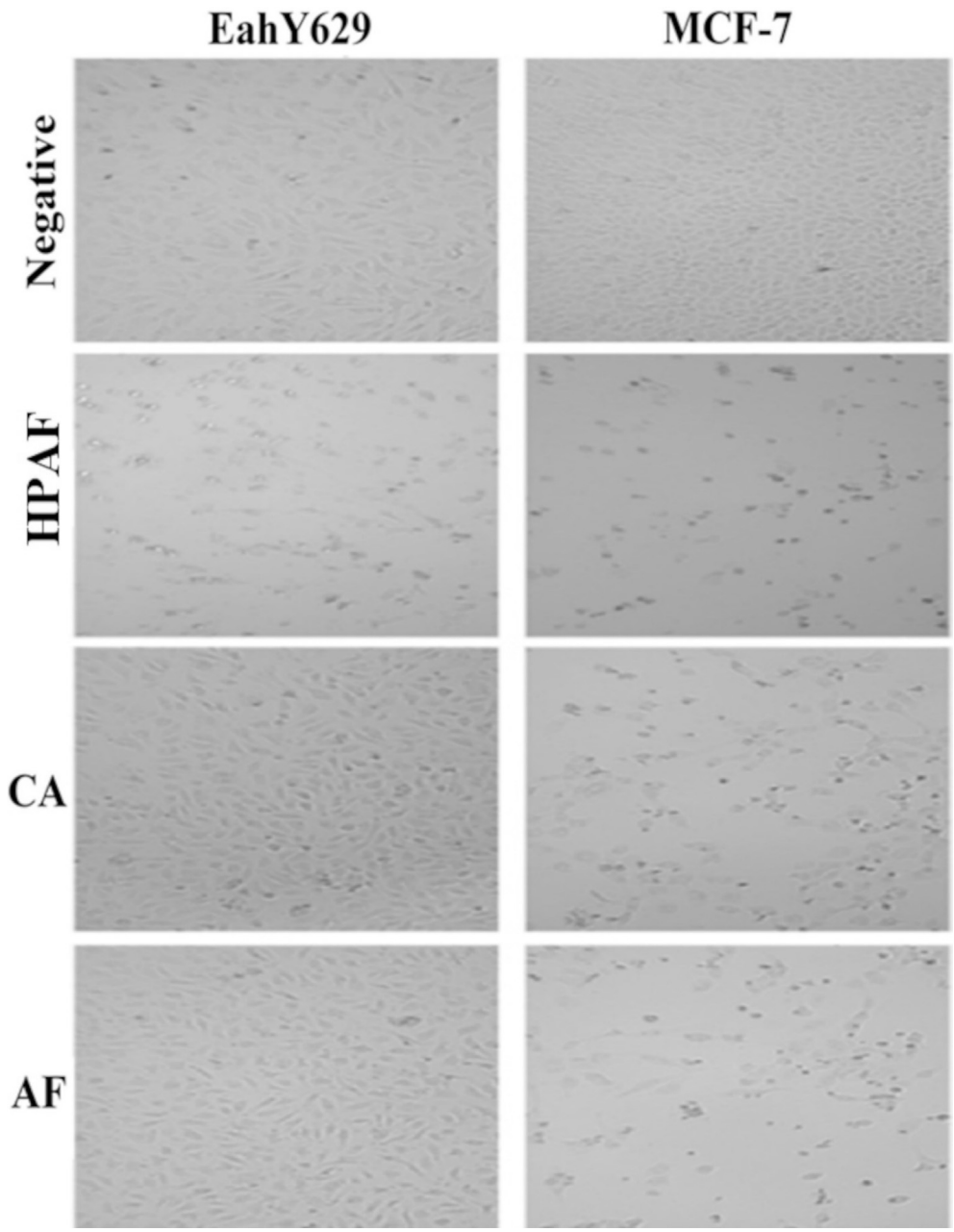

Fig. 1. The shapes of the cell lines (magnification ×40); human cancer cell lines, human endothelial cells (EA.hy926), and breast cancer cell lines (MCF-7) after treatment with HPAF, ordinary crude alkaloids (CA) and alkaloid fraction extracted by MNPs (AF).

when it found a low amount or, for some reason, difficult to extract with conventional techniques.

3.2. Antiangiogenesis activity of HPAF and crude alkaloids of L. camara

HPAF and crude methanolic extract of *L. camara* exhibited a profound antiangiogenic effect on *ex vivo* "rat aorta ring assay" (Table 2). HPAF was found to inhibit new vessels' sprouting in non-toxic doses (with IC_{50} = 1.56 μg/mL). The CA profoundly inhibited sprouting of microvessels for rat aortic ex-plants with IC_{50} = 43.62 μg/mL. Surprisingly it did not show any sign of cytotoxicity toward human endothelial cells EA. hy926. AF also demonstrated significant anti-angiogenic with IC_{50} = 109.05 μg/mL (also on safe

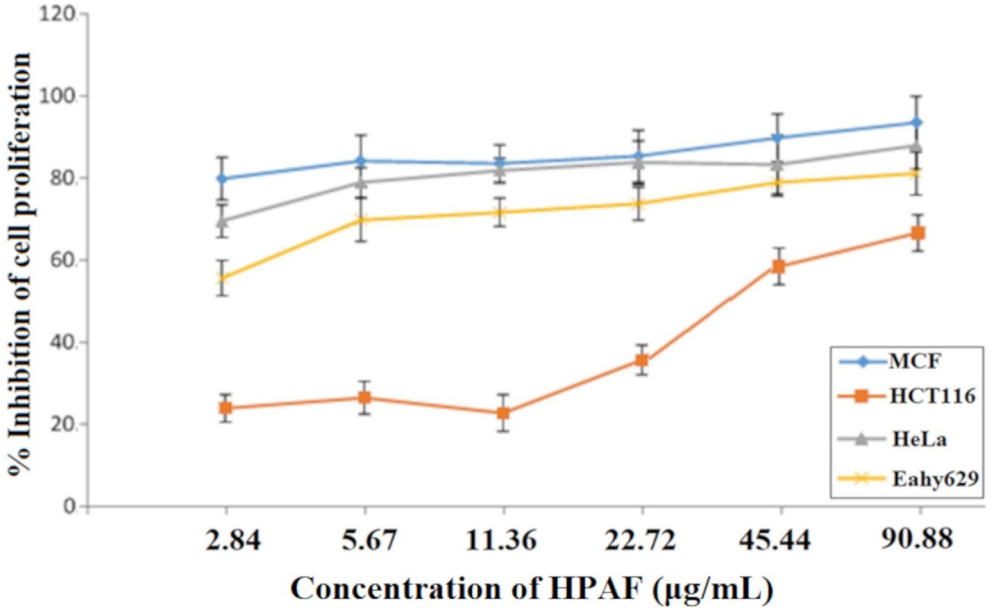

Fig. 2. The dose-dependent cytotoxic effect of HPAF on the four cell lines; HCT-116, MCF-7, HeLa, and EA. hy926.

doses), while imatinib (the standard antiangiogenic drug) showed inhibitory effect with $IC_{50} = 612.05$ µg/mL. The isolated highest peak (HPAF) and crude alkaloids excelled in curbing new blood vessels in explanted tissues. In summary, alkaloids extracted from *L. camara* represent promising antiangiogenic agents warranted for further study to develop a new drug for cancer and angiogenesis-related diseases. The effect of HPAF on the budding of microvessels for rat aortic are presented in Fig. 3. The graphical representation of the percentage inhibition of blood vessel growth in explanted tissue by different HPAF concentrations is presented in Fig. 4. The inhibition of microvessels formation is highly correlated with the HPAF concentrations ($R^2 = 0.986$, $p < 0001$). It is clearly noticeable that the inhibition of angiogenesis is dose-dependent at a very low concentration.

Alkaloids are among the most essential active ingredients in botanical herbs that have a biological effect. Some of these substances have also come into the therapeutic arena as a cancer treatment medication, such as vinblastine and vincristine (isolated from *Vinca rosea*) that interacts with tubulin [43], camptothecin (extracted from *Camptotheca acuminate*), a potent topoisomerase I inhibitor [44] and berberine from *Rhizoma coptidis* that has a chemo-preventive effect against colon cancer as well as an antiangiogenic effect [45].

Table 2
The results of the rat aorta ring assay presented as a mean percent inhibition of the alkaloids separated from *L.camara* in comparison with the standard drug Imatininb.

Substance	IC_{50} µg/mL
HPAF	1.56
Crude Alkaloid (CA)	43.62
Alkaloid fraction (AF)	109.05
Imatinib	450.74

The HPAF, CA, and AF were investigated for anticancer and antiangiogenic properties against different cancer cell lines. All tested samples showed a significant antiproliferative effect; however, HPAF exhibited a profound antiproliferative effect against all tested cancer cell lines. It showed an inhibitory effect far better than standard drugs. The breast cancer cell line was the most susceptible to HPAF with $IC_{50} = 0.027$ µg/mL. In another study, *L. camara* ethanolic extract displayed properties of cell death in the MCF-7 cell line by induction of the apoptosis and regulation of the Bcl-2 family [46]. These findings support the *L. camara* extract as a potential anti-breast cancer drug. Our result coincides with this study and suggests that the antiproliferative activity of the extract is attributed to the HPAF.

Angiogenesis is a biochemical mechanism by which new blood vessels are created from pre-existing vasculature. It plays a vital role in the progression of solid tumors [47]. Agents that can effectively inhibit tumor angiogenesis are more likely to have therapeutic value against tumor progression [48]. Curbing tumor angiogenesis will stop blood supply and oxygen

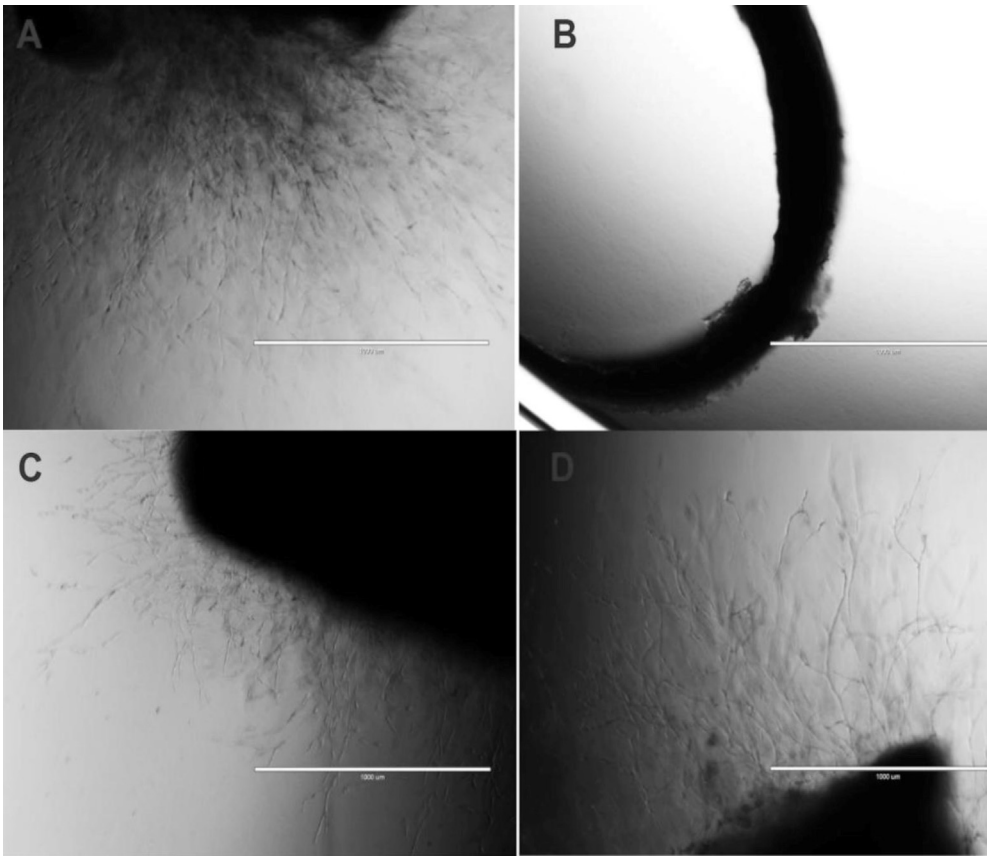

Fig. 3. Antiangiogenic effect of HPAF against sprouting of microvessels in rat aortic ex-plants (magnification ×40). A: Photomicrographic image of rat aortic ring of negative control showing extensive growth of microvessels, B: Photomicrographic image of rat aorta ring treated with 1090.53 μg/mL, C: Photomicrographic image of rat aorta ring treated with 136.31 μg/mL, D: Photomicrographic image of rat aorta ring treated with 17.08 μg/mL.

needed for growing tumors; besides, it cuts the cancer cell's avenues to metastasis to distant organs [48,49]. There are active alkaloids driven from plants that have potent antiangiogenic effects, for example, berberine, noscapine, brucine, evodiamine, sanguinarine, capsaicin, harmine, and pterogynidine, which block angiogenesis cascade in endothelial cells by down-regulation of STAT3 and β-catenin signaling, as well as Akt phosphorylation [15,49,50]. Sinomenine, brucine, and halofuginone can directly inhibit angiogenesis growth factors such as Smad protein, VEFG, TNF-α, and HIF-1α [50]. In previous work, the aqueous extract of *L. camara* was investigated for its effect on the angiogenesis process using *in vitro* and *in vivo* animal models; it showed remarkable antiangiogenic properties [51].

To our knowledge, this is the first reaserch that evaluates the antiangiogenic effect of alkaloids extracted from *L. camara*. The isolated HPAF profoundly halted new vessels' formation from ex-planted tissue (rat aorta) in non-toxic doses with IC_{50} = 1.56 μg/mL while imatinib (the standard anti-angiogenic drug) showed inhibitory effect with IC_{50} = 450.74 μg/mL. Interestingly, the activity is ascending from crude alkaloid, alkaloid fraction to isolated fraction, which suggests that the new alkaloids in HPAF are responsible for the antiangiogenic property of *L. camara*.

3.3. Limitations of the study

The chemical structure of HPAF is not determined in the present study due to the limited number of techniques that predict the planner structure of the molecules. Therefore, the purest alkaloid fraction was used. It needs more advanced techniques such as 2D NMR data, high-resolution MS, and X-ray, etc., to predict the three-dimensional structure of L. Also, the lowest two peaks of alkaloids in the HPLC diagram need more investigation to define the chemical properties of these

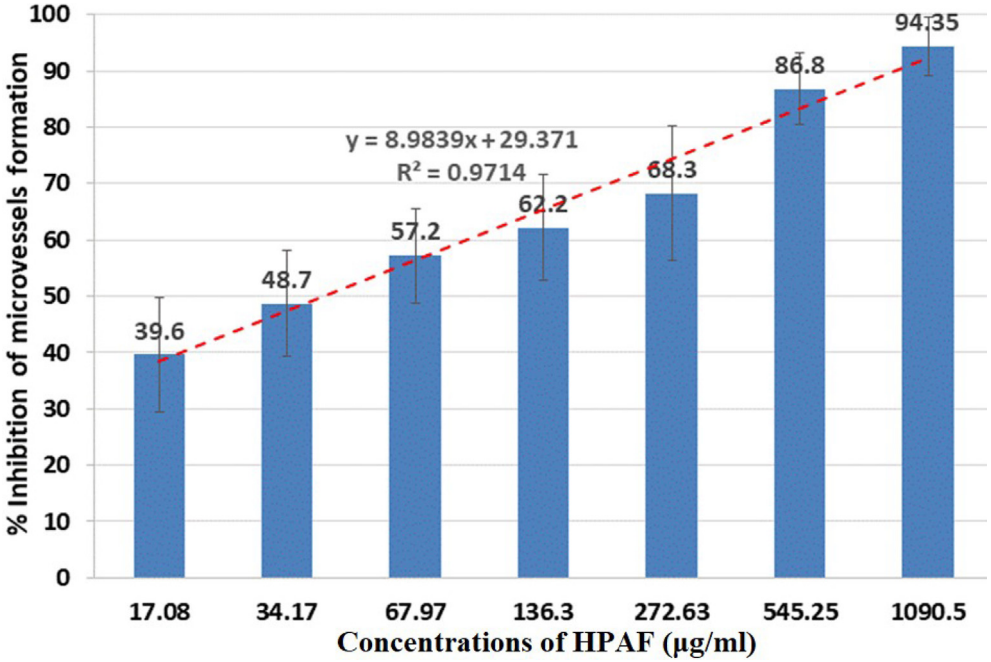

Fig. 4. Graphical representation of percentage inhibition of blood vessel growth from ex-planted tissue by different HPAF concentrations.

compounds. Because of the lack of information about the alkaloids in *L. camara*, it is impossible to discuss or suggest a mechanism for the biosynthesis of alkaloids in *L. camara*. Another factor is the ignored effect of time of shipping the samples from Iraq to Malaysia. Whether the storage time affects the chemical reactions between the components of the mixture of *L. camara* extract needs to be elucidated.

4. Conclusion

In the current work, a new alkaloid fraction (HPAF) was isolated from *L. camara* leaves, exploiting MNPs as a new technique for separating positively charged alkaloids. The HPAF, as well as crude alkaloids, were evaluated for their antiproliferative and antiangiogenic properties. It can be concluded that the separated alkaloid fraction has a selective cytotoxic effect against breast and cervical cancer cell lines, as well as potent antiangiogenic properties. Further mechanistic study on cancer cell, toxicological and preclinical investigation is warranted to develop a new cancer therapy drug.

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Author's contributions

The authors have participated equally in the preparation of the manuscript.

Declaration of competing interest

Concerning the submitted paper, the authors have no conflict of interest with any commercial or other affiliations.

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