Pak. J. Bot., 55(1): 307-311, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-1(4)

PROTECTIVE EFFECT OF ESSENTIAL OILS OF *OCIMUM BASILICUM*, *GALIUM ODORATUM,* AND *CYMBOPOGON CITRATUS* AGAINST OXIDATIVE DNA DAMAGE IN CULTURED HUMAN LYMPHOCYTE CELLS

OMAR F. KHABOUR[1*], KAREM H. ALZOUBI[2], SALWA F. M. HASSANEIN[3], HANIN MAKHLOUF[1] AND FARAH ALHASHIMI[1]

[1]Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, Jordan University of Science and Technology, Irbid, Jordan
[2]Department of Clinical Pharmacy, Jordan University of Science and Technology, Irbid, Jordan
[3]Department of Food Science and Nutrition, Taibah University, Almadinah, Saudi Arabia

Abstract

The potential of essential oils as antioxidants is gaining interest by researchers and medicinal industry. In this study, we investigated the protective effect of essential oils obtained from *Ocimum basilicum*, *Galium odoratum*, and *Cymbopogon citratus* against oxidative DNA damage in cultured human lymphocytes. DNA damage was assessed using 8-hydroxy deoxyguanosine assay (8-OHdG). The oil of *G. odoratum* showed significant decreases of 8-OHdG biomarker in cultured cells at all examined concentrations (0.1, 1 and 2 uL/mL) with magnitudes that range between 31-39% ($p<0.01$). With respect to *C. citratus* oil, a significant decrease of 8-OHdG was observed at 1 and 2 µL/mL concentrations with magnitudes of 34% and 44% respectively ($p<0.05$). Similarly, *O. basilicum* significantly decreased 8-OHdG at 1µL/mL and 2 µL/mL concentrations ($p<0.005$) with magnitude of decreases of about 62% at both concentrations. In conclusion, potent antioxidative DNA damage activity of examined essential oils was observed in cultured human lymphocytes.

Key words: Essential oil, DNA damage, Oxidative stress, 8-OHdG, Plant.

Introduction

Reactive oxygen species (ROS) are constantly generated during normal cellular activity (Franchina *et al.*, 2018; Pérez-Torres *et al*., 2021). However, an imbalance between the generation of ROS and the antioxidant capacity of cells can lead to oxidative stress (Alzoubi *et al.*, 2021; Andersson, 2018). Oxidative stress is associated with the development of chronic diseases, infection and exposure to chemicals including medications (Al-Sawalha *et al*., 2021; Barcia *et al.*, 2017; Incalza *et al.*, 2018; Khabour *et al.*, 2014; Rababa'h *et al*., 2019). Oxidative stress can cause severe damage to macromolecules and body tissues (Andersson, 2018; Franchina *et al.*, 2018). Oxidative DNA damage is a form of oxidative stress that is marked by the production of 8-OHdG biomarker in body cells (Al-Smadi *et al*., 2019; Aspinen *et al.*, 2016; Martins *et al.*, 2017; Tarboush *et al*., 2022). Accumulation of DNA damage in cells predisposes individuals to malignancies, cardiovascular diseases and premature aging (Khalil *et al*., 2021; Mayyas *et al*., 2020; Risques & Kennedy, 2018; Vijg, 2014).

In the last few decades, the total world population have shown increasing interest in plant research that is recognized as a source of discoveries of health improving agents (Yatoo *et al.*, 2017, Khabour & Hassanein, 2021; Hameed *et al*., 2022). Among such agents are essential oils, which are volatile, natural, complex compounds formed by aromatic plants as secondary metabolites (de Groot & Schmidt, 2016; Uma *et al.*, 2017; Baghalpour *et al*., 2021). The main group of essential oils are composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular weight and have strong odor (Lesage-Meessen *et al.*, 2015; Vieira *et al.*, 2018).

Ocimum basilicum, known as basil plant, is a rich source of essential oils that have several medicinal uses (Atiphasaworn *et al.*, 2017; Rodrigues *et al.*, 2017) and antioxidative properties (Tarchoune *et al.*, 2010). Similarly, the essential oil of *Galium odoratum*, a member of the *Rubiaceae* family (also known as sweet woodruff), has been shown to possess several biological and pharmacological activities such as antioxidant, antimicrobial, and antimutagenic activities (Kahkeshani *et al.*, 2013). The essential oil of *Cymbopogon citratus* (lemon grass) have been shown to carry important therapeutic potentials including being anti-cancer, anti-hypertensive, antioxidant, and anti-mutagenic (Boukhatem *et al.*, 2014; Campos *et al.*, 2014). In this study, we aimed at investigating the effect of essential oils of *O. basilicum*, *G. odoratum*, and *C. citratus* on oxidative DNA damage using the 8-hydroxydeoxyguanosine (8-OHdG) assay in cultured human lymphocytes. This assay has been shown to be useful for screening medicinal plants for their antioxidative properties (Alkofahi *et al.*, 2016; Anjum *et al*., 2019).

Materials and Method

Collection of plant materials: The used plant part to prepare oils (leaves) were collected from Almadina area, Saudi Arabia in the period from March to May / 2016. Plants were identified by a specialist in medicinal plants from the Biology Department at Taibah University, Saudi Arabia.

Essential oil extraction: Essential oils were extracted from 250 gram air dried leaves by hydro-distillation, using a Clevenger type apparatus as previously described (Carneiro *et al.*, 2017). The distilled essential oils were dehydrated with anhydrous sodium sulphate and then stored at -20°C in dark airtight bottles until further use.

Blood donors: Two healthy male volunteers (ages: 26 and 24 years) donated their blood to be used for lymphocyte cultures. Both volunteers were never smokers and non-alcoholic, not using any supplements or medications at least 6 months prior to participation in the study (Azab *et al*., 2019). Volunteers were checked for their hemoglobin level prior to blood withdraw to ensure their fitness for donation. Fifty mL of blood was obtained from volunteers each time they donated in sterile heparinized tubes. Volunteers gave written informed consent as required by the institutional ethics committee at Faculty of Applied Medical Sciences/Taibah University.

Blood cultures: The Euro clone Chromosome medium (Italy) was used to prepare cultured human lymphocytes using whole blood (Mhaidat *et al.,* 2016) with a ratio of blood to culture media of 1/9. Cultures were incubated at 37°C in CO_2 incubator for 70 h as previously described. About 4 cultures per donor were used for each drug concentration (i.e. n = 8 per each concentration) (Azab *et al.*, 2017; Esmadi *et al.*, 2016).

The 8-OHdG assay: Cultures from the above step were centrifuged at 2000 xg for 6 minutes and pellets were then washed in bovine serum-free chromosome medium. Cells were then then treated with different concentrations (0.1, 1 and 2 µL/mL) of the essential oil. Cisplatin (1 µg/mL, Sigma, Saint Louis, MI, USA) and Vitamin E (alpha tocopherol; 1 µg/mL; Acros Organics, Turkey) were used as controls to validate the assay (Khabour *et al.*, 2014). After six hours of incubation, tubes were centrifuged at 2000 xg and 0.1 mL of the supernatant was used for 8-OHdG assay. Untreated cultures were used as negative controls. An ELISA kit obtained from Sigma-Aldrich (Saint Louis, MI, USA) was used for the measurement of the 8-OHdG as described by the bulletin that was provided in the kit. Changes in the absorbance were analyzed at 405 nm using a plate reader (BioTek, model: ELx800, Winooski, VT, USA).

Statistical analysis: Statistical comparisons were performed using GraphPad Prism statistical software (version 4). ANOVA followed by Tukey posthoc test were used for the three group analysis. A $p<0.05$ was used as a threshold for statistical significant.

Results

In this study, oils extracted from the three medicinal plants were screened for anti-mutagenic activity using the 8-OHdG assay and cultured human lymphocytes. Cisplatin (1 µg/mL), which is known to induce oxidative stress, and Vitamin E (1 µg/mL) that has potent antioxidant activity, were used as controls. Cisplatin induced an increase in 8-OHdG by about 85%, whereas Vitamin E reduced 8-OHdG by about 40% ($p<0.01$).

Figure 1 shows the anti-mutagenic activity of *C. citratus* oil in cultured human lymphocytes. *C. citratus* oil significantly decreased 8-OHdG biomarkers at 1µL/mL and 2 µL/mL concentrations ($p<0.05$). However, a considerable but not significant decrease was observed at 0.1 µL/mL concentration ($p>0.05$). The magnitude of decrease at 0.1 µL/mL, 1 µL/mL and 2 µL/mL were about 29%, 34% and 44%, respectively.

The oil of *G. odoratum* induced significant decreases of 8-OHdG biomarker at all examined concentrations ($p<0.01$, Fig. 2). The magnitude of decrease at 0.1 µL/mL, 1 µL/mL and 2 µL/mL were about 39%, 32% and 31% respectively. This finding indicates a saturation effect that was observed at 0.1 µL/mL concentration.

With respect to *O. basilicum*, significant decreases in 8-OHdG biomarkers was detected at 1µL/mL and 2 µL/mL concentrations ($p<0.005$, Fig. 3) with magnitude of decreases equal to 62% in both cases. Thus, a saturation effect was observed at 1µL/mL concentration. On the other hand, the 0.1 µL/mL was found to be not effective in decreasing 8-OHdG biomarker in cultured blood lymphocytes.

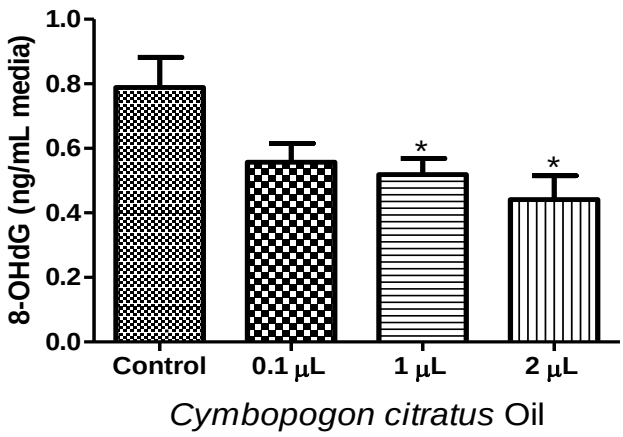

Fig. 1. Antioxidative DNA damage activity of *Cymbopogon citratus* essential oil.
Cultured human lymphocytes were treated with different concentrations of *C. citratus* essential oil (0.1, 1, and 2 uL/mL). The 8-OHdG was measured using ELISA technique. *C. citratus* essential oil concentrations of 1, and 2 uL/mL significantly reduced 8-OHdG levels, indicating reduced oxidative DNA damage in human cultures lymphocytes. Data were expressed as mean ± SEM. * indicates significant difference from the control group. ANOVA p value <0.05.

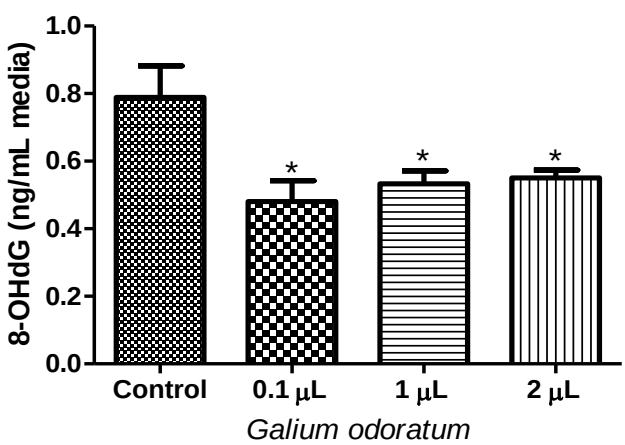

Fig. 2. Antioxidative DNA damage activity of *Galium odoratum* essential oil.
Cultured human lymphocytes were treated with different concentrations of *Galium odoratum* essential oil (0.1, 1, and 2 uL/mL). At all three tested essential oil concerntrations, 8-OHdG was significantly reduced. The 8-OHdG was measured using ELISA technique. Data were expressed as mean ± SEM. * indicates significant difference from the control group. ANOVA p value <0.01.

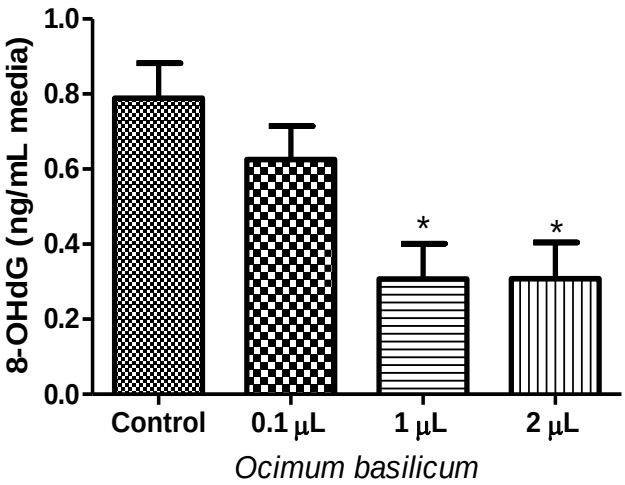

Fig. 3. Antioxidative DNA damage activity of *Ocimum basilicum* essential oil.
Cultured human lymphocytes were treated with different concentrations of *Ocimum basilicum* essential oil (0.1, 1, and 2 uL/mL). The 8-OHdG was measured using ELISA technique. Data were expressed as mean ± SEM. * indicates significant difference from the control group. ANOVA p value <0.001.

Discussion

In this study, we investigated the antioxidative DNA damage potential of three essential oils extracted from *O. basilicum*, *G. odoratum*, and *C. citratus* using cultured human lymphocytes. The results showed strong antioxidative DNA damage effect of the three examined essential oils with slight variations.

The *O. basilicum* also known as great basil is herb native to Africa and Asia that belongs to Lamiaceae (mints) plant family (Kaya *et al.*, 2008). The plant is rich in phyto-nutrients that are used as food flavors. In addition, *O. basilicum* has several medicinal uses including treatment of viral and bacterial infection, treatment of gastrointestinal diseases, headaches, diabetes mellitus, weight control, kidney diseases and cancer (Atiphasaworn *et al.*, 2017; Bhatti *et al.*, 2017; Rodrigues *et al.*, 2017; Singh, 1999). The results showed strong activity of essential oil obtained from the leaves of *O. basilicum* against oxidative DNA damage. This findings is consistent with the reported strong antioxidative property of this herb (Atiphasaworn *et al.*, 2017; Tenore *et al.*, 2017; Zlotek *et al.*, 2016; Khalil *et al*., 2021; Rahman *et al*., 2022). Extract from *O. basilicum* has been shown to protect HepG2 human cells from oxidative DNA damage (Thirugnanasampandan & Jayakumar, 2011). Similar results were reported using *E. coli* (Beric *et al.*, 2008) and cultured breast cell lines (Al-Ali *et al.*, 2013). The antioxidative property of *O. basilicum* could be due to the presence of flavonoids and anthocyanins compounds that are rich in its leaves (Ghasemzadeh *et al.*, 2016; Vlase *et al.*, 2014). Further fractionation of *O. basilicum* essential oil is recommended to identify the active ingredients leading to antioxidative DNA damage properties of the oil.

G. odoratum is another herb that belongs to the family Rubiaceae and it is native to Europe and Asia. *G. odoratum* has been shown to possess medicinal properties (Kahkeshani *et al.*, 2013; Wojnicz *et al.*, 2012). Current results showed strong antioxidative DNA damage effect of the essential oil extracted from its leaves. The antioxidative property of *G. odoratum* has been shown previously using animal models (Kahkeshani *et al.*, 2013). The antioxidative properties of *G. odoratum* could be due to coumarins and flavonoids compounds that are abundant in its leaves (Baser *et al.*, 2004).

The last examined essential oil was extracted from *C. citratus*, which is a perennial plant native to the Middle East and Asia. Among the medicinal uses of lemon grass are antimicrobial infections, anti-inflammatory, antidepressant and diuretic properties (Boukhatem *et al.*, 2014; Campos *et al.*, 2014). In the current study, results suggest a strong effect of the essential oil extracted from lemon grass against oxidative DNA damage. This is consistent with the previously reported antioxidative property of the plant (Boukhatem *et al.*, 2014; Campos *et al.*, 2014; Rahim *et al.*, 2014; Somparn *et al.*, 2014). Moreover, *C. citratus* essential oil has been shown to protect cells against N-methyl-N-nitrosurea (MNU)-induced DNA damage (Bidinotto *et al.*, 2012; Bidinotto *et al.*, 2011; Rao *et al.*, 2009; Suaeyun *et al.*, 1997). Further investigations are required to identify the bioactive compounds in the extracted oil.

Among the limitations of the study is that antioxidative DNA properties of examined essential oils were only tested using *In vitro* cultured cells. Thus, the present findings need to be confirmed using *In vivo* animal models.

In conclusion, the current study reported a strong activity of essential oils derived from *O. basilicum*, *G. odoratum*, and *C. citratus* against oxidative DNA damage using cultured human lymphocytes.

Acknowledgments

Authors would like to thank the Deanship of Research at Jordan University of Science and Technology for supporting the study.

References

Al-Ali, K.H., H.A. El-Beshbishy, A.A. El-Badry and M. Alkhalaf. 2013. Cytotoxic activity of methanolic extract of Mentha longifolia and Ocimum basilicum against human breast cancer. *Pak. J. Biol. Sci.*, 16: 1744-1750.

Alkofahi, A.S., K.H. Alzoubi, O.F. Khabour and N.M. Mhaidat. 2016. Screening of selected medicinal plants from Jordan for their protective properties against oxidative DNA damage. *Ind. Crops & Prod.*, 88: 106-111.

Al-Sawalha, N.A., I.D. Pokkunuri, K.H. Alzoubi, O.F. Khabour and B.N. Almomani. 2021. Waterpipe tobacco smoke exposure during lactation-susceptibility of reproductive hormones and oxidative stress parameters in male progeny rats. *Reprod. Sci*., 28:37-42.

Al-Smadi, M.L., R. Mansour, A. Mahasneh, O.F. Khabour, M.M. Masadeh and K.H. Alzoubi. 2019. Synthesis, characterization, antimicrobial activity, and genotoxicity assessment of two heterocyclic compounds containing 1,2,3-Selena- or 1,2,3-Thiadiazole Rings. *Molecules*, 24: 4082.

Alzoubi, K.H., O.F. Khabour, R.M. Al-Awad and Z.O. Aburashed. 2021. Every-other day fasting prevents memory impairment induced by high fat-diet: Role of oxidative stress. *Physiol. Behav*., 229: 113263.

Andersson, K.E. 2018. Oxidative stress and its possible relation to lower urinary tract functional pathology. *BJU Int.*, 121: 527-533.

Anjum S., R. Khadim, S.Z.A. Shah, A. Hamid, S.A. Jan, Z.K. Shinwari, N. Ahmad and A. Ghafoor. 2019. Biochemical characterization of geographically diverse mentha species from Azad Jammu and Kashmir. *Fresenius Environmental Bulletin*, 28(2A): 1336-1344.

Aspinen, S., J. Harju, P. Juvonen, T. Selander, H. Kokki, K. Pulkki and M.J. Eskelinen. 2016. The plasma 8-OHdG levels and oxidative stress following cholecystectomy: a randomised multicentre study of patients with minilaparotomy cholecystectomy versus laparoscopic cholecystectomy. *Scand. J. Gastroenterol.*, 51: 1507-1511.

Atiphasaworn, P., S. Monggoot, E. Gentekaki, S. Brooks and P. Pripdeevech. 2017. Antibacterial and antioxidant constituents of extracts of endophytic fungi isolated from *Ocimum basilicum* var. *thyrsiflora* leaves. *Curr. Microbiol.*, 74: 1185-1193.

Azab, B., A. Alassaf, A. Abu-Humdan, Z. Dardas, H. Almousa, M. Alsalem, O. Khabour, H. Hammad, T. Saleh and A. Awidi. 2019. Genotoxicity of cisplatin and carboplatin in cultured human lymphocytes: a comparative study. *Interdiscip Toxicol*., 12: 93-97.

Azab, M., O.F. Khabour, K.H. Alzoubi, H. Hawamdeh, M. Quttina and L. Nassar. 2017. Assessment of genotoxicity of pyrethrin in cultured human lymphocytes. *Drug & Chem. Toxicol.*, 40: 251-255.

Baghalpour, N., S.A. Ayatollahi, N. Naderi, T. Hosseinabadi, Y. Taheri, J. Mahroo-Bakhtiyari, Z.K. Shinwari, A.T. Khalil and J. Sharifi-Rad. 2021 Antinociceptive and anti-inflammatory studies on Tradescantia zebrine. *Pak. J. Bot*., 53(1): 357-365.

Barcia, J.M., S. Portoles, L. Portoles, A.C. Urdaneta, V. Ausina, G.M. Perez-Pastor, F.J. Romero and V.M. Villar. 2017. Does oxidative stress induced by alcohol consumption affect orthodontic treatment outcome? *Front. In Physiol.*, 8: 22.

Baser, K.H.C., T. Özek, N. Kırımer, D. Deliorman and F. Ergun. 2004. Composition of the essential oils of *Galium aparine* L. and *Galium odoratum* (L.) Scop. from Turkey. *J. Essential Oil Res.*, 16: 305-307.

Beric, T., B. Nikolic, J. Stanojevic, B. Vukovic-Gacic and J. Knezevic-Vukcevic. 2008. Protective effect of basil (*Ocimum basilicum* L.) against oxidative DNA damage and mutagenesis. *Food & Chem. Toxicol.*, 46: 724-732.

Bhatti, H.A., Y. Tehseen, K. Maryam, M. Uroos, B.S. Siddiqui, A. Hameed and J. Iqbal. 2017. Identification of new potent inhibitor of aldose reductase from *Ocimum basilicum*. *Bioorganic Chem.*, 75: 62-70.

Bidinotto, L.T., C.A. Costa, M. Costa, M.A. Rodrigues and L.F. Barbisan. 2012. Modifying effects of lemongrass essential oil on specific tissue response to the carcinogen N-methyl-N-nitrosurea in female BALB/c mice. *J. Med. Food*, 15: 161-168.

Bidinotto, L.T., C.A. Costa, D.M. Salvadori, M. Costa, M.A. Rodrigues and L.F. Barbisan. 2011. Protective effects of lemongrass (*Cymbopogon citratus* STAPF) essential oil on DNA damage and carcinogenesis in female Balb/C mice. *J. Appl. Toxicol. : JAT*, 31: 536-544.

Boukhatem, M.N., M.A. Ferhat, A. Kameli, F. Saidi and H.T. Kebir. 2014. Lemon grass (*Cymbopogon citratus*) essential oil as a potent anti-inflammatory and antifungal drugs. *The Libyan J. Med.*, 9: 25431.

Campos, J., G. Schmeda-Hirschmann, E. Leiva, L. Guzman, R. Orrego, P. Fernandez, M. Gonzalez, C. Radojkovic, F.A. Zuniga, L. Lamperti, E. Pastene and C. Aguayo. 2014. Lemon grass (*Cymbopogon citratus* (D.C) Stapf) polyphenols protect human umbilical vein endothelial cell (HUVECs) from oxidative damage induced by high glucose, hydrogen peroxide and oxidised low-density lipoprotein. *Food Chem.*, 151: 175-181.

Carneiro, N.S., C.C.F. Alves, J.M. Alves, M.B. Egea, C.H.G. Martins, T.S. Silva, L.C. Bretanha, M.P. Balleste, G.A. Micke, E.V. Silveira and M.L.D. Miranda. 2017. Chemical composition, antioxidant and antibacterial activities of essential oils from leaves and flowers of *Eugenia klotzschiana* Berg (Myrtaceae). *Anais da Academia Brasileira de Ciencias*, 89: 1907-1915.

de Groot, A. and E. Schmidt. 2016. Essential Oils, Part V: Peppermint Oil, Lavender Oil, and Lemongrass Oil. *Dermatitis: Contact, Atopic, Occupational, Drug*, 27: 325-332.

Esmadi, F.T., O.F. Khabour, K. Abbas, A.E. Mohammad, R.T. Obeidat and D. Mfady. 2016. Synthesis, characterization and biological activity of some unsymmetrical Schiff base transition metal complexes. *Drug & Chem. Toxicol.*, 39: 41-47.

Franchina, D.G., C. Dostert and D. Brenner. 2018. Reactive oxygen species: Involvement in T cell signaling and metabolism. *Trends in Immunol*., 39: 489-502.

Ghasemzadeh, A., S. Ashkani, A. Baghdadi, A. Pazoki, H.Z. Jaafar and A. Rahmat. 2016. Improvement in flavonoids and phenolic acids production and pharmaceutical quality of sweet basil (*Ocimum basilicum* L.) by ultraviolet-B irradiation. *Molecules*, 21: 1203.

Hameed, S., A.T. Khalil, M. Ali, J. Iqbal, L. Rahman, M. Numan, S. Khamlich, M. Maaza, I. Ullah, B.A. Abbasi, F. Alasmari and Z.K. Shinwari. 2021. Precursor effects on the physical, biological and catalytic properties of *Fagonia indica* Burm.f *Microscopy Research and Technique*. https://doi.org/10.1002/jemt.23867

Incalza, M.A., R. D'Oria, A. Natalicchio, S. Perrini, L. Laviola and F. Giorgino. 2018. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vascular Pharm.*, 100: 1-19.

Kahkeshani, N., B. Farahanikia, P. Mahdaviani, A. Abdolghaffari, G. Hassanzadeh, M. Abdollahi and M. Khanavi. 2013. Antioxidant and burn healing potential of *Galium odoratum* extracts. *Res. Pharm. Sci.*, 8: 197-203.

Kaya, I., N. Yigit and M. Benli. 2008. Antimicrobial activity of various extracts of *Ocimum basilicum* L. and observation of the inhibition effect on bacterial cells by use of scanning electron microscopy. *African Journal of Traditional, Complementary, and Alternative Medicines : AJTCAM*, 5: 363-369.

Khabour, O.F., K.H. Alzoubi, D.S. Mfady, M. Alasseiri andT.F. Hasheesh. 2014. Tempol protects human lymphocytes from genotoxicity induced by cisplatin. *International Journal of Clinical and Experimental Medicine*, 7: 982-988.

Khabour, O.F. and S.F.M. Hassanein. 2021. Use of vitamin/zinc supplements, medicinal plants, and immune boosting drinks during COVID-19 pandemic: A pilot study from Benha city, Egypt. *Heliyon*, 7: e06538. doi: 10.1016/ j.heliyon.2021.e06538.

Khalil, A.T., M.D. Khan, S. Razzaque, S. Afridi, I. Ullah, J. Iqbal, S. Tasneem, A. Shah, Z.K. Shinwari and M. Ayaz. 2021.Single precursor-based synthesis of transition metal sulfide nanoparticles and evaluation of their antimicrobial, antioxidant and cytotoxic potentials. Applied Nanoscience. DOI: 10.1007/s13204-021-02030-z

Lesage-Meessen, L., M. Bou, J.C. Sigoillot, C.B. Faulds and A. Lomascolo. 2015. Essential oils and distilled straws of lavender and lavandin: a review of current use and potential application in white biotechnology. *Applied Microbiology and Biotechnology*, 99: 3375-3385.

Martins, G.V., A.P.M. Tavares, E. Fortunato and M.G.F. Sales. 2017. Paper-based sensing device for electrochemical detection of oxidative stress biomarker 8-Hydroxy-2'-deoxyguanosine (8-OHdG) in Point-of-Care. *Scientific reports*, 7: 14558.

Mayyas, F., H. Aldawod, K.H. Alzoubi, O. Khabour, A. Shihadeh and T. Eissenberg. 2020. Comparison of the cardiac effects of electronic cigarette aerosol exposure with waterpipe and combustible cigarette smoke exposure in rats. *Life Sci*., 251: 117644..

Mhaidat, N.M., K.H. Alzoubi, O.F. Khabour, K.Z. Alawneh, L.A. Raffee, E.S. Alsatari, E.I. Hussein and K.E. Bani-Hani. 2016. Assessment of genotoxicity of vincristine, vinblastine and vinorelbine in human cultured lymphocytes: a comparative study. *Balkan Journal of Medical Genetics : BJMG*, 19: 13-20.

Pérez-Torres, I., V. Castrejón-Téllez, M.E. Soto, M.E. Rubio-Ruiz, L. Manzano-Pech and V. Guarner-Lans. 2021. Oxidative stress, plant natural antioxidants, and obesity. *Int. J. Mol Sci*, 22: 1786.

Rababa'h, A.M., K.H. Alzoubi, S. Baydoun and O.F. Khabour. 2019. Levosimendan prevents memory impairment induced by diabetes in rats: role of oxidative stress. *Curr. Alzheimer Res*, 16: 1300-1308.

Rahim, S.M., E.M. Taha, M.S. Al-janabi, B.I. Al-douri, K.D. Simon and A.G. Mazlan. 2014. Hepatoprotective effect of Cymbopogon citratus aqueous extract against hydrogen peroxide-induced liver injury in male rats. *African Journal of Traditional, Complementary, and Alternative Medicines : AJTCAM*, 11: 447-451.

Rahman, H., M. Shah, Z.K. Shinwari, W. Ali, N.Z. Khan, M. Aasim, N.S. Bibi and M. Ayaz. 2022. Total phenolic-flavonoids contents, anti-leishmanial, antimicrobial and antioxidant potentials of Pakistani tea brands and tea plant Camellia sinensis. *Pak. J. Bot*., 54(2): DOI: http://dx.doi.org/10.30848/PJB2022-2(42)

Rao, B.S., R. Shanbhoge, B.N. Rao, S.K. Adiga, D. Upadhya, B.K. Aithal and M.R. Kumar. 2009. Preventive efficacy of hydroalcoholic extract of Cymbopogon citratus against radiation-induced DNA damage on V79 cells and free radical scavenging ability against radicals generated in vitro. *Human & Experimental toxicology*, 28: 195-202.

Risques, R.A. and S.R. Kennedy. 2018. Aging and the rise of somatic cancer-associated mutations in normal tissues. *PLoS genetics*, 14: e1007108.

Rodrigues, L.B., A. Martins, J. Ribeiro-Filho, F. Cesario, E.C. FF, T.R. de Albuquerque, M.N.M. Fernandes, B.A.F. da Silva, L.J. Quintans Junior, A.A.S. Araujo, P.D.P. Menezes, P.S. Nunes, I.G. Matos, H.D.M. Coutinho, A. Goncalves Wanderley and I.R.A. de Menezes. 2017. Anti-inflammatory activity of the essential oil obtained from *Ocimum basilicum* complexed with beta-cyclodextrin (beta-CD) in mice. *Food and Chemical toxicology: An International Journal Published for the British Industrial Biological Research Association*, 109: 836-846.

Singh, S. 1999. Evaluation of gastric anti-ulcer activity of fixed oil of *Ocimum basilicum* Linn. and its possible mechanism of action. *Indian journal of Experimental Biology*, 37: 253-257.

Somparn, N., S. Saenthaweesuk, J. Naowaboot and A. Thaeomor. 2014. Effects of Cymbopogon citratus Stapf water extract on rat antioxidant defense system. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet*, (97 Suppl 8): S57-63.

Suaeyun, R., T. Kinouchi, H. Arimochi, U. Vinitketkumnuen and Y. Ohnishi. 1997. Inhibitory effects of lemon grass (*Cymbopogon citratus* Stapf) on formation of azoxymethane-induced DNA adducts and aberrant crypt foci in the rat colon. *Carcinogenesis*, 18: 949-955.

Tarboush, N.A., D.H. Almomani, O.F. Khabour and M.I. Azzam. 2022. Genotoxicity of glyphosate on cultured human lymphocytes. *Int. J. Toxicol*., 41: 126-131.

Tarchoune, I., C. Sgherri, R. Izzo, M. Lachaal, Z. Ouerghi and F. Navari-Izzo. 2010. Antioxidative responses of *Ocimum basilicum* to sodium chloride or sodium sulphate salinization. *Plant Physiology and Biochemistry : PPB*., 48: 772-777.

Tenore, G.C., P. Campiglia, R. Ciampaglia, L. Izzo and E. Novellino. 2017. Antioxidant and antimicrobial properties of traditional green and purple "Napoletano" basil cultivars (*Ocimum basilicum* L.) from Campania region (Italy). *Natural Product Research*, 31: 2067-2071.

Thirugnanasampandan, R. and R. Jayakumar. 2011. Protection of cadmium chloride induced DNA damage by Lamiaceae plants. *Asian Pacific Journal of Tropical Biomedicine*, 1: 391-394.

Uma, K., X. Huang and B.A. Kumar. 2017. Antifungal effect of plant extract and essential oil. *Chinese Journal of Integrative Medicine*, 23: 233-239.

Vieira, A.J., F.P. Beserra, M.C. Souza, B.M. Totti and A.L. Rozza. 2018. Limonene: Aroma of innovation in health and disease. *Chemico-biological Interactions*, 283: 97-106.

Vijg, J. 2014. Somatic mutations, genome mosaicism, cancer and aging. *Current Opinion in Genetics & Development*, 26: 141-149.

Vlase, L., D. Benedec, D. Hanganu, G. Damian, I. Csillag, B. Sevastre, A.C. Mot, R. Silaghi-Dumitrescu and I. Tilea. 2014. Evaluation of antioxidant and antimicrobial activities and phenolic profile for *Hyssopus officinalis*, *Ocimum basilicum* and *Teucrium chamaedrys*. *Molecules*, 19: 5490-5507.

Wojnicz, D., A.Z. Kucharska, A. Sokol-Letowska, M. Kicia and D. Tichaczek-Goska. 2012. Medicinal plants extracts affect virulence factors expression and biofilm formation by the uropathogenic *Escherichia coli*. *Urological Research*, 40: 683-697.

Yatoo, M.I., U. Dimri, A. Gopalakrishnan, K. Karthik, M. Gopi, R. Khandia, M. Saminathan, A. Saxena, M. Alagawany, M.R. Farag, A. Munjal and K. Dhama. 2017. Beneficial health applications and medicinal values of Pedicularis plants: A review. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, 95: 1301-1313.

Zlotek, U., S. Mikulska, M. Nagajek and M. Swieca. 2016. The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L.) extracts. *Saudi Journal of Biological Sciences*, 23: 628-633.

(Received for publication 30 May 2018)