ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 15, Issue, 06, pp.25023-25026, June, 2023
DOI: https://doi.org/10.24941/ijcr.45337.06.2023

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

THE ANTIPROLIFERATIVE EFFECT OF ESSENTIAL OILS IN CANCER CELL LINES

***Flores-Encarnación, M.[1*], Hernández-Hernández F.C.[1], Aguilar-Gutiérrez G.R.[2] and Cabrera-Maldonado C.[3]**

[1]Laboratorio de Microbiología Molecular y Celular. Edif. EMA1-421. Biomedicina, Facultad de Medicina, Benemérita Universidad Autónoma de Puebla. Puebla. Puebla, México
[2]CISEI, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, México
[3]Facultad de Ciencias Químicas. Benemérita Universidad Autónoma de Puebla. Puebla, Puebla. México

ARTICLE INFO

Article History:
Received 24th March, 2023
Received in revised form
14th April, 2023
Accepted 20th May, 2023
Published online 30th June, 2023

Key words:

Essential Oil, Antiproliferative, Cancer, Cell Line, *T. vulgaris*.

****Corresponding Author:***
Flores-Encarnación, M

ABSTRACT

Essential oils are volatile aromatic compounds present in the leaves, stems, and fruit shells. They are abundant in nature and have monoterpene compounds with antibacterial, antioxidant, and anti-inflammatory properties. In recent years, interest in essential oils has increased, since their antiproliferative properties have been studied. This work shows some aspects related to the antiproliferative effect of essential oils in cancer cell lines.

Citation: ***Flores-Encarnación, M., Hernández-Hernández F.C., Aguilar-Gutiérrez G.R. and Cabrera-Maldonado C.*. 2023.** "The antiproliferative effect of essential oils in cancer cell lines*". International Journal of Current Research*, 15, (06), 25023-25026

INTRODUCTION

It has been reported the use of biologically active organic compounds which are extracted from plant species with ability to eliminate pathogenic microorganisms (Daferera *et al*., 2003; Flores-Encarnación *et al*., 2016; Marston *et al*., 2016). Different essential oils with antibacterial properties are known. Some oils have been used in food preservation, pharmaceutical, agronomic, aromatherapy and fragrance industries (Saranra and Devi, 2017). The cinnamon, clove, geranium, lemon orange, rosemary, aniseed, eucaliptus, camphor oils have showed antibacterial activity (Boskovica *et al*., 2015; Prabuseenivasan *et al*., 2006). Thyme has been used as an antiseptic, antimicrobial, medication, astringent, anthelmintic, medicinal drug. It is incredibly useful in cases of infections by Gram-positive and Gram-negative bacteria, fungi and yeasts so, assorted intestinal infestations, like hookworms and ascarids (Borugă *et al*., 2014; Reddy *et al*., 2014). It has been reported that *T. vulgaris* inhibits the growth of pathogenic microorganisms such as *Candida albicans*, *Pseudomonas aeruginosa*, uropathogenic *Escherichia coli*, *Vibrio parahaemolyticus*, *V. fluvialis*, *Mycobacterium* sp., *Streptococcus pneumoniae*, *S. mutans*, *Staphylococcus aureus*, among others (Billinger *et al*., 2009; Djeghboub *et al*., 2018; Flores-Encarnación *et al*., 2018; Flores- Encarnación *et al*., 2020; Oramadike and Ogunbanwo, 2017; Rojas- Armas *et al*., 2015).

In recent years, different works have reported that *T. vulgaris* contains phenolic compounds with high antioxidant activity, inclusive several preclinical studies pointing to the anticancer potential of T.vulgaris have been described (Bentayeb *et al*., 2014; Kubatka *et al*., 2019). Therefore, this work shows some aspects related to the antiproliferative effect of essential oils in cancer cell lines.

ESSENTIAL OILS AND THEIR ANTIBACTERIAL MECHANISMS: Since ancient times, plants have played a fundamental role in the life of man. With the advances in the chemistry and pharmaceutical sciences, the uses of plants as broad-spectrum treatments have increased (Celis *et al*., 2008). Essential oils of plants have been widely used for the treatment of various diseases which is why many medicinal plants have attracted scientific interest. Essential oils have antioxidant and anti-inflammatory properties and have been associated with modulation of various genetic pathways (Khoury *et al.*, 2016; Patra and Baek, 2016; Wu *et al*., 2009). The essential oils are aromatic substances obtained from plant materials. They are secondary metabolites that provide a defense function or attraction in plant (Butkiené *et al*., 2015; Flores-Encarnación *et al*., 2016; Solórzano-Santos and Miranda-Novales, 2012). Other secondary metabolites include flavonoids, phenols, terpenes, alkaloids, lectins and polypeptides (Citarasu, 2010; Sánchez *et al*., 2009).

Essential oils are characterized by a complex mixture of compounds belonging to different classes of organic chemistry: hydrocarbons, phenols, terpenes, alcohols, aldehydes, ketones, esters, ethers (Butkiené *et al*., 2015; Hassan *et al*., 2016; Kordali *et al*., 2005). It has been reported that *T. vulgaris*, *Cinnamomum verum* (cinnamon) and *Lippia berlandieri* (oregano) have antioxidant properties related with phenolic compounds, such as carvacrol and thymol and these can be used under certain conditions as fungicides and bactericides (Abdalá and Roozen, 2011; Burt, 2004; Kordali *et al*., 2005). Some phenolic compounds derived from plant extracts have been reported as viable alternatives to antibiotics for the treatment of infectious agents in aquaculture (Citarasu, 2010). Essential oils have been used against a considerable number of pests and because their low mammalian toxicity and general availability, they have been considered as active ingredients in some botanical pesticides (Flores-Encarnación *et al*., 2019; Mohan *et al.*, 2011). At present, different antibacterial mechanisms have been reported for essential oils. It is known that essential oils have the ability to penetrate the lipid membrane of bacteria, making it more permeable and with it causing leaking ions and cytoplasm and bacterial lysis (O'Bryan *et al*., 2015). Other effects that have been observed in microorganisms by the action of essential oils are: 1. Destabilization of bacterial membrane by changes in membrane structure; 2. Alteration of the cell permeability; 3. Disturbance on ionic gradients; 4. Modification of bacterial quorum-sensing; 5. Leakage of potassium ions from cells; 6. Alteration of membrane potential (proton translocation); 7. Changes in pH gradient and ATP production of bacterial cell; 8. Interleaving into double-stranded DNA; 9. Blocking the bacterial respiratory activity (Cowan, 1999; Flores-Encarnación *et al*., 2016; Flores-Encarnación *et al*., 2020; O'Bryan *et al*., 2015; Rajendran *et al.*, 2014).

THE ANTIPROLIFERATIVE EFFECT OF ESSENTIAL OILS

According to oficial data from the World Health Organization, cancer is the second leading cause of death in the world population and it is estimated that 9.6 million people died from this disease in 2018 (Niksic *et al*., 2021). There were an estimated 18.1 million cancer cases around the world in 2020. Of these, 9.3 million cases were in men and 8.8 million in women (WCRFI, 2020). It has been reported that different essential oils have antimicrobial, antioxidant, and even anti-inflammatory properties (Borugă *et al.*, 2014, Khoury *et al.*, 2016; Patra and Baek, 2016; Saranra and Devi, 2017). In addition to that, certain essential oils have been labeled as promising anticancer agents and are currently being investigated for their cytotoxic and antiproliferative activities in cancer cell lines or experimental animals (Edris, 2007; Kubatka *et al*., 2019; Niksic *et al*., 2021). For some time, novel tumor-specific therapies have been required maintaining a high degree of efficacy and less toxic. In this context, it has been proposed to use anticancer drugs from natural products, representing a valuable source for the identification and development of novel treatment options for cancer (Newman and Cragg, 2007; Sertel *et al.*, 2011). So, research has focused on the health effects of phytochemicals and plant-derived extracts. Ait M'barek *et al*., (2007) reported that thyme essential oil has anticancer activities and it was investigated *in vitro* using the human ovarian adenocarcinoma cell line. Those authors and others proposed that thyme essential oil cytotoxicity might be due to its lipophilic compounds that accumulate in cancer cell membranes and increase their permeability, resulting in leakage of enzymes and metabolites, besides that thyme essential oil could also shown immune-stimulatory effects (Ait M'barek *et al*., 2007; Halat *et al*., 2022; Sertel *et al*., 2011; Sikkema *et al.*, 1995). Thyme possesses numerous compounds, especially the monoterpenoid phenols carvacrol and thymol, which exerted anticancer effects in various types of cell lines mimicking human cancers and they demonstrated their potential as chemopreventive or anticancer agents in different types of cancers (Elbe *et al*., 2020; Pakdemï Rlï *et al.*, 2020). The anticancer action mechanisms of monoterpenoid compounds include: induction of cell death by apoptosis and/or necrosis, inhibition of cell growth (antiproliferative effect), augmentation of ROS generation, depolarization of mitochondrial membrane, activation of Bax proapoptotic mitochondrial proteins, inhibition of angiogenesis, interaction with caspase or poly-ADP ribose polymerase, diminution of tumorigenesis by modulating the activity of carcinogen metabolizing enzymes, antimutagenic, antiproliferative, antioxidant properties, cell cycle arrest, and loss of key organelle functions (Ahmad *et al*., 2021; Blowman *et al*., 2018; Halat *et al*., 2022; Islam *et al.*, 2019; Niksic *et al*., 2021). Ribeiro *et al*., (2020) reported the cytotoxic/anti-proliferative activities in vitro of ten commercially available essential oils using a human skin keratinocyte cell line and a human fetal epithelial colon cell line. They observed that the Chinese cinnamon showed toxicity at all the tested concentrations on both cell lines, while lemongrass presented cytotoxicity (at all the tested concentrations) on human skin keratinocyte cell line. Basil, German chamomile and clove essential oils showed cytotoxicity at the highest concentration of 250 μg mL^{-1} on the human fetal epithelial colon cell line. They observed also that the ajowan and palmarosa showed no cytotoxicity at concentrations ≤ 125 μg mL^{-1} and all the other essential oils presented no cytotoxicity at 32 and 16 μg mL^{-1}. According to the research findings, essential oils possess anticancer potential against mouth, breast, lung, prostate, liver, colon, and brain cancer and even in leukemia (Gautam *et al.*, 2014). So, different studies has indicated that the specific components of essential oils increase the cytotoxic activity of chemotherapeutic drugs (for example: docetaxel, paclitaxel, 5-fuorouracil) on different cell lines and thus open the possibility of reducing their dose while providing the same effect (Carnesecchi *et al.*, 2001; Niksic *et al*., 2021). More than 60% of anticancer compounds are derived from plants, microorganisms, and marine sources. Today, medicinal plants are being considered safe due to their low side effects relative to chemical drugs. The anticancer plants contain a wide range of compounds, such as colchicine, vincristine, and podophyllotoxin, which are mitotic inhibitors binding to mitotic spindle tubular proteins and possessing alkaloids such as vinca alkaloids (*Vinca rosea*) that are used in chemotherapy (Srivastava *et al*., 2005; Tabatabaei *et al*., 2018). Plants of the Lamiaceae family can be a rich source of phytochemicals such as phytosterols, flavonoids, carotenoids, and terpenoids that act as antioxidants, annihilating free radicals and stimulating the immune system. These compounds form DNA adducts inhibiting the activity of carcinogens and also blocking metabolic pathways of cancer (Craig, 1999; Tabatabaei *et al*., 2018).

CONCLUSION

Essential oils are substances that have been used since ancient times. In recent years, the properties of them have been studied, proving to be compounds with anti-inflammatory, antioxidant and potent antimicrobial activities. In addition to this, essential oils show antiproliferative properties, data that have been obtained in cell lines associated with cancer. Among the essential oils, *T. vulgaris*, known as thyme, seems to be an alternative that could be used in the future to prevent the development of abnormal cells or prevent their proliferation. It is necessary to carry out more studies about the antiproliferative potential in cells associated with cancer.

ACKNOWLEDGEMENTS

Thanks to Facultad de Medicina-BUAP and Grupo de Académicos de Puebla SC for the facilities provided for the development of this work.

REFERENCES

Abdalá A.E. and Roozen J.P. 2011.. The effects of stabilized extracts of sage and oregano on the oxidation of salad dressings. Eur. Food Res. Technol. 212:551-560.

Ahmad A., Saeed M. and Ansari I.A. 2021. Molecular insights on chemopreventive and anticancer potential of carvacrol: implications from solid carcinomas. J. Food Biochem. 45:e14010.

Ait M'barek L., Ait Mouse H., Jaâfari A., Aboufatima R., Benharref A., Kamal M., Bénard J., El Abbadi N., Bensalah M., Gamouh A.,

Chait A., Dalal A. and Zyad A. 2007. Cytotoxic effect of essential oil of thyme *Thymus broussonettii* on the IGR-OV1 tumor cells resistant to chemotherapy. Braz. J. Med. Biol. Res. 40:1537-1544.

Bentayeb K., Vera, P., Rubio C. and Nerín C. 2014. The additive properties of oxygen radical absorbance capacity ORAC assay: the case of essential oils. Food Chem. 148:204-208.

Billinger M.E., Olivier K.N., Viboud C., de Oca R.M., Steiner C., Holland S.M., Prevots D.R. 2009. Non tuberculous mycobacteria associated lung disease in hospitalized persons, United States, 1998-2005. Emerg. Infect. Dis. 15:1562-1569.

Blowman K., Magalhães M., Lemos M.F., Cabral C. and Pires I.M. 2018 Anticancer properties of essential oils and other natural products. Evid. Based Complement. Altern. Med. 2018:1-12.

Borugă O., Jianu C., Mişcă C., Goleţ I., Gruia A.T., Horhat F.G. 2014. *Thymus vulgaris* essential oil: chemical composition and antimicrobial activity. J. Med. Life. 7:56-60.

Boskovica M., Zdravkovica N., Ivanovica J., Janjica J., Djordjevica J., Starcevica M., Baltica M.Z. 2015. Antimicrobial activity of thyme *Thymus vulgaris* and oregano *Origanum vulgare* essential oils against some food-borne microorganisms. Procedia Food Sci. 5:18-21.

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. Inter. J. Food Microbiol. 94:223-253.

Butkiené R., Bidiené J. and Judzentiene A. 2015. Variations of secundary metabolites essential oils in various plant organs of *Juniperus communis* L. wild growing in Lithuania. Baltic Forestry. 21:59-64.

Carnesecchi S., Schneider Y., Ceraline J., Duranton B., Gosse F., Seiler N. and Raul F. 2001. Geraniol, a component of plant essential oils, inhibits growth and polyamine biosynthesis in human colon cancer cells. J. Pharmacol. Exp. Ter. 298:197-200.

Celis Á., Mendoza C., Pachón M., Cardona J., Delgado W. and Cuca L.E. 2008. Extractos vegetales utilizados como biocontroladores con énfasis en la familia *Piperaceae.* Una revisión. Agron. Colombiana. 26:97-106.

Citarasu T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. Aquacult. Inter. 18:403-414.

Cowan M.M. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12:564-565.

Craig W.J. 1999. Health-promoting properties of common herbs. Amer. J. Clin. Nutrit. 70:491s-499s.

Daferera D.J., Ziogas B.N., Polissiou M.G. 2003. The effectiveness of plant essential oils in the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop. Protect. 22:39-44.

Djeghboub S., Oumouna M., Benyahia N. 2017. Evaluation of the anti-bacterial activity of thyme *Thymus vulgaris* and rosemary *Romarinus officinalis* essential oils on *Pseudomonas aeruginosa*. In: Kallel A., Ksibi M., Ben Dhia H., Khélifi N. eds Recent Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions. EMCEI. Advances in Science, Technology & Innovation IEREK Interdisciplinary Series for Sustainable Development. Springer, Cham.

Edris A.E. 2007. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. Int. J. Devot. Pharmacol. Toxicol. Eval. Nat. Prod. Deriv. 21:308-323.

Elbe H., Yigitturk G., Cavusoglu T., Uyanikgil Y. and Ozturk F. 2020. Apoptotic effects of thymol, a novel monoterpene phenol, on different types of cancer. Bratisl. Lek. Listy. 121:122-128.

Flores-Encarnación, M., Espino-Benítez A.S., Aguilar-Gutiérrez G.R., Martínez-Flores L.D., Xicohtencatl-Cortes J., Carreño-López R., Cabrera-Maldonado C. 2020. The effect of *Thymus vulgaris* on the respiratory activity of uropathogenic *Escherichia coli*. Inter. J. Res. Studies in Biosc. 8:1-6.

Flores-Encarnación M., Morales-Báez J.R., Aguilar-Gutiérrez G.R., Cabrera-Maldonado C., Silvia del Carmen García-García. 2019. The effect of *Thymus vulgaris* essential oil on the viability of eukariotic cells.Inter. J. Curr. Res. 11:8001-8006.

Flores-Encarnación M., Nava-Nolazco R. M., Aguilar-Gutiérrez G. R., Carreño-López R. and García-García S. C. 2018. The effect of *Thymus vulgaris* on growth and biofilm formation of uropathogenic *Escherichia coli*. Afr. J. Microbiol. Res. 12:237-242.

Flores-Encarnación M., Nava-Nolazco R.M., Carreño-López R., Aguilar-Gutiérrez G.R., García-García S.C., Cabrera-Maldonado C. 2016. The antibacterial effect of plant-based essential oils. Int. J. Res. Studies Biosci. 4: 1-6.

Gautam N., Mantha A.K. and Mittal S. 2014. Essential oils and their constituents as anticancer agents: a mechanistic view. Biomed. Res. Int. 2014: 1-23.

Halat D.H., Krayem M., Khaled S. and Younes S. 2022. A Focused insight into thyme: biological, chemical, and therapeutic properties of an indigenous mediterranean herb. Nutrients. 14:2104-2126.

Hassan W., Gul S., Rehman S., Noreen H., Shah Z., Mohammadzai I. and Zaman B. 2016. Chemical composition, essential oil characterization and antimicrobial activity of *Carum copticum*. Vitam. Miner. 5:2.

Islam M.T., Khalipha A.B.R., Bagchi R., Mondal M., Smrity S.Z., Uddin S.J., Shilpi J.A. and Rouf R. 2019. Anticancer activity of thymol: a literature-based review and docking study with emphasis on its anticancer mechanisms. IUBMB Life. 71:9-19.

Khoury R.E., Atoui A., Verheecke C., Maroun R., Khoury A.E., and Mathieu F. 2016. Essential oils modulate gene expression and ochratoxin A production in *Aspergillus carbonarius*. Toxins. 8:1-14.

Kordali S., Kotan R., Mavi A., Cakir A. Ala A. and Yildirim A. 2005. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of turkish *Artemisia absinthium*, *A. dracunculus*, *A. santonicum*, and *A. spicigera* essential oils. J. Agric. Food Chem. 53:9452- 9458.

Kubatka P., Uramova S., Kello M., Kajo K., Samec M., Jasek K., Vybohova D., Liskova A., Mojzis J., Adamkov M., Zubor P., Smejkal K., Svajdlenka E., Solar P., Samuel S.M., Zulli A., Kassayova M., Lasabova Z., Kwon T.K., Pec M., Danko J. and Büsselberg D. 2019. Anticancer activities of *Thymus vulgaris* L. in experimental breast carcinoma in vivo and in vitro. Int. J. Mol. Sci. 20:1749-1778.

Marston H.D., Dixon D.M., Knisely J.M., Palmore T.N., Fauci A.S. 2016. Antimicrobial resistance. JAMA. 316:1193-1204.

Mohan M., Haider S.Z., Andola H.C. and Purohit V.K. 2011. Essential oils as green pesticides: for sustainable agriculture. RJPBCS. 2:100-106.

Newman D.J. and Cragg G.M. 2007. Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 70:461-477.

Niksic H., Becic F., Koric E., Gusic I., Omeragic E., Muratovic S., Miladinovic B. and Duric K. 2021. Cytotoxicity screening of *Thymus vulgaris* L. essential oil in brine shrimp nauplii and cancer cell lines. Scientifc Reports. 11:13178.

O'Bryan C.A., Pendleton S.J., Crandall P.G. and Ricke S.C. 2015. Potential of plant essential oils and their components in animal agriculture- in vitro studies on antibacterial mode of action. Frontiers Vet. Sci.2:1-8.

Oramadike C.E. and Ogunbanwo S.T. 2017. Antagonistic activity of *Thymus vulgaris* extracts against *Vibrio* species isolated from seafoods. J. Food Sci. and Technol. 54:1199-1205.

PakdemïRlï A., Karaca C., Sever T., Daskin E., LeblebïCï A., YïG˘ ïTba¸si T., Ba¸sbinar Y. 2020. Carvacrol alters soluble factors in HCT-116 and HT-29 cell lines. Turk. J. Med. Sci. 50:271-276.

Patra J.K. and Baek K.H. 2016. Antibacterial activity and action mechanism of the essential oil from *Enteromorpha linza* L. against foodborne pathogenic bacteria. Molecules. 21:1-11.

Prabuseenivasan S., Jayakumar M. and Ignacimuthu S. 2006. In vitro antibacterial activity of some plant essential oils. BMC Compl. Alternat. Med. 6:39-47.

Rajendran M.P., Pallaiyan B.B. and Selvaraj N. 2014. Chemical composition, antibacterial and antioxidant profile of essential oil from *Murraya koenigii* L. leaves. AJP. 4:200-214.

Reddy V P., Kandisa R.V., Varsha P.V. and Satyam S. 2014. Review on *Thymus vulgaris* traditional uses and pharmacological properties. Med. Aromat. Plants. 3:164-167.

Ribeiro S.O., Fontaine V., Mathieu V., Zhiri A., Baudoux D., Stévigny C. and Souard F. 2020. Antibacterial and cytotoxic activities of ten commercially available essential oils. Antibiotics. 9:0717.

Rojas-Armas J., Ortiz-Sánchez J., Jáuregui-Maldonado J., Ruiz-Quiroz J., Almonacid Román R. 2015. Aceite esencial de *Thymus vulgaris* L tomillo, su combinación con EDTA contra *Candida albicans* y formulación de una crema. Anal. Facultad de Medicina. 76:235-240.

Sánchez Y., Pino O., Correa T.M., Naranjo E. and Iglesia A. 2009. Estudio químico y microbiológico del aceite esencial de *Piper auritum* Kunth caisimón de anís. Rev. Prot. Veg. 24:39-46.

Saranra P. and Devi V.D. 2017. Essential oils and its antibacterial properties- a review. Life Sci. Arch. 3:9941011.

Sertel S., Eichhorn T., Plinkert P.K. and Efferth T. 2011. Cytotoxicity of *Thymus vulgaris* essential oil towards human oral cavity squamous cell carcinoma. Anticancer Res. 31:81-88.

Sikkema J., de Bont J.A. and Poolman B. 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev. 59:201-222.

Solórzano-Santos F. y Miranda-Novales M. 2012. Essential oils from aromatic herbs antimicrobial agents. Curr. Opinion Biotechnol. 23:136-141.

Srivastava V., Negi A.S., Kumar J., Gupta M., Khanuja S.P. 2005. Plant-based anticancer molecules: a chemical and biological profile of some important leads. Bioorganic Med. Chem. 13:5892-5908.

Tabatabaei S.M., Kianinodeh F., Nasiri M., Tightiz N., Asadipour M. and Gohari M. 2018. In vitro inhibition of mcf-7 human breast cancer cells by essential oils of *Rosmarinus officinalis*, *Thymus vulgaris* L., and *Lavender* x *intermedia*. Arch. Breast Cancer. 5:81-89.

World Cancer Research Fund International WCRFI: Worldwide cancer data. https://www.wcrf.org/cancer-trends/worldwide-cancer-data 2020.

Wu V.C.H., Qiu X., Delos-Reyes B.G., Lin C.S. and Pan Y. 2009. Application of cranberry concentrate *Vaccinium macrocarpon* to control *Escherichia coli* O157:H7 in ground beef and its antimicrobial mechanism related to the down regulated slp, hdeA and cfa. Food Microbiol. 26:32-38.

****