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Anti-cervical cancer potentials and GC-MS analysis of *Cymbopogon citratus* (DC.) Stapf and *Origanum vulgare* Linn.Extracts

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Abstract

Cervical cancer is the fourth leading cause of death among women worldwide and many conventional drugs have several side effects and poor selectivity in cancer treatment. In this study, the cytotoxic potentials of *C. citratus*(CC) and *O. vulgare*(OV) extracts were assessed as a probable source of natural products for the treatment of cervical cancer. The crude extracts and fractions were challenged against cervical (HeLa) cancer cell line using MTT assay. The phytochemical components present in the extracts were analyzed using gas chromatography-mass spectrometry (GC-MS).The CC crude exhibited the highest inhibition on the growth of HeLa (CC_{50}=43.43±0.05 µg/mL) as compared with OV crude (170.2±0.11 µg/mL). The CC fractions' potentials were in the order of n-hexane (3.81±0.22 µg/mL) >dichloromethane (DCM) (5.44±0.16 µg/mL)>aqueous (38.57±0.12 µg/mL)>ethylacetate (50.24±0.05 µg/mL). Similarly, OV fractions' potentials were in the order of n-hexane (57.95±0.12 µg/mL)>DCM (61.63±0.07 µg/mL)>ethylacetate (172.4±0.21 µg/mL)>aqueous (176±0.14 µg/mL).These results were compared with vincristine (standard) with aCC_{50} value of 0.75±0.14 µg/mL.The major compounds present in CC aqueous extract with their percentage composition includeCyclohexasiloxane,dodecamethyl-(20.79%),Cycloheptasiloxane,tetradecamethyl-(17.93%), Cyclopentasiloxane, decamethyl-(15.04%), Cyclooctasiloxane, hexadecamethyl-(8.98%). OV aqueous extract revealed Hexadecanoic acid, methyl ester(24.71%), 9-Octadecenoic acid (Z)-, methyl ester(22.52%), Methyl stearate(15.40%), 9-Octadecenoic acid (Z)-, methyl ester(11.56%), as its major compounds.Our results suggest that the CC and OV plants are a favorable source of natural products with promising properties for anti-cervical drug discovery.

Keywords: Cervical; Cancer; Phytochemicals; Extracts; Fractions; Chromatography

1. Introduction

Cervical cancer prevalence has increased to alarmingly high levels worldwide, with low- to middle-income countries reporting a 4-fold higher prevalence of the disease in 2010[1]. In countries classified as low on the Human Development Index (HDI), the disease is ranked as the second most prevalent type of cancer and the second largest cause of cancer-related mortality amongst women after breast cancer [2]. Cervical cancer is the most common type of cancer diagnosed and the main reason why people die from cancer in Africa. There are 60.9 million women in Nigeria who are 15 years of age or older and at risk of acquiring cervical cancer. According to current statistics, 7,968 women die from cervical cancer each year while 12,075 women receive a diagnosis. Human papillomavirus (HPV)-16/18 infection in the cervical

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region is predicted to affect 3.5% of women in the general population at any given time, and 66.9% of invasive cervical malignancies are caused by HPVs 16 or 18 [3]. Girls between the ages of 9 and 13 can receive the vaccine to prevent the illness, but it is not financially feasible [4]. Similarly, the other conventional treatments that are now accessible, such as surgery, radiation, and chemotherapy, are likewise out of reach [5].Furthermore, novel synthetic chemotherapeutic drugs created in the previous decade that are currently being used in clinical settings have fallen short of expectations despite the high expense of development[6]. According to Bansal and Malhotra [7], these synthetic chemotherapeutic drugs frequently have severe adverse effects on normal cells (unselective). Therefore, there is a continuing need for new, potent, and affordable anticancer medicines with little adverse effects on healthy cells. This required the use of novel cytotoxic agents derived from medicinal plants.

In many countries throughout the world, medicinal plants are a frequent alternative to conventional cancer therapy, making them essential to human survival. It has been demonstrated that several agents, including vinblastine, vincristine, irinotecan, topotecan, camptothecin, taxol, and podophyllotoxin derived from various plants, had anticancer effects [8, 9]. *Oreganum vulgare* and C*. citratus* are medicinal perennial herbs with a wide range of pharmacological applications in traditional medicine, complementary and alternative therapies, and modern medicine. In order to assess their therapeutic properties against cancer, especially in Nigeria where cancer cases are on the rise, these edible herbs' potential pharmacological properties must be made known to the scientific community [10, 11]. These herbs are present in almost all of our home gardens and markets and are consumed throughout the world as a spice and prophylactic.There have been few investigations on *O. vulgare* and *C. citratus*' ability to prevent human skin, liver, stomach, lung, prostate, and colon cancer[12, 13]. To the best of our knowledge, this is the first study to describe the GC-MS-analysed phytochemical components and anti-cervical potentials of *O. vulgare* and *C. citratus* aqueous crude extracts and their fractions using HeLa cell line.

2. Material and methods

2.1. Collection of Plants

The plant *C. citratus*, known as lemongrass, and *O. vulgare*, known as Oregano,were collected from Dadin Kowa, Jos South LGA, Plateau State. The plant was identified and authenticated by Dr. Agyeno and a voucher number (FFJ/2019/240) was issued at the Herbarium of the Department of Plant Science and Biotechnology, Faculty of Natural Science University of Jos, Nigeria and deposited for future reference.

2.2. Extraction

The plant parts collected were dried under a shade and ground into a coarse powder at room temperature. Maceration was carried out on 400 g each of plant material in an aqueous solution for 72 hours and was stirred occasionally. Filtration was carried out and the extracts were dried using a freeze dryer. The extracts were stored at 4 °C until analysis.To obtain fractions of different polarity from the crude extract, it was re-dissolved in 250 mL of distilled water and partitioned (3×) with an equal volume of n-hexane, dichloromethane (DCM), and ethyl acetate (EtOAce) and aqueous. Fractions were concentrated under reduced pressure.

2.3. MTT assay to determine the extracts' effect on cell proliferation

2.3.1. Cell culture

HeLa cells were gotten from the WHO reference polio laboratory, University College Hospital, Ibadan, Nigeria. Eagle's minimum essential medium (EMEM) supplemented with 10% Fetal Bovine Serum (FBS), penicillin (100 units/mL), streptomycin (100 mg/mL), L-glutamine (2 mM), 0.07% $NaHCO_3$, and 1% non-essential amino acids and vitamin solution were used for the cell culture. The cultures were sustained in a humidified condition with 5% CO_2 at 37 °C and split every two weeks.

2.3.2. Cytotoxicity assay

Using the methods described by [14], the ability of the cells cleaving to MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], Sigma, Chem), by the action of mitochondrial enzyme succinate dehydrogenase was evaluated. The monolayers of the cells grown in 96 well-microtitre plates reached confluency in 24 hours. Pre-solubilization of each crude extract in dimethyl-sulphoxide (DMSO) at 37 °C and 10-fold dilutions were serially done to give concentrations of 1000 to 0.01 µg/mL. Incubation of cells with varying extracts concentration was then done at 37 °C in a CO_2 environment, CTX (positive control), and negative control (growth medium alone) in triplicate for 72 hours. Furthermore, the viability of the cell was evaluated microscopically for the cytopathic effect (CPE) if it is present or

absent. When the 72 hours of treatment elapsed, the supernatants were taken out from the wells, and MTT solution (25 µL, 2 mg dissolved in 1 mL of PBS) was applied per well. The plates were incubated at 37 °C for 2 hours to dissolve the formazan crystals before DMSO (75 µL) was applied per well. The microtitre plates were agitated for 15 min, and the optical density was quantified by a multi-well spectrophotometer (Multiskan, Thermo Fisher Scientific, Waltham, MA) at 492 nm. The extract concentration required to reduce cell viability by half was identified as the 50% cytotoxic concentration (CC_{50}). A non-linear regression curve included in the GraphPad Prism software was used to compute the CC_{50} value.

2.3.3. GC-MS Analysis

The aqueous crude extracts of*C. citratus* and*O. vulgare* were analyzed with GC (Agilent Technologies 7800, USA) coupled with MS (Agilent Technologies 5975, USA) to determine their phytochemical constituents. The HP5MS capillary column (30 m×0.32 mm×0.25 µL) was used under the following conditions: oven temperature coded from 80 °C for 2 min, then progressively increased to 240 °C for 6 min; injector temperature (250 °C), carrier gas (Helium), flow rate is 1 mL/min; the volume of the injection sample was 1 µL; scan ranges from 50–500 and mode of analysis is splitless. The relative quantity of every component was computed by the comparison between its average peak area to the total area. The Identification details of the separated volatile molecules were carried out through retention indices and mass spectrometry by comparing using a database of National Institute Standard and Technology (NIST), library 2008.

3. Results

3.1. Anti-cervical effects of *C. citratus* and *O. vulgare* aqueous crude and fractions

The result obtained showed that the crude aqueous extract of *C. citratus*had aCC_{50} value of 43.43±0.05µg/mL while *O. vulgare* crude extract had an IC_{50} value of 170.2±0.11µg/mL (Figure 1). The n-hexane fraction of *C. citratus* had the highest anti-cervical effect (3.81±0.22µg/mL) followed by the DCM fraction (5.44±0.16µg/mL) as compared with the standard drug Vincristine (0.75±0.14µg/mL). Similarly, the n-hexane fraction of *O. vulgare* had the highest anti-cervical effect (57.95±0.12µg/mL) followed by the DCM fraction (61.63±0.07µg/mL) as presented in Table 1.

3.2. GC-MS Analysis

*C. citratus*leaves aqueous extract revealed the presence of seventeen major bioactive molecules as shown in Table 2 and the abundance of the compounds in *C. citratus* leaf aqueous extract ranges between 0.95 and 20.79. Cyclooctasiloxane, hexadecamethyl- had the highest molecular weight of 593.2 g/mol with a retention time of 12.680 minutes while Propionic acid, 3-(m-aminobenzoyl)-2-methyl- had the lowest molecular weight of 207.2 g/mol with a retention time of 9.299 minutes.

Gas chromatography-mass spectrometry (GC–MS) analysis of *O. vulgare*leaves aqueous extract revealed the presence of thirteen major bioactive compounds as shown in Table 3. Methyl stearate had the highest molecular weight (298.5 g/mol) with a retention time of 16.541 minutes while 2-Octen-1-ol, (E)- had the lowest molecular weight of 128.21 g/mol with a retention time of 5.941 minutes.

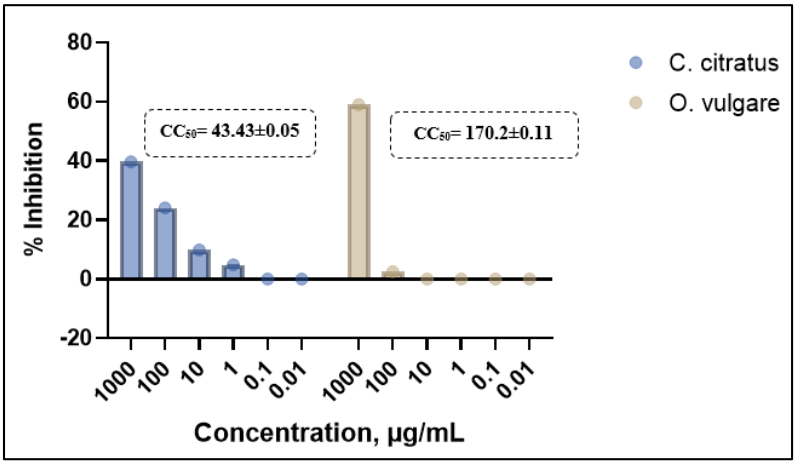

Figure 1 Percentage inhibition and CC_{50} of *C. citratus* and *O. vulgare* aqueous crude extracts

Table 1 Anti-cervical activity of *O. vulgare* and *C. citratus* fractions

Plant	Fractions	Anti-cervicalactivity (HeLa) CC_{50} ±SEM (µg/mL, n=3)
C. citratus	Hexane	3.81±0.22
	DCM	5.44±0.16
	EtOAc	50.24±0.05
	Aqueous	38.57±0.12
O. vulgare	Hexane	57.95±0.12
	DCM	61.63±0.07
	EtOAc	172.4±0.21
	Aqueous	176±0.14
Vincristine		0.75±0.14

aHeLa = human cervix adenocarcinoma; bMTT method, with the cells incubated with the test samples for 72 h.

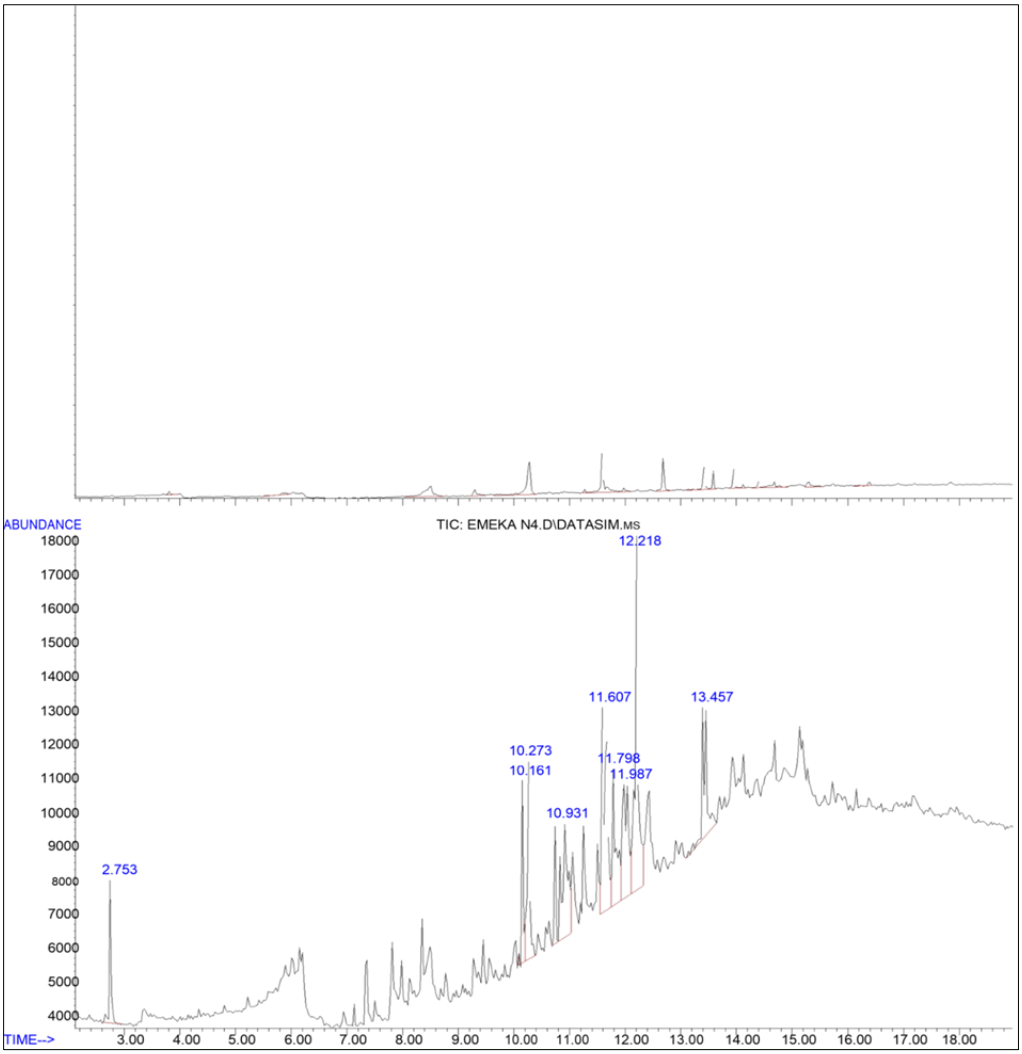

Figure 2 Chromatogram of the aqueous crude extract of *C. citratus*

Table 2 Moleculesfrom the Aqueous Extract of*Cymbopogon citratus* leaves obtained by GC-MS

Peak	Family	Retention Time	Area (%)	Compound	Molecular Formula
1	Organo-silicons	3.834	1.22	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$
2	Organo-silicons	5.863	1.57	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$
3	Organo-silicons	8.511	15.04	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$
4	Alkyl-phenylketones	9.299	3.64	Propionic acid, 3-(m-aminobenzoyl)-2-methyl-	$C_{11}H_{13}NO_3$
5	Organo-silicons	10.285	20.79	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$
6	Aromatic compound	11.271	0.95	1,2-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$
7	Organo-silicons	11.581	17.93	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$
8	Aromatic compound	11.976	2.05	(E)-2-bromobutyloxychalcone	$C_{19}H_{19}BrO_2$
9	Organo-silicons	12.680	8.98	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$
10	Fatty acid esters	13.412	6.86	Fumaric acid, 2-hexyl undecylester	$C_{21}H_{38}O_4$
11	Aromatic compound	13.609	4.27	1,2-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$
12	Organo-silicons	13.947	5.05	Silane, diethylpentadecyloxy(2-phenylethoxy)-	$C_{27}H_{50}O_2Si$
13	Acetanilides	14.145	1.04	Acetamide, N-[4-(trimethylsilyl)phenyl]-	$C_{21}H_{17}NOSi$
14	Alkaloid	14.398	2.11	1H-Indole-2-carboxylic acid, 6 (4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-isopropyl ester	$C_{21}H_{25}NO_5$
15	Benzodioxoles	14.680	4.31	N-methyl-N-acetyl-3,4-methylenedioxybenzylamine	$C_{11}H_{13}NO_3$
16	Aromatic compound	15.300	3.08	1,4-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$
17	Aromatic compound	16.398	1.12	1,2-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$

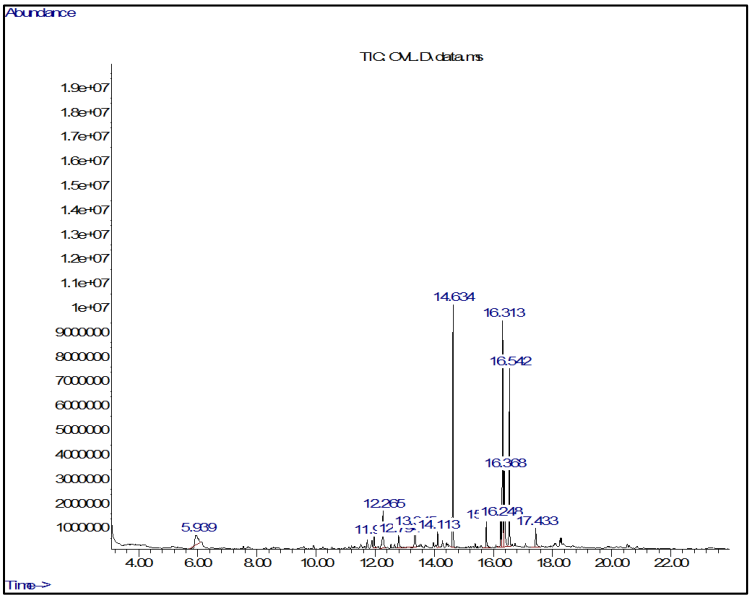

Figure 3 Chromatogram of the aqueous crude extract of *O. vulgare*

Table 3 Moleculesfrom the Aqueous Extracts of*Origanum vulgare* leaves obtained by GC-MS

Peak	Family	Retention Time	Area (%)	Compound	MW, g/mol	Molecular Formula
1	Alcohols	5.941	3.65	2-Octen-1-ol, (E)-	128.21	$C_8H_{16}O$
2	Alkanes	11.965	1.52	Cyclohexane, 1,5-diethenyl-2,3-dimethyl-, (1.alpha., 2.alpha., 3.alpha., 5.alpha.)-	164.29	$C_{12}H_{20}$
3	Alkenes	12.266	5.64	Albene	162.27	$C_{12}H_{18}$
4	Ketones	12.795	2.35	(Z)-2-6-Dimethylocta-2,5,7-trien-4-one	150.2	$C_{10}H_{14}O$
5.	Amines	13.345	2.59	3,5,7-Tramino-1-azaadamantane	182.27	$C_9H_{18}N_4$
6.	Ketones	14.113	1.67	1H-Inden-1-one, 2,3,3a,4,5,7a-hexa hydro-4,4a,7a-trimethyl-	178.27	$C_{12}H_{18}O$
7	Fatty acid methyl esters	14.632	24.71	Hexadecanoic acid, methyl ester	270.5	$C_{17}H_{18}O_2$
8	Sesquiterpene epoxides	15.763	3.03	Trans-Z-alpha.-Bisabolene epoxides	220.35	$C_{15}H_{24}O$
9	Fatty acid methyl esters	16.245	2.79	9,12-Octadecadienoic acid, methyl ester, (E,E)-	294.5	$C_{19}H_{34}O_2$
10	Fatty acid methyl esters	16.313	22.52	9-Octadecenoic acid (Z)-, methyl ester	296.5	$C_{19}H_{36}O_2$
11	Fatty acid methyl esters	16.370	11.56	9-Octadecenoic acid (Z)-, methyl ester	296.5	$C_{19}H_{36}O_2$
12	Fatty acids	16.541	15.40	Methyl stearate	298.5	$C_{19}H_{38}O_2$
13	Alkenes	17.433	2.55	.alpha-Farnesene	204.35	$C_{15}H_{24}$

4. Discussion

4.1. Anti-cervical activity of O. vulgare and C. citratus crude and fractions

In a variety of cell lines, the amount of formazan generated is directly related to the cell number [15]. The American National Cancer Institute (NCI) defines cytotoxicity for crude extracts as a CC_{50}<30 µg/mL in a preliminary assessment after a 72-hour exposure interval [16]. The result obtained showed that *C. citratus* and *O. vulgare* aqueous crude extracts had cytotoxicity that is above the normal range (Figure 1). Bioactivity-guided fractionation revealed that the cytotoxic molecules of *C. citratus* lie in the n-hexane and DCM fractions with CC_{50} far below 30 µg/mL on Hela cells as compared with the standard drug, Vincristine (Table 1). Similarly, the cytotoxic molecules of *O. Vulgare* lie in the n-hexane and DCM fractionsbut are above30 µg/mL as also shown in Table 1. Researchers have investigated *C. citratus*' anti-cancer abilities. Its unique oil which usually lies in the non-polar region (hexane/dichloromethane) has been shown to offer a strong barrier against certain malignancies. When directly injected, *Cymbopogon* citrate oil inhibits cancer tumours in a dosage-dependent manner, meaning the higher the oil dose, the better the outcome, according to animal studies. The trial's findings suggest that the oil has a potentially practical anti-cancer effect, reducing the viability of tumour cells by inducing the apoptotic process [17, 18].

4.2. GC-MS analysis

Due to the anti-cervical effect of *O. vulgare* and *C. citratus*, their components were identified by GC-MS. We found that the predominant class of molecules in *C. citratus* aqueous crude extract are the organo-silicons (Table 2), while*O. Vulgare*aqueous crude extractis the fatty acid methyl esters (Table 3). These bioactive molecules have been reported to play crucial roles in human disease and general metabolisms. Hexadecanoic acid, methyl ester also known as palmitic acid has many beneficial effects in preventing cancer [19] and improving immune function [20].Similarly, 9-

Octadecenoic acid and its methyl esters have a beneficial effect in the prevention of tumor growth [21]. No clear specific biological activity has been reported for the organo-silicon compounds identified in *C. citratus*, however, this class of compounds might also play a crucial role in the plant's metabolism and other health benefits to humans in traditional medicine.The identification of Fumaric acid, 2-hexyl undecylester from *C. citratus* leaf aqueous extract corroborates the findings of Abdullah et al. [22] who identified this compound from *Mangiferaindica* kernel as one of the bioactive components against breast cancer.

5. Conclusion

C. citratus and O. vulgare are well-known medicinal plants in traditional medicines. The leaves are used for the management of malaria, liver disease, stress, inflammation, hypertension, influenza, digestive problems, kidney problems, and cancer. Our results suggest that the *C. Citrates* and *O. vulgare* plants are a favourable sources of natural products with promising properties for anti-cervical drug discovery.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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