Malaysian Journal of Microbiology, Vol 14(5) 2018, pp. 387-392
DOI: http://dx.doi.org/10.21161/mjm.114417

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Antibacterial activity and toxicity of Duckweed, *Lemna minor* L. (Arales: Lemnaceae) from Malaysia

Li Peng Tan[1*], Ruhil Hayati Hamdan[1], Maizan Mohamed[1], Siew Shean Choong[1], Yean Yean Chan[2], Seng Hua Lee[3]

[1]Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Pengkalan Chepa, 16100 Kota Bharu, Kelantan, Malaysia.
[2]Department of Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, 16150 Kota Bharu, Kelantan.
[3]Institute of Tropical Forestry and Forest Products, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
Email: li.peng@umk.edu.my; leeseng@upm.edu.my

Received 6 October 2017; Received in revised form 13 June 2018; Accepted 27 June 2018

ABSTRACT

Aims: New therapeutics are needed to ease the prevailing waterborne disease, and one of the alternatives is by exploring the natural compounds with antimicrobial properties. Duckweed, *Lemna* sp. is recorded as a medicinal herb that known to have antifungal and antibacterial activities towards several fungi and bacteria. Suitability of duckweed (*Lemna minor*) as an antibacterial resource against selected waterborne bacteria were evaluated in terms of its antibacterial activity and toxicity.
Methodology and results: Antibacterial activity of the duckweed methanolic extract was tested against 11 selected waterborne bacteria using disc diffusion, minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) assay. Brine shrimp lethality assay was used to determine the toxicity of this extract. The lethal concentrations of plant extract resulting in 50% mortality of the brine shrimp (LC50) were then determined.
Conclusion, significance and impact of study: Results showed that duckweed extract exhibited bacteriostatic and bactericidal against the selected bacteria activity at the concentration of MIC = 1.8-2.0 mg/mL and MBC ≥ 2.0 mg/mL. This study shows that methanolic extract of *L. minor* may contain bioactive compounds against bacteria and potential therapeutic effect. The crude extract is slightly toxic and may not safe to be used in high concentration but is valuable in further study as a potential antitumor agent.

Keywords: Antimicrobial resistance; bacteriostatic; bactericidal; Brine shrimp; therapeutic

INTRODUCTION

According to World Health Organization, waterborne disease is a global burden which is estimated to cause more than 2.2 million deaths per year, making it the leading cause of disease and death around the world (Prüss-Üstün and Corvalán, 2006). Human infections caused by pathogens transmitted from fish and aquatic environment are quite common (Novotny *et al.*, 2010). There are often bacterial species facultatively pathogenic for both fish and human beings. Antibiotics are once considered the most promising solution for combating diseases caused by microbes (Gould and Bal, 2013). Nevertheless, these drugs have begun to lose its usefulness as bacteria resistance towards the antibiotics has aroused due to the misuse and over prescription of the drugs (Davies and Davies, 2010).

Antimicrobial resistance (AMR) is now a global threat that causes economic lost due to increased mortality and morbidity as a result of medicine ineffectiveness and infections persistent, thus increasing the risk of spread to others. New therapeutics are needed to ease this problem, and one of the alternatives is by exploring the efficacies of natural compounds with antimicrobial properties (Bérdy, 2012). Among 28,000 plant species that are currently available for medicine, fewer than 13% of them are regularly cited as being used in studies for regulatory publications (Lufkin, 2017). Metabolites from plants, especially the ones with presence of secondary metabolites, including flavonoids, alkaloids, tannins, and saponins are significantly important for their potential biological activities against microbes (Pandith, 2012). Exploitation of plants to identify new antibiotics is needed to sustain the effect of antimicrobial treatments in the future (Van der Waaij and Nord, 2000). In traditional medicine, whole plants or mixtures of plants are used rather than isolated compounds. Crude extract of plants are also often studied and tested before proceeding

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 14(5) 2018, pp. 387- 392
DOI: http://dx.doi.org/10.21161/mjm.114417

further into specific phytoconstituents. There is evidence that crude plants extracts are often pharmacologically more active than their pure active compounds at an equivalent dose due to the synergistic effects and additive effects of various components present in the whole extracts (Lal *et al.*, 2007; Rasoanaivo *et al.*, 2011).

Duckweed, *Lemna* sp. has been recorded as a medicinal herb and is known to have antifungal and antibacterial activities towards several fungi and bacteria in previous studies (Duke *et al.*, 2002; Gulcin *et al.*, 2010; Almahy, 2015). This aquatic plant is of interest due to its rapid growth rate in warm environment, to the extent of forming a mat on water surface and become pest for other aquatic plants or animals if the growth of duckweed is not controlled (Tkalec *et al.*, 1998; Almahy, 2015). Although it is known to have the antimicrobial properties, apart from being animal feed, *Lemna* is not commonly utilized for therapeutic purposes (Yilmaz *et al.*, 2004). Additives value can be given to this plant if it is proven to have antibacterial activity towards selected waterborne bacteria, but safe to be used for both animals and human-beings. Therefore, the main objective of this study is to determine the suitability of *Lemna minor* to be used as an antibacterial resource against selected waterborne bacteria by evaluating its antibacterial activity and toxicity.

MATERIALS AND METHODS

Plant collection and extract preparation

The fresh plants were collected from a drainage system located in an industry area in Port Klang, Selangor. Extraction was carried out as described previously by Gulcin *et al*. (2004). The collected duckweed sample was cleaned under tap water and dried in oven. Twenty-five grams of dried duckweed sample was ground into fine powder composition using a grinder and then mixed with 500 mL of methanol. The obtained extracts were filtered through Whatman No. 1 paper. Residue will be re-extracted by repeating the same procedures until extraction solvents became colorless. The filtrate was collected and placed in an oven set at 40 °C to allow the methanol to be fully evaporated. The extract was then transferred into a Schott bottle and stored at -20 °C until further use (Gulcin, 2005).

Collection of bacteria

The bacterial species used in the present study were *Aeromonas hydrophila*, *Pseudomonas putida*, *Vibrio cholerae* Bengal, *V. cholerae* El-Tor, *V. cholerae* Non, *V. alginolyticus*, *Staphylococcus aureus*, *Streptococcus agalactiae* (isolates from human and fish), *Citrobacter freundii* and *Escherichia coli*. *Vibrio cholera* and *S. agalactiae* (human) isolates were obtained in pure cultures from Department of Parasitology and Microbiology, Universiti Sains Malaysia; *S. aureus* and *E. coli* were obtained in pure cultures from Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, while other bacteria were isolated from fish.

Determination of antibacterial activity

Disc diffusion assay

The extract was re-dissolved using methanol in the concentration of 100 mg/mL and 20 µL of this extract was loaded onto sterile Whatman No. 1 membrane filter paper discs (6 mm diameter) and left to dry. A suspension of bacteria according to 0.5 McFarland standard was prepared and spread onto the surface of Muller-Hinton agar (MHA) plates. Paper discs containing the plant extract were carefully placed on the surface of each plate. Oxytetracycline (OTC) was served as positive control and solvent as negative control. The plates were left at 4 °C for an hour to allow diffusion of extracts before incubated for 24 h at 30-37 °C. Microbial inhibition was indicated by measuring the diameter of the clear zone around the discs and recorded as diameter on inhibition zone in millimetre (mm). The test was done in three replicates. The strength of activity was divided into three categories according to for inhibition zone diameters, namely strong (≥ 20 mm), moderate (10 to 19 mm) and weak (1 to 9 mm) (Bonjar, 2004).

Minimum Inhibition Concentration (MIC)

The antibacterial activity of duckweed methanolic extract was determined using microdilution method. One-hundred milligrams of the extract were dissolved in 1 mL DMSO for stock preparation. One millilitre of the stock extract was added into 9 mL of 5% DMSO to give a crude extract concentration of 10 mg/mL. A two-fold serial dilution started with the concentration of 2 mg/mL was carried out for the extract in the test wells which contain 100 µL Trypticase soy broth (TSB) each. Five microliters of each bacterial suspension (10^5 CFU) was added to each well. Control wells were prepared with culture medium and bacterial suspension only. The plates were sealed and incubated for 24 h at 30-37 °C. The assays were prepared in triplicates to determine the MIC of the extract towards each bacterium.

Minimum Bactericidal Concentration (MBC)

MBC of the extract was determined by sub-culturing 50 µL of the suspensions from the wells which did not show any growth during MIC assays. The MBC was defined as the lowest concentration of sample which completely killed the bacteria.

Determination of toxicity

Brine Shrimp Lethality Assay (BSLA) – Ten milligrams of the extract were dissolved in 1 mL of DMSO. Extract solution was transferred into each vials in 5, 50 and 500 µL. Each of these vials was then topped up with saline to obtain the final concentrations of 10, 100 and 1000 µg/mL respectively at the volume of 5 mL. One vial was supplemented with 1% DMSO to serve as negative control. Brine shrimp cysts, *Artemia salina* were hatched

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 14(5) 2018, pp. 387- 392
DOI: http://dx.doi.org/10.21161/mjm.114417

in artificial sea water (38 g NaCl in 1 L of distilled water) under constant aeration and light source for 24 h. Ten nauplii were transferred into each of the vial with prepared extract solution. The vials were maintained under illumination. Survived nauplii were counted macroscopically after 24 h. The test was repeated three times and the average mortality rate was adjusted using Abbott's formula. The lethal concentrations of plant extract resulting in 50% mortality of the brine shrimp (LC_{50}) were then determined using Probit analysis.

RESULTS AND DISCUSSION

Disc diffusion assay

In the present study, methanolic extracts of duckweed was tested for its antibacterial activity through disc diffusion methods. Among all 11 bacteria tested, none of these bacteria are susceptible to the duckweed methanolic extracts as inhibition zones were not observed for all the replicates conducted.

Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The test was then extended to MIC and MBC assay. Results shows that inhibitory of bacterial growth occurred at the concentration of 1.8 - 2.0 mg/mL for all the bacteria tested (Table 1). While bactericidal effect of duckweed methanolic extracts occurred at the concentration of 2.0 mg/mL for *S. aureus* and all the Gram-negative bacteria tested (Table 1). *Streptococcus agalactiae* with the highest MIC did not killed by the extracts at the concentration of 2.0 mg/mL, but their colony growth at this concentration are reduced compared to the others lower concentrations used. This suggested that MBC of the duckweed extract against *S. agalactiae* are positive yet required higher concentration, which is > 2.0 mg/mL. According to the Meyer's Toxicity Index, extracts are considered as toxic when the LC_{50} < 1000 µg/mL (Meyer *et al.*, 1982). Clarkson's Toxicity Criterion (Clarkson *et al*., 2004) on the other hand indicate that the current result from the brine shrimp lethality bioassay, methanolic extracts of duckweed with LC_{50} = 140.64 µg/mL is medium toxic (Figure 1). According to Gulcin *et al.* (2010) and Effiong and Sanni (2010), *Lemna* sp. generally consists of tannins and flavonoids or steroids. These chemical compounds are generally believed to be the major active compounds against the bacteria (Dahiya and Purkayastha, 2012; Bhat and Al-Daihan, 2014).

Increasing burden of antibacterial resistance species to the current available antibacterial drugs has led researcher seeking possible alternative from the nature to cater this situation. Compounds that possess antimicrobial properties such tannins, terpenoids, alkaloids, and flavonoids are mainly extracted from plants (Cowan, 1999). It seems very likely, therefore, that the extracts of duckweed may contains antibacterial compounds that can inhibit bacteria by a different mechanism than that of currently used antibacterial drugs and may have therapeutic value as an antibiotic against waterborne bacterial strains. The potential of duckweed extracts as an antibacterial agent had been tested against numbers of bacteria and fungi. Results from the previous studies showed that duckweed extracts are a promising candidate for inhibiting the growth of various bacteria (Gulcin *et al*., 2010; Zhang *et al*., 2010). However, in the current study, methanolic extracts of local duckweed did not show any of the inhibitory effect on the bacteria tested under the disc diffusion assay. This result was contradicted with the previous study utilising the same extracting solvent, methanol, which has been recommended as the best among few solvents was used.

Table 1: MIC and MBC of methanolic extracts of duckweed on different waterborne bacteria.

Bacteria species	MIC (mg/mL)	MBC (mg/mL)
Gram-positive		
Staphylococcus aureus	1.8	2.0
Streptococcus agalactiae (Fish)	2.0	>2.0
Streptococcus agalactiae (Human)	2.0	>2.0
Gram-negative		2.0
Aeromonas hydrophila	1.9	2.0
Citrobacter freundii	1.8	2.0
Escherichia coli	1.9	2.0
Pseudomonas putida	1.9	2.0
Vibrio cholerae Bengal	1.9	
Vibrio cholerae El-tor	1.9	2.0
Vibrio cholerae Non	1.9	2.0
Vibrio alginolyticus	1.8	2.0

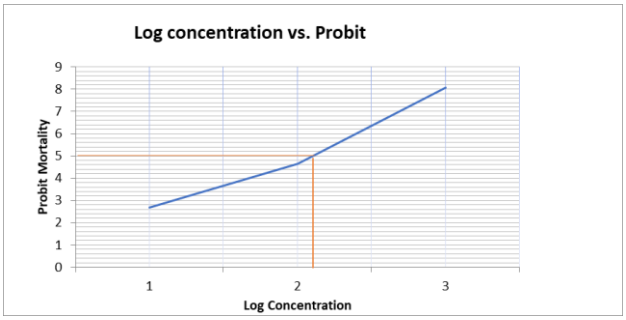

Figure 1: Toxicity of methanolic extracts of duckweed on brine shrimp nauplii.

In this study, focus was on waterborne bacteria whilst *S. aureus*, a foodborne bacterium was included as a control, as several studies were done on the antibacterial effect against this bacterium and positive results were obtained. Thus, this bacterium is used to determine that the negative results against the selected waterborne bacteria are not due to the species or nature of these

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

bacteria. The absence of inhibition zone for all bacteria during the disc diffusion assay was unexpected. However. as previously reported, the absence of an inhibition zone did not necessarily indicate that the compound was inactive, especially for less polar compounds that are scarcely soluble or insoluble in water, such as duckweed extracts, thus diffuse more slowly and uniformly into the culture medium (Mann and Markham, 1998; Moreno *et al*., 2006). This was shown when MIC and MBC results are positive for the duckweed extract against all bacteria at ≥ 2.0 mg/mL. Therefore, it appears that the diffusion method could not reliably screen the antimicrobial activity of plant extracts due to different solubility and diffusion levels of these natural antimicrobial compounds (Klančnik *et al*., 2010). The sensitivity of disc diffusion is comparative lower than the microdilution method to determine antibacterial activity. However, diffusion methods are still commonly used because of their simplicity and low cost, despite the low reproducibility and robustness of these methods.

Besides solvent used and bacterial species, the antimicrobial activity of duckweed extract is suspected largely influenced by the locality of this plant harvested. It is known that phytochemical of similar plants that grow out in different geographical locations may vary (Mai *et al*., 2001). According to Liu *et al*. (2016), environmental factors for instance altitude, annual sunshine and annual temperature may influence types and contents of active substances in plants. A number of studies have been carried out on the antibacterial effects of essential oils and plant extracts indicated that plant extracts or essential oils of the same species collected from different geographical regions could show significant variations in their ability to suppress bacterial growth (Mikulásová *et al*., 2011; Karahan *et al*., 2016; Stanković *et al*., 2017). Therefore, the low bacteriostatic effect of the duckweed crude extract might highly due to the geographical and environmental factors.

Methanolic extract of duckweed in this study showed weak antimicrobial activity (MIC = 1.8-2.0 mg/mL) against both Gram-negative and Gram-positive bacteria. According to Fankam *et al.* (2015), MICs values above 625 µg/mL are considered weak antibacterial activity for a plant extract, while the moderate values ranged from 100 ≤ MIC ≤ 625 µg/mL. For a significant antibacterial activity, the MICs values shall be lied below 100 µg/mL. It is known that lipopolysaccharides (LPS) consists of lipid A, the core polysaccharide, and the O-side chain, which provides the "quid" that allows Gram-negative bacteria to be more resistant to essential oil and other natural extracts with antimicrobial activity (Nazzaro *et al*., 2013). Natural compounds from plants exhibit various mechanisms against microorganisms viz. inhibit cell wall synthesis, accumulate in bacterial membranes causing energy depletion, or interfere the permeability of cell membrane which had a consequence a permeability increase and loss of cellular constitutes, membrane disruption and changes the structure and function of key cellular constituents, resulting in mutation, cell damage, and death (Kang *et al*., 2011). Although, antibacterial mechanisms of *L. minor* against various microorganisms was not fully illustrate, we suggest that may be more than one of the mechanisms mentioned above playing important roles in disrupting the Gram-positive and work equally well on Gram-negative bacteria.

Brine Shrimp Lethality Assay (BSLA)

Although duckweed is widely used as an animal feed due to its fast growing rate and high protein content, the toxicity or side effect of this plant are under study (Ziegler *et al*., 2016). The methanolic extracts of duckweed show positive results towards brine shrimp, this indicates the extracts are biologically active. Brine shrimp is an effective alternative model for predicting the toxicity of plant extracts (Hamidi *et al*., 2014). According to the comparison table on toxicity class of plant made by Hamidi *et al.* (2014), positive correlation between LC_{50} result of brine shrimp and LD_{50} of animal models is confirmed. The current result on toxicity of methanolic duckweed extracts (LC_{50} = 140.64 µg/mL) is considered slightly toxic towards human as it is LC_{50} > 82.27 µg/mL for BSLA or equivalent to LD_{50} > 8193.00 mg/kg for mice model. Nevertheless, further investigation of the phytochemical compound by using *in vitro* method should be pursued to indicate the exact constituents that are potential to be antitumor agents while relative safe for normal cells.

CONCLUSION

The results obtained from this study reveal that *L. minor* extract has a weak antimicrobial activity against both Gram-positive and Gram-negative bacteria. The locality where the plant was collected is suspected to be the reason that lead to this variation compared to the previous studies. The present study also showed that *L. minor* extract is considered slightly toxic towards human while being biologically active. Therefore, further study on each of the phytochemical compound of this plant extract is warranted to determine its potential as antitumor agents.

ACKNOWLEDGEMENTS

The authors wish to thanks for the financial support provided by SGJP-MyRA Keserakanan USM, Research Code: R/MyRA/A06.00/01351A/002/2017/000396.

REFERENCES

Almahy, H. A. (2015). Antibacterial activity of methanol extracts of the leaves of *Lemna minor* against eight different bacterial species. *International Journal of Pharmaceutics* **5, 46-50.**

Bérdy, J. (2012). Thoughts and facts about antibiotics: where we are now and where we are heading. *The Journal of Antibiotics* **65, 385-395.**

Bhat, R. S. and Al-Daihan, S. (2014). Phytochemical constituents and antibacterial activity of some green

leafy vegetables. *Asian Pacific Journal of Tropical Biomedicine* **4,189-193.**

Bonjar, S. (2004). Evaluation of antibacterial properties of some medicinal plants used in Iran. *Journal of Ethnopharmacology* **94, 301-305.**

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., … Folb, P. I. (2004). *In vitro* antiplasmodial activity of medicinal plants native to or naturalized in South Africa. *Journal of Ethnopharmacology* **92, 177-191.**

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews* **12, 564-582.**

Dahiya, P. and Purkayastha, S. (2012). Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. *Indian Journal of Pharmaceutical Sciences* **74, 443-450.**

Davies, J. and Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews* **74, 417-433**.

Duke, J. A., Bogenschutz-Godwin, M. J., DuCellier, J. and Duke, P. A. K. (2002). Handbook of Medicinal Herbs. 2nd Edn. CRC Press LLC, Florida USA. **pp. 237-238.**

Effiong, B.N. and Sanni, A. (2010). Antifungal properties and phytochemical screening of crude extract of *Lemna pauciscostata* (Helgelm) against fish feed spoilage fungi. *Life Science Journal* **7, 1-4.**

Fankam, A. G., Kuiate, J. R. and Kuete, V. (2015). Antibacterial and antibiotic resistance modifying activity of the extracts from *Allanblackia gabonensis*, *Combretum molle* and *Gladiolus quartinianus* against Gram-negative bacteria including multi-drug resistant phenotypes. BMC Complement. *BMC Complementary and Alternative Medicine* **15, 1-12.**

Gould, I. M. and Bal, A. M. (2013). New antibiotic agents in the pipeline and how they can help overcome microbial resistance. *Virulence* **4, 185-191.**

Gulcin, I. (2005). The antioxidant and radical scavenging activities of black pepper (*Piper nigrum*) seeds. *International Journal of Food Sciences and Nutrition* **56, 491-499.**

Gulcin, I., Irfan Küfrevioglu, Ö., Oktay, M. and Büyükokuroglu, M. E. (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *Journal of Ethnopharmacology* **90, 205-215.**

Gulcin, I., Kirecci, E., Akkemik, E., Topal, F. and Hisar, O. (2010). Antioxidant, antibacterial, and anticandidal activities of an aquatic plant: duckweed (*Lemna minor* L. Lemnaceae). *Turkish Journal of Biology* **34, 175-188.**

Hamidi, M., Jovanova, B. and Panovska, T. (2014). Toxicological evaluation of the plant products using brine shrimp (*Artemia salina* L.) model. *Macedonian Pharmaceutical Bulletin* **60, 9-18.**

Kang, C., Hah, D., Kim, C., Kim, Y., Kim, E. and Kim, J. (2011). Evaluation of antimicrobial activity of the methanol extracts from 8 traditional medicinal plants. *Toxicological Research* **27, 31-36.**

Karahan F., Avsar, C., Ozyigit, I. I. and Berber, I. (2016). Antimicrobial and antioxidant activities of medicinal plant *Glycyrrhiza glabra* var. *glandulifera* from different habitats. *Biotechnology and Biotechnological Equipment* **30, 797-804.**

Klančnik, A., Piskernik, S., Jeršek, B. and Možina, S. S. (2010). Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *Journal of Microbiological Methods* **81, 121-126.**

Lal, A. A., Murthy, P. B. and Pillai, K. S. (2007). Screening of hepatoprotective effect of a herbal mixture against CCl4 induced hepatotoxicity in Swiss albino mice. *Journal of Environmental Biology* **28, 201–207.**

Liu, W., Yin, D. X., Li, N., Hou, X. G., Wang, D. M., Li, D. W. and Liu, J. J. (2016). Influence of environmental factors on the active substance production and antioxidant activity in *Potentilla fruticosa* L. and its quality assessment. *Scientific Reports* **6, 1-18.**

Lufkin, B. (2017). 'Super plants. In 33 ideas that will change the world'. *BBC Future.* http://www.bbc.com/future/gallery/20170905-33-ideas-that-will-change-the-world [Retrieved on 8 September 2017]

Mai, H. D., Van-Dufat, H. T., Miche, S., Tillequin, F., Bastien, D. and Sevenet, T. (2001). A new diprenyl coumarin and alkaloids from the bark of *Zanthoxylum dimorphophyllum* (Rutaceae). *Zeitschrift für Naturforschung C* **56, 492-494.**

Mann, C. M. and Markham, J. L. (1998). A new method for determining the minimum inhibitory concentration of essential oils. *Journal of Applied Microbiology* **84, 538-544.**

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. and McLaughlin, J. L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica* **45, 31-34.**

Mikulásová, M., Vaverková, S., Habánová, M. and Lokalít, R. (2011). Antimicrobial effect of essential oils from plants collected from different localities. *Acta Fytotechnica et Zootechina* **2, 29-31.**

Moreno, S., Scheyer, T., Romano, C. S. and Vojnov, A. A. (2006). Antioxidant and antimicrobial activities of

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

rosemary extracts linked to their polyphenol composition. *Free Radical Research* **40, 223-231.**

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R. and De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals (Basel)* **6, 1451-1474.**

Novotny L., Halouzka, R., Matlova, L., Vavra, O., Dvorska, L., Bartos, M. and Pavlik, I. (2010). Morphology and distribution of granulomatous inflammation in fresh water ornamental fish infected with mycobacteria. *Journal of Fish Diseases* **33, 947-955.**

Pandith, J. I. (2012). Phytochemical screening of certain plant species of Agra city. *Journal of Drug Delivery and Therapeutics* **2, 135-138.**

Prüss-Üstün A. and Corvalán, C. (2006). Preventing disease through healthy environments - Towards an estimate of the environmental burden of disease. WHO Press, World Health Organization, Geneva, Switzerland. **pp. 34.**

Rasoanaivo, P., Wright, C. W., Willcox, W. L. and Gilbert, B. (2011). Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions. *Malaria Journal* **10, 1-12.**

Stanković, M., Ćurčić, S., Zlatić, N. and Bojović, B. (2017). Ecological variability of the phenolic compounds of *Olea europaea* L. leaves from natural habitats and cultivated conditions. *Biotechnology and Biotechnological Equipment* **31, 499-504.**

Tkalec, M., Željka, V. C. and Regula, I. (1998). The effect of oil industry ‘‘high density brines’’ on duckweed *Lemna minor* L. *Chemosphere* **37, 2703-2715**.

Van der Waaij, D. and Nord, C. E. (2000). Development and persistence of multi-resistance to antibiotics in bacteria; an analysis and a new approach to this urgent problem. *International Journal of Antimicrobial Agents* **16, 191-197.**

Yilmaz, E., Akyurt, I. and Gunal, G. (2004). Use of duckweed, *Lemna minor*, as a protein feedstuff in practical diets for common carp, *Cyprinus carpio*, fry. *Turkish Journal of Fisheries and Aquatic Sciences* **4, 105-109.**

Zhang, Y., Hu, Y., Yang, B., Ma, F., Lu, P., Li, L., Wan, C., Rayner, S. and Chen, S. (2010). Duckweed (*Lemna minor*) as a model plant system for the study of human microbial pathogenesis. *PLoS One* **5, e13527.**

Ziegler, P., Sree, K. S. and Appenroth, K. J. (2016). Duckweeds for water remediation and toxicity testing. *Toxicological and Environmental Chemistry* **10, 1127-1154.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538