ORIGINAL ARTICLE

Antibacterial and Cytotoxic Activities of Ducrosia *anethifolia*: A Potential Biomedicine Against Selected Human Pathogens and Cancer Cell Lines

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ABSTRACT

Background and Objective: *Recently, many scientific researchers are focusing on investigating the antimicrobial activity of medicinal plants against different gram positive and gram negative bacteria as well as against some cancer cell lines as an alternative substituent to the available drugs in the market. This study was designed to investigate the chemical composition of Ducrosia anethifolia solvent extracts with their potential as antibacterial and anticancer biomedical agents*

Methods: *Different extracts of Ducrosia anethifolia were analyzed to reveal their major chemical composition using the gas chromatography-mass spectrometry (GC-MS). These compounds were further tested for their antibacterial (agar well diffusion technique) and cytotoxic activities (MTT assay).*

Results: *In-vitro antibacterial assay against selected human pathogens indicated highest susceptibility of Bacillus subtilis organic and aquatic extracts of Ducrosia fruit and leaves (15.5 mm inhibition zone) followed by Staphylococcus aureus (15mm) mainly with acetone fruit extracts. Negligible or no effect against was observed against the gram negative bacteria tested in this study. On the other hand, cytotoxic activity of methanol and water fruit and leaves Ducrosia extracts were tested on K562, HL60 and TPH1 (human adenoma leukemia) and MDA and MCF7 (human breast carcinoma) cell lines. Potent effect was recorded with water leave extract on K562 and MCF7 in a concentration varying between 4-5µl of extract/100 µl of cell line medium.*

Conclusion: *Potent therapeutic effect was observed with Ducrosia anethifolia crude extracts that may add further to new promising natural therapeutic agents.*

KEYWORDS: *Antibacterial, Cytotoxic activity, Ducrosia anethifolia, Biomedicine, Cell lines.*

INTRODUCTION

Flora of Saudi Arabia includes a large number of plant species making upto estimated 2243 species in total[1]. Medicinal plants represent about 254 species and considered as most economic plants.[2] In such a tremendous amount of variation, diversified labeled benefit from their natural ingredients in the manufacturing of agents for the treatment of various diseases has been extensively experimented, especially after the increased side effects of synthetic pharmaceutical and more importantly increased resistance. Thus scientists started to go back to nature by using medicinal plants with an interest of acting as a safe source of pharmaceutical therapy focusing, in particular, on those diseases which are incurable and difficult to be completely eliminated such as cancer; one of the most troubling diseases in the past few decades all over the world. It is a multi factorial disease contributing to the uncontrolled growth and invasion of abnormal cells, leading to tumor formation.[3] Cancer could be attributed to either the change in food habits, use of tobacco and alcohol, chronic infections, exposure to harmful radiations and chemicals, or more widely due to change in lifestyle and environmental pollution[4]. The recent estimates revealed the number of new cancer cases and cancer-related deaths has increased by 11% and 7.9%, respectively, in the year 2017 as compared to 2012[5]. Less developed countries suffer more serious effects from this disease due to the lack of diagnostic techniques, standard methods of treatment, and higher treatment cost.[6] Here comes the potential of bioactive plants providing new and novel products for disease treatment and prevention which overcomes the side effect of the synthetic products. The antitumor area has the greatest impact of plant derived drugs, where drugs like vinblastine, vincristine, taxol, and camp to the cin have improved the chemotherapy of some cancers, in addition to reducing the increased multidrug resistance induced with cancer

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chemotherapy. Furthermore, these plant derived therapeutics have been extensively studied and used as antimicrobial agent. During few recent decades and mostly as a result of their diversity, versatility and safety in comparison with the synthetic materials, natural products from plants have attained special interest among academic and industrial scientific communities.[7,8] Today we know that the essential oils and various plant extracts have a broad spectrum activity against Gram-positive and Gram negative pathogenesis and they also perform the antifungal activity.[9,10]

In this paper, the impact of natural extracts from the plant (Alhza) *Ducrosia anethifolia* (DC) Boiss Apiacae, an aromatic plant with medicinal properties, was studied. It is characterized by its branches growing from the base of wood in a length of about 35 cm and green leaves, with a long gray oval shape, and sharp appendages terminal. This plant grows in solid sandy soil as well as in rocky environments, especially near water rafting places.[11,12] It is a plant thought to be medicinal; in fact, it is used since ancient times to treat certain diseases such as chest ailments, gynecological diseases, infectious diseases and has dermatological uses; additionally it is used as an antimicrobial, antifungal, anti-depressant and anti-anxiety agent.[13-17] A literature survey showed no previous reports of the analysis of the volatile compound from *Ducrosia anethifolia* crude extracts. According to the best of our knowledge, there are no published reports on antimicrobial activity of crude extracts of *this plant*. Hence, the present study was designed to preliminary investigate the chemical composition of *Ducrosia anethifolia* solvent extracts with their potential as antibacterial and anticancer biomedical agents.

METHODS

Plant Material

The plant was locally collected from the province (Thadeq), 180 km North of Riyadh after taking the approval from institutionl Ethical Review Board and was identified at Plant Taxonomy Laboratory, Botany Department, King Saud University, Riyadh, Saudi Arabia.

Extraction Method

The aerial parts of the plant (leaves and fruits) were collected, washed with sterile distilled water and kept to dry at room temperature at shade. The dried samples were then grounded into powder and 08g of each sample were extracted with 120 ml of the organic solvents i-e acetone and methanol separately in addition to aqueous extraction with sterile distilled water. Samples were kept in a rotary shaker for 3 days at 120rpm and 28˚C. The extracts were further filtered through Whatman filter paper number: 1 and let stand at room temperature for complete evaporation. Dried extracts were then re-dissolved in sterile distilled water, filtered through Millipore syringe filter units of 0.45µm pore size (Millipore, USA) and then stored in sterile micro-centrifuge tubes at -4˚C for further use.

Bacterial Isolates

The bacterial strains tested in this paper were all provided from the Microbiology Department, King Khaled University Hospital, Riyadh. These isolates were divided into gram positive strains such as *Staphylococcus aureus* ATCC 25923, methicillin resistant *Staphylococcus aureus* MRSA ATCC 12498, *Bacillus subtilis* (ATCC 6633) and *Enterococcus feacalis* ATCC 29122 and gram negative namely: *Escherichia coli* ATCC 25966, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Salmonella* sp. and *Serratia* sp. all these isolates were cultured on nutrient agar (Oxoid), and 0.5 MacFrland turbidity of each bacterial suspension was prepared in 5ml nutrient broth tubes for the antibacterial assay.

In-vitro Antibacterial Assay

In reference to Berghe and Vlietinek[18], the antibacterial activity of dried *Ducrosia* leaves and fruit extracts (DL,DF) were performed using the well diffusion technique, where each bacterial suspension was inoculated on Muller Hinton agar plates (Oxoid) with sterile cotton swabs. Plates were then perforated with a sterile cork borer of 6 mm diameter. Wells were then loaded with 70µl of each of the extracts in study. Plates were rested at room temperature for 30 min and then incubated at 37˚C for 18- 24 hrs. All experiments were repeated in duplicate and average of the inhibition zone around each well was calculated in mm and tabulated (Table-1). Data were compared to positive standard antibiotic disc Meropenem (10µg) (Oxoid) and to the organic solvents methanol and acetone as negative control.

GC-MS Analysis

Chemical composition of *Ducrosia* dried methanol and acetone leaves (DL) and fruits (DF) extracts was determined using the GC-MS spectrum. An Agilent 7890A chromatograph, coupled with Agilent 5975C mass spectrometer (Agilent Technologies, USA), operating at 70 eV ionization energy, 0.5 s/scan and a mass range of 35-500, equipped with a HP-5MS (J & W) capillary column (30 m × 0.25 mm, 0.25 µm film thickness composed of 5% phenylmethylsiloxane) programmed as above with helium as the carrier gas at the flow rate of 0.9 mL/min and a split ratio of 1:60. GC-MS of standards solvents was performed simultaneously and was performed at King Saud University, Central Laboratory.

Cytotoxic Activity

K562, HL60 and TPH1 (human adenoma leukemia) together with MDA and MCF7 (human breast

carcinoma) cell lines were obtained from King Abdullah International Research Center, Riyadh, KSA. All cell lines were maintained in DMEM (Invitrogen) supplemented with 10% FBS and 100 µg/ ml Streptomycin in a humidified 5% CO_2 at 37˚C. Cell viability following to exposure to both dried leaves (methanol and water) extracts was estimated using the MTT reduction assay[19]. 100µl of all cell lines were loaded on 96 well plates at a density of $10X10^3$ cells/ml for (K562, HL60 and THP1), and $3X10^3$ for (MDA and MCF7) cells/ml, whereas the blank wells were loaded only with the growth medium. Extracts in study were added from 1µl to 9 µl. Plates were incubated in 5% CO_2 at 37˚C for 48 hrs. Following incubation, plates were read in a plate reader (Molecular device, USA) at 590 nm. Each assay was repeated twice, results were calculated from a calibration curve by linear regression using Microsoft excel.

RESULTS

Antibacterial Activity

The total six Ducrosia leaves (DL) and fruit (DF) extracts were tested for their antibacterial activity against some selected human pathogens listing *Staphylococcus aureus* ATCC 25923, methicillin resistant *Staphylococcus aureus* MRSA ATCC 12498, *Bacillus subtilis* (ATCC 6633) and *Enterococcus feacalis* ATCC 29122 and gram negative namely: *Escherichia coli* ATCC 25966, *Pseudomonas aeruginosa* ATCC 27853 , *Klebsiella pneumoniae* ATCC 700603, *Salmonella* sp. and *Serratia* sp. Higher activity was noted with both acetone and methanolic fruit extracts against the gram positive *Bacillus subtilis* (15-15.5 mm) which appeared to be more sensitive to the extracts in study followed by *Staphylococcus aureus* (12.5 mm) mainly with acetone DL extracts and MRSA with a 0.6-0.9 mm inhibition zone but with DL acetone extract; less or negligible effect was observed against gram negative strains tested in the present study (Fig:1). Data were tabulated and mean of inhibition zone was recorded (Table-1). Compared data with both positive and negative controls were not shown.

Table- 1: *Ducrosia anethifolia (L) antibacterial activity.*

Organisms	Ducrosia Fruit Extracts Average Inhibition Zone in mm			Durcorsia Leaves Extracts Average Inhibition Zone in mm		
	A	*M*	*W*	*A*	*M*	*W*
S. aureus	12.5	_	_	_	_	_
MRSA	11	9	_	_	_	_
B. subtilis	15.5	15	_	11	12	_
E. feacalis	_	_	_	_	_	_
E. coli	_	_	_	_	_	_
P. aeruginosa	_	_	_	_	_	_
K. pneumonia	_	_	_	_	_	_
Salmonella sp.	_	_	_	_	_	_
Serratia sp.	_	_	_	_	_	_

A: acetone extract M: methanol extract W: water extract

GC-MS Analysis

The major chemical constituent obtained from the GC-MS spectra of DL and DF methanol and acetone extracts was determined as tetradecenol. It is an unbranched aliphatic compound belonging to the monoterpenes and to which the bacterial growth inhibition of mainly gram positive bacteria in study is highly attributed (Figs: 2-5).

In Vitro Cytotoxic Assay

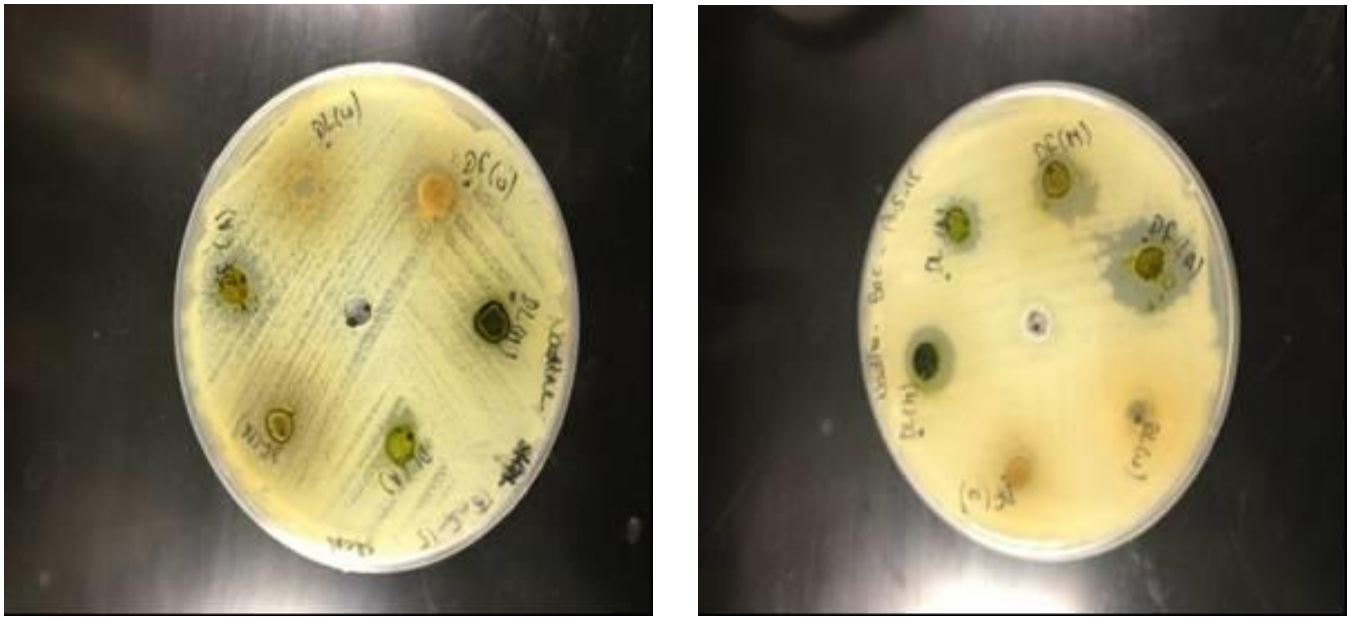
In addition to the remarkable antibacterial effect of *Ducrosia anethifolia* organic extracts mainly against gram positive bacteria correlated mainly to the presence of tetradecenol, a potent cytotoxic activity of *Ducrosia* leaves water extract with K562 and MCF7 in a concentration varying between 4-5 µl of extract/100 µl of cell line medium was noted (Figs: 6, 7).

DISCUSSION

Plants are the major source of newer drugs; they have led to the discovery of botanical medicine mainly in the development of anti-oxidants, anti-cancer and other anti-infective agents, and continue to contribute to new leads in the clinical trials. Scientists have accumulated a lot of information during the past two decades through in vivo and in vitro studies.[15,20] The aim of using natural products with therapeutic potential is nowadays a common interest to all scientific researchers; indicating the beginning of a new chemotherapeutic era leading to the discovery of new natural antibiotics as well as other curative agents including anti-cancerous or chemotherapeutic alternatives.[21,22] The present work, up to our knowledge, is the first to study the antibacterial and the cytotoxic activity of *Ducrosia anethifolia* (Boiss.) leaves and fruit organic extracts using methanol and acetone as well as water, against some human bacterial pathogens and types of human colon and leukemia cell lines.

These natural extracts owe their biological activity to their synergistic relation with different compounds present. Often, these components individually purified are not effective as when they are combined with other compounds[22-24]. However, the mode of action is still not very clear, but most previous studies have indicated that the bacteriostatic and bacteriocidic effect of natural compounds is mainly attributed to the bacterial cell wall integrity and disturbance.[21]

This could be explained by the perturbation of the lipid fraction in the bacterial plasma membrane caused by the high presence of tetradecenol and hence modifications in the cell membrane integrity.[25,26] In fact, as a result of the lipophilic nature of tetradecenol which easily can cross the cell membrane through the aqueous phase and get into the cell structures resulting in increased fluidity and permeability of the cell wall, disturbance of membrane embedded proteins and



A **B**

Fig. 1: *The antibacterial assay revealing the inhibition zone around each Ducrosia extracts well on the agar surface. A: showing the inhibition of Ducrosia fruit acetone extract on Staphylococcus aureus. B: indicating the effect of both Ducrosia extracts fruit and leaves, acetone and methanol on Bacillus subtilis.*
Df: Ducrosia fruit *DL: Ducrosia leaves* *A: Acetone* *M: Methanol* *W: Water*

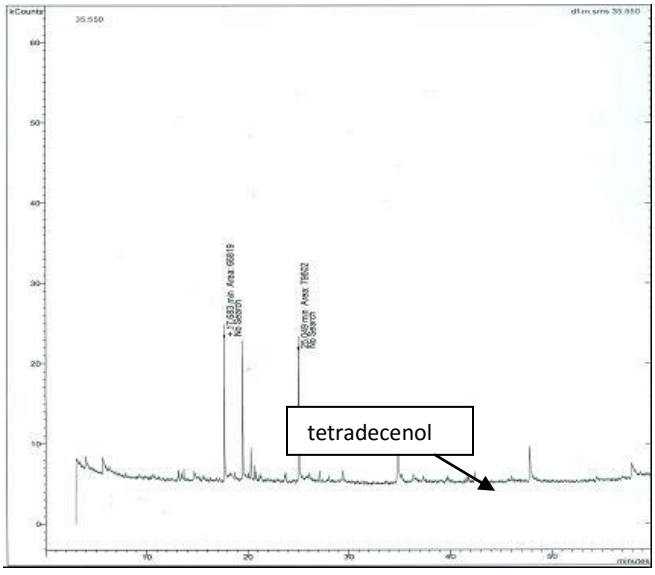


Fig. 2: *GC-MS Ducrosia leaves methanol extract, revealing the presence of tetradecenol at 17.6 min peak area.*

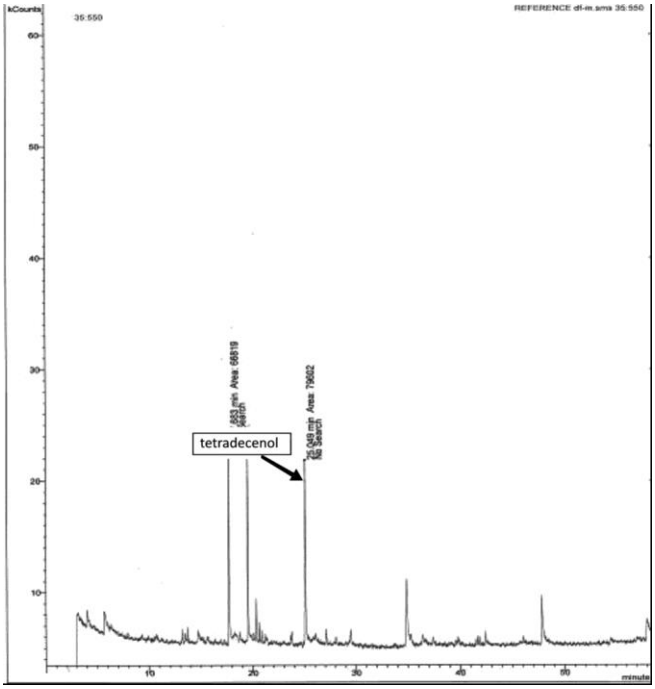


Fig. 3: *GC-MS Ducrosia fruit methanol extract showing the peaks related to tetradecenol.*

alteration of ion transport process.[27,28] However, the mechanism of action is still poorly determined. It was suggested that lipid constituents of the bacterial cell membrane are critical for their function thus any imbalance in the lipid structure or even the net ion surface charge will lead to an increased permeability of the bacterial cell wall and consequently a potent antibacterial effect of the extract in study[26,29] Our results were consistent with many previous articles which indicate higher bacterial activity against gram positive pathogenic bacteria than against gram negative bacteria.[26]

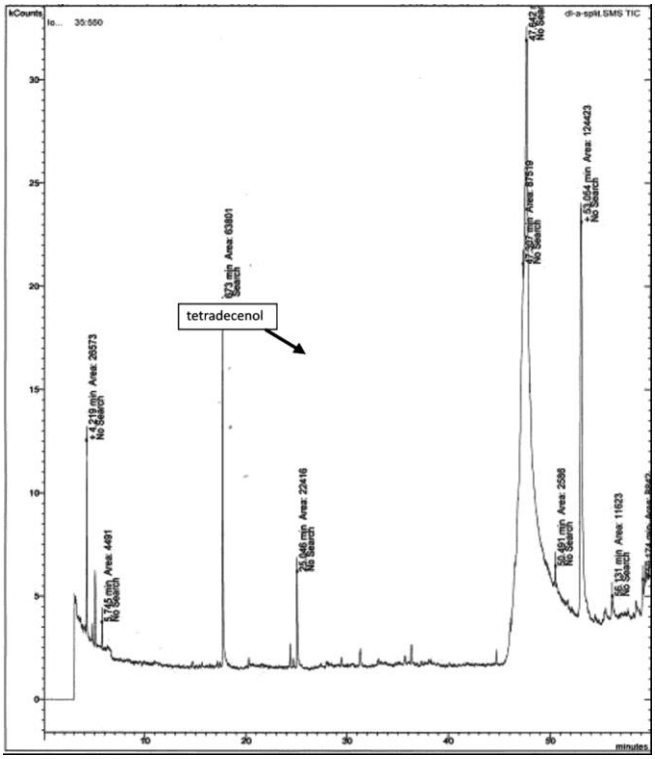


Fig. 4: *GC-MS analysis for Ducrosia leaves acetone extract with a peak related to the tetradecenol compound.*

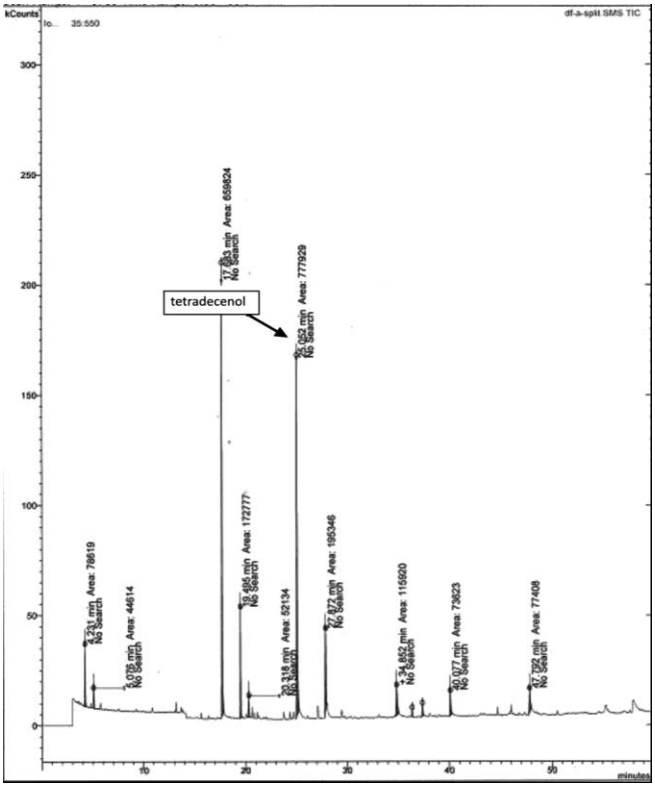


Fig. 5: *GC-MS analysis for Ducrosia fruit acetone extract with the presence of the chemical constituent tetradecenol.*

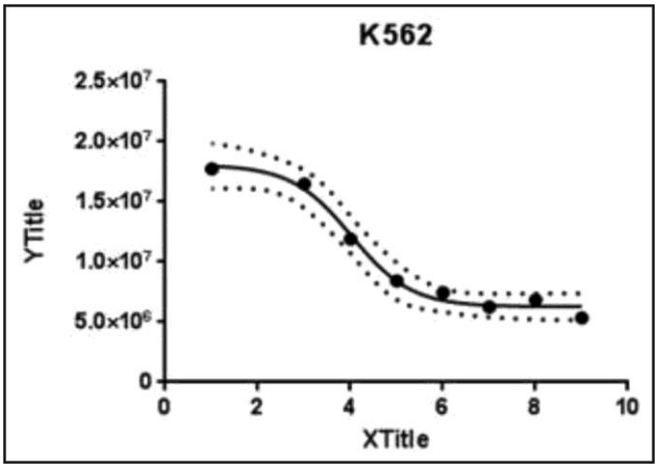


Fig. 6: *DL W extract against K562 showing a good cytotoxic activity ranging from 4 to 6 µg of the extract in study 100 ml of cell line medium.*
X axis: the extract concentration.
Y axis: number of cells

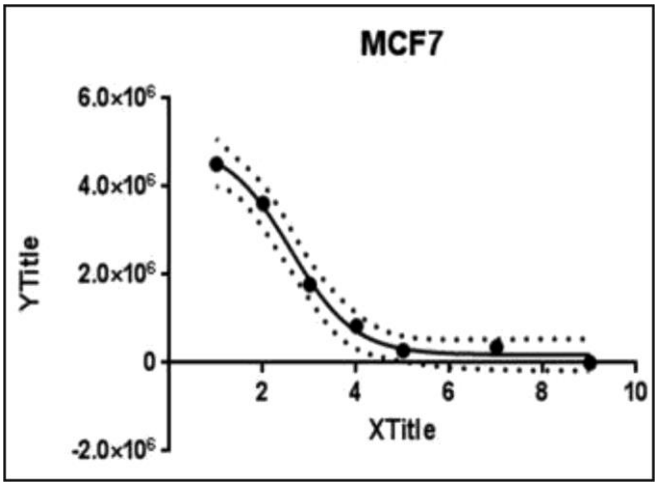


Fig. 7: *DL W extract activity against MCF7 indicating a potent cytotoxic activity with a crude extract concentration ranging from 4-6 µg/ 100ml.*
X axis: the extract concentration.
Y axis: number of cells

In addition to that, oxygenated aliphatic compounds such as tetradecenol, present in Apiaceae plants such as *Ducrosia* sp., have not only a remarkable antibacterial activity particularly against gram positive bacteria but also a significant cytotoxic activity.[31] This goes in agreement with our finding where better cytotoxic activity of *Ducrosia* leaves water extract was observed with K562 and MCF7 in a concentration varying between 4-5µl of extract/100 µl of cell line medium (Fig: 6 and 7). The potent cytotoxic activity was more pronounced with MCF7, HL60, K562 cell lines when treated with the aqueous DL extract compared to the methanolic extract. Significant decline in viability at 3 µl of the water extract in

comparison to the untreated control cells, whereas with DL methanol extract a drastic decrease in cell lines proliferation was observed; suggesting that DL water extract could be used as a promising cytotoxic agent (Fig: 6 and 7).

Similar effect was observed with *D. anethifolia* essential oils extracts when screened on K562 leukemia cell lines[31]. Due to the high heterogeneous compositions of *D. anethifolia*, and in accordance with previous studies stating that natural compounds from aromatic plants can act not only as anticancer agents but can reduce the patients anxiety overcome the nausea vomiting effect prone to chemotherapy, it was noted in the present work that DL extracts have anticancer properties or/and can improve the life quality of the cancer patient by decreasing cancer therapy side effects.[5,32] Changing as such the history of many types of cancer[34] where many anticancer drugs have been introduced between 1940(s) and 2006, all were derived from plants and targeted mainly the cell cycle progression.[35] Vinca alkaloids viz Vinblastine and Vincristine derived from *Catharanthus roseus* are used to treat leukemia, bladder and testicular Cancer.[36] Various mechanisms of actions are involved in cancer treatment, although is difficult to define a unique mechanism[37], but it could be mainly explained either by activating the detoxification enzymes or by DNA repair modulation, preventing metastasis or by inhibiting angiogenesis.[32] However, further attempts are still to be studied determining the cytotoxic activity of natural compounds and reveal the chemotherapeutic mechanistic approach.

CONCLUSION

The *Ducrosia anethifolia* leaves and fruits extracts, when screened for the first time against gram positive and gram negative bacteria and against K562, HL60 and TPH1 (human adenoma leukemia) together with MDA and MCF7 (human breast carcinoma) cell lines, show potent antibacterial and cytotoxic effects giving new promising hope in the chemotherapeutic field with aromatic plants.

LIMITATIONS OF STUDY

Though the authors of the study have provided extensive results on the topic, however, few limitations have to be mentioned. First sample size was not enough to proceed further with the anticancer activity as well as to collect each chemical constituent of the crude extracts and study their antibacterial effects due to the hardness of collecting the plant from arid areas. Second the cell permeability of the bacterial strains tested in this study couldn't be determined. Future projects on this component may yield more proficient results.

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AUTHOR'S CONTRIBUTION

MAW: Conception and design of study, Final approval of manuscript.
NM: Acquisition of data, drafting and critical revision of manuscript.
HZ: Acquisition of data and substantial contribution in experimental work.
AM: Analyzed and interpretation of data.
All authors read and approved the work.

CONFLICT OF INTEREST

None to declare.

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