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Antimicrobial activity and toxicity of *Eucalyptus globulus* Labill. essential oil against vaginal microorganisms

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ABSTRACT

The antimicrobial potential of commercial essential oil (EO) of *Eucalyptus globulus* L. was evaluated against six bacterial vaginal isolates (*E. coli* 1 and *E. coli* 2, *S. aureus* 1 and *S. aureus* 2, *P. aeruginosa* and *P. mirabilis*) and two isolates of *Candida* strains (*C. albicans* 1 and *C. albicans* 2). The antimicrobial activity was assessed through double-dilution micro-plate assay to determine the minimum inhibitory (MIC) and minimum bactericidal/fungicidal (MBC/MFC) concentration. In addition, brine shrimp toxicity assay was performed in order to determine acute toxicity of the examined EO. The isolated strains of pathogens have shown strain specificity to the tested EO. Certain pathogens resistance was noticed toward the tested antibiotic, as well. *E. coli* isolates showed resistance to the tested antibiotics but did not show resistance against the *Eucalyptus* EO (*E. coli* 1 MIC/MBC 12.5/12.5 µL/mL; *E. coli* 2 MIC/MBC 25/25 µL/mL). Moreover, the *Eucalyptus* EO showed effectiveness in application against *S. aureus* 2 (MIC/MBC 6.25/6.25 µL/mL), and *C. albicans* 1 strains (MIC/MFC 6.25/6.25 µL/mL). Furthermore, brine shrimp lethality bioassay revealed the *Eucalyptus* oil toxicity at an LC_{50} value of 2.66 mg/mL. The chemical composition of the separated essential oil was analyzed by gas chromatography combined with mass spectrometry (GC-MS) showing eucalyptol (59.63%), *p-cymene* (15.55%) and DL-limonene (14.90%) as dominant constituents. Although a number of toxicology trials are needed, these results provide scientific support to examination of *Eucalyptus* EO as an antimicrobial agent in alternative treatment of multiresistant human pathogens of vaginal origin.

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1. Introduction

Medicinal plants have been known for millennia as a rich source of therapeutic agents for the treatment and prevention of various diseases such as malaria, typhoid fever, schistosomiasis, onchocerciasis, lymphatic filariasis, African trypanosomiasis and dengue (Wansi et al., 2019; Mohammadhosseini et al., 2017). Moreover, these powerful sources of bioactive metabolites, alongside the different practices in terms of preparing and applying herbal remedies that have been developed by certain ethnic groups throughout the centuries, have become the most useful database for the evaluation of new pharmaceuticals (Jarić et al., 2007). The study of ethnobotanical heritage in Balkan region has shown comparatively strong connections between the local community and natural resources of wild-growing medicinal herbs (*Achillea millefolium* L., *Hedera helix* L., *Matricharia chamomilla* L. etc.) that represent a large portion of its floristic richness (Zlatković et al., 2014). Due to the increasing popularity and extensive usage of phytotherapy all over the globe (Sarker and Nahar, 2018a), essential oils, originated from medicinal plants, are becoming more significant not only in pharmacology

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and medicine, but also in economy (Mohammadhosseini et al., 2019, Wansi et al., 2018). For medical purposes, whole components (Frezza et al., 2017) are used in the production of drugs due to a number of positive pharmacological effects involving antioxidant property, prevention of hypertension, inhibition of acetylcholine (Yeom et al., 2015) as well as various antimicrobial effects (Karaman et al., 2017). Moreover, essential oils are commonly consumed as a spice in the food along with cosmetics and perfumery industries due to their color and fragrance (Sarker and Nahar, 2018). Vaginitis and vulvovaginitis cover a group of inflammatory conditions with heterogeneous etiology which cause various problems such as redness, itching, pain, discharge, cervicitis, bleeding mucous membranes of the vagina and could have an impact on outcome of pregnancy (Bogavac et al., 2012). They usually develop as a consequence of bacterial, fungal (particularly *Candida albicans* and non-*albicans* species) and protozoal infections (mostly *Trichomonas vaginalis*) (Pierce and Hart, 1992; Bortz et al., 2013). Therapy of choice includes the administration of antibiotics in combination with a vaginal suppository according to the results of the antibiogram (Bortz et al., 2013). Considering the fact that antimicrobial properties of plants and their secondary metabolites are well-known, vaginal infections could be treated with EOs (Vermani and Garg, 2002; Naeini et al., 2009; Carson and Hammer, 2011). EOs contain monoterpenes, sesquiterpenes and their related alcohols, as active substances, which inhibit transmission of bacteria, especially *Staphylococcus* sp., *Streptococcus* sp. and *Candida sp.* yeasts (Warnke et al., 2009; Carson and Hammer, 2011). The leaves of *Eucalyptus globulus* L. (Family: Myrtaceae; Genus: *Eucalyptus*) (Fig. 1) are the principal source of *Eucalyptus* oil (1.0-2.4%) which has antibacterial effects on pathogenic bacteria in the respiratory tract (Salari et al., 2006). The aim of the current study was to evaluate antimicrobial efficacy of commercial *Eucalyptus* oil against isolated bacterial and yeast strains of the women suffering from vaginitis and to determine their inhibitory effects and their toxicity, as well.

Fig. 1. *Eucalyptus globulus* L.

2. Experimental

2.1. Bacterial and yeasts strains

Six bacterial strains involving *E. coli* 1, *E. coli* 2, *S. aureus* 1, *S. aureus* 2, *P. aeruginosa* and *P. mirabilis* along with two yeasts Candida human isolates (*C. albicans*1 and *C. albicans*2) were used as test microorganisms. All isolates were obtained from women with symptoms of vaginal infection at regular gynecological checkup examination at the Department of Obstetrics and Gynecology, Clinical Centre of Vojvodina, Faculty of Medicine, University of Novi Sad. This protocol was approved by the Ethical Board of Clinical Centre of Vojvodina. Stocks of isolated bacterial cultures were conserved on Müeller-Hinton agar (MHA) and yeast strains on Malt agar (MA), both at 37 °C. Inoculum used in antimicrobial assay (approximately 1.5×10^8 CFU/mL for bacteria and 1.5 x 10^6 for yeast) was prepared from overnight cultures, according to McFarland standard procedures.

2.2. Essential oil

The commercial *Eucalyptus* oil (EO) was obtained from the MeiLab Manufacturer, Belgrade, Serbia.

2.3. Antimicrobial assay

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of EO was assayed by standard CLSI procedure (Clinical Laboratory Standards Institute, 2008) using 96-well microtiter plate (Spektar, Čačak, Serbia). Microdilution test was performed in Müeller-Hinton broth (MHB, Torlak, Belgrade, Serbia) for bacterial isolates and Malt broth (MB, Torlak, Belgrade, Serbia) for yeasts. Inoculated broth was mixed with double dilutions of the EO and incubated at 37 °C/24 h for bacteria, and 37 °C/48 h for yeasts. Antimicrobial testing was done in triplicates (1.25-50 µL/mL) in comparison to negative (not inoculated with bacteria/yeast) and positive control (not treated with EO). Finally, 100 µL from each microplate well was transferred to the MHA plate for bacteria and MA plate for *C. albicans*. After incubation at 37 °C overnight, the number of viable microorganisms was determined. MBC represents the reduction of 99.9% or more of the initial inoculum, while the MIC is the lowest concentration of EO that being resulted as a non-visible growth in petri dishes. Streptomycin, ampicillin, tetracycline and cefuroxime (Torlak, Belgrade, Serbia) as well as one antimycotic-nystatin (Hemofarm, Vršac, Serbia) were used in antibiogram test.

2.4. Brine shrimp toxicity assay

Artemia salina bioassay was performed according to Meyer et al. (1982). Toxicity of EO was calculated by series of double dilutions in five repetitions with a control: 25 µL of 1.0% oil solvent dimethyl sulfoxide (DMSO) and 225 µL ASW with larvae as control. Toxicity was calculated after 24 h by counting dead nauplii under a stereomicroscope (Zeiss, Germany). Results were expressed as LC_{50} (lethal concentration required to kill 50% of the population of the tested organisms-brine shrimp *Artemia salina*). Abbott's equation was applied for calculation of the mortality rate

according to the following equation (Eqn. 1):

$$\text{Mortality (\%)} = ((n_{test} - n_{control})/n_{control}) \times 100 \quad \text{(Eqn. 1)}$$

Where n_{test} and $n_{control}$ are the numbers of dead larvae in the first test and control tests, respectively.

2.5. Gas chromatography-mass spectrometry (GC/MS)

Gas chromatography-mass spectrometry (GC/MS) was used to determine the active substances from the present EO. Analyses were performed on Agilent capillary gas chromatograph coupled with mass spectrometer (MSD) (model GC Agilent 7890A; MS 5975C). Preparation of EO for this analysis includes 1:10 w/v dilution in dichloromethane. Retention indices (RI) were used for the identification of the EO components by comparing the obtained spectral data from analyzed EOs and RI from literature data.

2.6. Statistical analyses

One-way ANOVA with post-hoc Tukey HSD test at the sensitivity level $p < 0.01$ was used to determine significant differences between activities of EO against each bacterial strain and among activity of tested antibiotics. LC_{50} value for the brine shrimp lethality bioassay was derived from linear regression analysis using the Origin program (OriginLab version 8.0, Corporation, Northampton, MA, USA).

3. Results and Discussion

3.1. Antimicrobial activity

Antimicrobial activity of EO was evaluated by MIC and MBC/MFC values (Table 1) where lower value points out the stronger effect. According to the lowest obtained MIC and MBC values of *Eucalyptus* oil (6.2 µL/mL), *S. aureus* 2 strain showed the highest susceptibility. Great variation in antibacterial activities of EO against investigated strains of bacteria were present and obtained in a range from 6.2 to 25 µL/mL indicating strong strain specificities (Table 1). The most sensitive strains were *E. coli* 1, *S aureus* 2 and *C. albicans* 1. Multiresistance was determined according to antibiotic resistance tests on clinical bacterial isolates *P. aeruginosa*, *E. coli* 1 and *E. coli* 2 (Table 2). *P. aeruginosa* strain proved to be the most resistant strain amongst all examined strains. This was also proved by susceptibility testing of bacterial strains to common antibiotics (Table 2). Moreover, strains of gram-negative *E. coli* 1 and *E. coli* 2 showed a prominent-significant activity (12.5-25 µL/mL). Amongst all the investigated antibiotics, tetracycline and streptomycin showed high activity against three bacterial strains including *S. aureus* 1 *S. aureus* 2 and *P. mirabilis*. The obtained results of antimicrobial activity of *Eucalyptus* EO are in accordance with the previously published data regarding the sensitivity of *S. aureus* and *E. coli* strains (Meyer et al., 1982; Trivediand Hotchandani, 2004, Mulyaningsih et al., 2010; Damjanović et al., 2011; Bachir and Benali, 2012). Moreover, the present study, together with the previous analysis, support the antibacterial properties of *E. globulus* L. essential oils and suggest them as potent antibacterial agents (Sartorelli et al., 2007; Noumi et al., 2011). In addition, both *Candida* strains showed sensitivity to the analyzed EO, while *C. albicans*1 strain presented higher sensitivity (Table 1). This result is in accordance with previous research data (Mulyaningsih et al., 2010; Damjanović et al., 2011). Furthermore, EO showed stronger effect against vaginal *C. albicans* strains comparing to previous obtained study where effect of EO against oral *C. albicans* strains was examined (MIC = 9.7 µL/mL) (Noumi et al., 2011).

Table 1
MIC and MBC/MFC values (µL/mL) of EO against bacterial and fungal strains.

***E. globulus* Labill.**		
Bacterial strains*		
	MIC	**MBC**
E. coli 1	12.5 [b]	12.5 [b]
E. coli 2	25 [c]	25 [c]
S. aureus 1	25 [c]	25 [c]
S. aureus 2	6.2 [a]	6.2 [a]
P. aeruginosa	ND	ND
P. mirabilis	25 [c]	25 [c]
Fungal strains*		
	MIC	**MFC**
C. albicans 1	6.2 [a]	6.2 a
C. albicans 2	12.5 [b]	12.5 [b]

*strain number, ND: not detected, **values are expressed in µL/mL; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; MFC: minimum fungicidal concentration; a,b,c-significant differences between antimicrobial activity within strain of bacteria were determined by Tukey HSD test at $p<0.01$. In each column, different letters mean significant difference.

3.2. Brine shrimp toxicity assay

Results for toxicity assay LC_{50} values were based on the relationship between mortality(%) and oil concentration after 24 h and *Eucalyptus* oil exhibited an LC_{50} of 2.66 mg/mL (Fig. 2). Although *Eucalyptus* EO is mostly known as antiseptic (Trivedi and Hotchandani, 2004), toxicological data on the systematic use of *Eucalyptus* EO are lacking. Based on the results of effectiveness of Eucalyptus EO against methicillin resistant *S. aureus* wound infection (Sherry et al., 2001), toxicity testing is necessary for further clinical studies. Thus, we used *A. salina* lethality test since it is considered as a rapid and effective method for preliminary assessment of toxicity (Sahgal et al., 2010). Good correlation between the *in vivo* tests in mice and the in vitro tests with *A. salina* were previously reported ($r = 0.85$, $p<0.05$), indicating that brine shrimp toxicity assay is the appropriate test for determining acute toxicity (Parra et al., 2001). In accordance with this study, *A. salina* method could also be used in evaluation of EO toxicity for predicting local

Table 2
MIC and MBC values of antibiotics and antimycotic (µg/mL).

Tested strains*	Antibiotics								Antimicotic	
	(µg/mL)									
	Streptomycin		Ampicillin		Tetracycline		Cefuroxime		Nystatin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
E. coli 1	ND	ND	ND	ND	ND	ND	ND	ND	NT	
E. coli 2	ND	ND	ND	ND	ND	ND	512 [b]	ND	NT	
S. aureus 1	128 [a]	256 [b]	128 [a]	128 [a]	64 [a]	128 [c]	512 [a]	ND	NT	
S. aureus 2	128 [a]	256 [b]	ND	ND	128 [b]	256 [c]	ND	ND	NT	
P. aeruginosa	ND	ND	ND	ND	ND	ND	ND	ND	NT	
P. mirabilis	128 [a]	256 [b]	128 [a]	256 [b]	128 [b]	256 [c]	512 [a]	ND	NT	
C. albicans 1	NT	NT	NT	NT	NT	NT	NT	NT	64 [a]	64 [a]
C. albicans 2	NT	NT	NT	NT	NT	NT	NT	NT	64 [a]	128 [b]

*strain number, nd-not detected, nt-not tested, MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; MFC: minimum fungicidal concentration a,b,c - in each column different letters mean significant differences ($p<0.01$), Tukey HSD test.

vaginal acute toxicity. Thus, we recommend further toxicity testing of *Eucalyptus* EO before application.

3.3. Chemical composition

In the investigated *Eucalyptus* EO, 16 components (99.79%) were identified. The major classes of compounds were oxygenated hydrocarbons (60.57%), aromatic monoterpenes (15.55%) and cyclic terpenes (15.33%), among which main constituents were eucalyptol (1,8-cineole), *p-cymene* and DL-Limonene (Table 3). The GC/MS profile of EO exhibits that the hydrocarbon monoterpenes and oxygenated hydrocarbons were the main compounds in investigated EO. Dominant compounds were eucalyptol (1,8-cineole), *p-cymene* and DL-limonene, which is in accordance with other studies

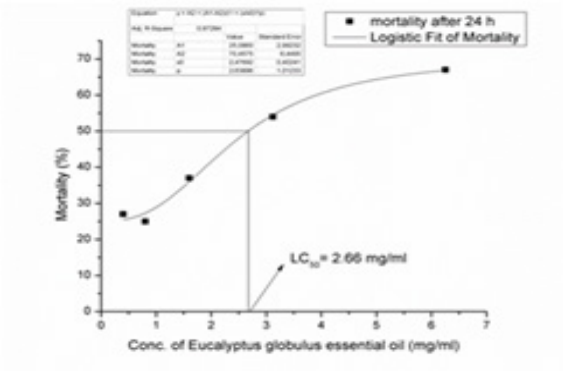

Fig. 2. Relationship between mortality (%) and *E. globulusL.* EO concentration after 24 h.

(Silva et al., 2003; Mulyaningsih et al.,2010). According to the previous studies (Knobloch et al., 1988; Griffin et al., 1989), hydrocarbons tend to be relatively inactive, related to their limited hydrogen binding capacity and water solubility, thus hydrocarbon monoterpenes were characterized like weak antimicrobial agents. In contrast, oxygenated hydrocarbons like terpinen-4-ol, 1,8 cineole (eucalyptol) and linalool have been reported to significantly contribute to antibacterial activity due to their adverse effect on the structural integrity of the bacterial cell wall (Hinou et al., 1989; Cristoph et al., 2000). Notwithstanding, eucalyptol, one of the major constituent of Eucalyptus EO, as individual component tested to Gram-positive and Gram-negative bacteria, exhibited the lower antimicrobial activity in comparison to EO (Raman et al., 1995; Tzakou et al., 2001; Mulyaningsih et al., 2010). It is most likely that synergistic effects of minor components play an important role in antimicrobial activities (Burt, 2004).

4. Concluding remarks

Table 3
Chemical composition of EO from E. *globulus* L.

Peak No.	Compound	R.I.[a]	*E. globulus* EO (%)
	Monoterpene hydrocarbons		8.34
1.	Thuja-2,4(10)-diene	8.40	5.19
2.	β-Myrcene	8.97	1.23
3.	β-Pinene	9.03	1.12
4.	*cis*-Ocimene	9.46	0.17
5.	γ-Terpinene	10.24	0.63
6.	Aromatic monoterpenes		15.55
7.	*p*-Cymene	9.70	15.55
8.	Cyclic terpenes		15.33
9.	α-Phellandrene	9.41	0.24
10.	DL-Limonene	9.78	14.90
11.	*trans*-Pinocarveol	12.00	0.19
12.	Oxygenated hydrocarbons		60.57
13.	Eucalyptol	9.93	59.63
14.	β-Linalool	10.84	0.26
15	*p*-Menth-1-en-4-ol	12.68	0.27
16.	*p*-Menth-1-en-8-ol	12.91	0.41

[a]Retention indices relative to C_9-C_{24} *n*-alkanes on the HP 5MS column.

Isolated strains of vaginal microbial pathogens have shown strong strain specificity to the tested EO. *Eucalyptus* EO showed activity against Gram negative strains *E. coli* 1 and *E. coli* 2 which indicates *Eucalyptus* EO as a promising alternative for the treatment of vaginal infections caused by *E. coli*. Therefore, we could recommend *Eucalyptus* EO for further testing as potential antiseptic agent with local application in humans after detailed toxicity evaluation. Since the obtained results pointed out the resistance of clinical isolates of Gram-negative bacteria to conventional antibiotics, investigation of *Eucalyptus* EO as the possible alternative agent, in the treatment of vaginal infections, should draw more attention in the future.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

Bachir, R. G., Benali, M., 2012. Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. Asian Pac. J. Trop. Biomed. 2(9), 739-742.

Bogavac, M., Lakic, N., Simin, N., Nikolic, A., Sudji, J., Bozin, B., 2012. Bacterial vaginosis and biomarkers of oxidative stress in amniotic fluid. J. Matern.-Fetal Neonatal Med. 25(7), 1050-1054.

Bortz, J., Levinson, R. S., Kirschner, M., Cuca, R.C., 2013. U.S. Patent Application No. 13/557,527.

Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. Int. J. Food Microbiol. 94(3), 223-253.

Carson, C. F., Hammer, K.A., 2011. Chemistry and Bioactivity of Essential Oils. Lipids and Essential Oils as Antimicrobial Agents, pp. 203-238.

Christoph, F., Kaulfers, P.M., Stahl-Biskup, E., 2000. A comparative study of the *in vitro* antimicrobial activity of tea tree oils sp. with special reference to the activity of β-triketones. Planta Med. 66(06), 556-560.

Clinical Laboratory Standards Institute, 2008. M02-A11-Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard, 11th ED. Wayne PA, USA: Clinical and Laboratory Standard Institute. CLSI Document.; M07-A9-Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard, 9th Ed. Wayne, PA, USA: Clinical and Laboratory Standard Institute. CLSI Document.

Damjanović-Vratnica, B., Đakov, T., Šuković, D., Damjanović, J., 2011. Antimicrobial effect of essential oil isolated from *Eucalyptus globulus* Labill. from Montenegro. Czech J. Food Sci. 29(3), 277-284.

Frezza, C., Venditti, A., Serafini, I., Carassiti, A., Foddai, S., Bianco, A., Serafini, M., 2017. Phytochemical characteristics of *Galeopsisladanum* subsp. *angustifolia*(Ehrh. ex Hoffm.) Gaudin collected in Abruzzo region (Central Italy) with chemotaxonomic and ethnopharmacological implications. Trends Phytochem. Res. 1(2), 61-68.

Griffin, S. G., Wyllie, S. G., Markham, J. L., Leach, D. N., 1999. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. Flav. Frag. J. 14(5), 322-332.

Hinou, J.B., Harvala, C.E., Hinou, E.B., 1989. Antimicrobial activity screening of 32 common constituents of essential oils. Pharmazie 44(4), 302.

Jarić, S., Popović, Z., Mačukanović-Jocić, M., Djurdjević, L., Mijatović, M., Karadžić, B., Pavlović, P. 2007. An ethnobotanical study on the usage of wild medicinal herbs from Kopaonik Mountain (Central Serbia). J. Ethnopharmacol. 111(1), 160-175.

Knobloch, K., Pauli, A., Iberl, B., Weis, N., Weigand, H., 1988. Mode of action of essential oil components on whole cells of bacteria and fungi in plate tests. In Bioflavour 87, 287-299.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. J., McLaughlin, J. L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45(05), 31-34.

Mohammadhosseini, M., Sarker, S.D., Akbarzadeh, A., 2017. Chemical composition of the essential oils and extracts of *Achillea* species and their biological activities: A review. J. Ethnopharmacol. 199, 257-315.

Mohammadhosseini, M., Venditti, A., Sarker, S.D., Nahar, L., Akbarzadeh, A., 2019. The genus *Ferula:* Ethnobotany, phytochemistry and bioactivities – A review. Ind. Crop Prod. 129, 350-394.

Mordi, N., Ismail, S., Mansor, S.M., 2010. Brine shrimp lethality and acute oral toxicity studies on *Swietenia mahagoni* (Linn.) Jacq. seed methanolic extract. Pharmacognosy Res. 2(4), 215.

Mulyaningsih, S., Sporer, F., Zimmermann, S., Reichling, J., Wink, M., 2010. Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. Phytomedicine 17(13), 1061-1066.

Naeini, A., Khosravi, A.R., Chitsaz, M., Shokri, H., Kamlnejad, M., 2009. Anti-*Candida albicans* activity of some Iranian plants used in traditional medicine. J. Mycol. Méd. 19(3), 168-172.

Noumi, E., Snoussi, M., Hajlaoui, H., Trabelsi, N., Ksouri, R., Valentin, E., Bakhrouf, A., 2011. Chemical composition, antioxidant and antifungal potential of *Melaleuca alternifolia* (tea tree) and *Eucalyptus globulus* essential oils against oral *Candida species*. J. Med. Plants Res. 5(17), 4147-4156.

Parra, A.L., Yhebra, R.S., Sardiñas, I.G., Buela, L.I., 2001. Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD_{50} value) in mice, to determine oral acute toxicity of plant extracts. Phytomedicine 8(5), 395-400.

Pierce, A.M.,Hart, CA., 1992. Vulvovaginitis: causes

and management. Arch. Dis. Child. 67(4), 509-512.

Raman, A., Weir, U., & Bloomfield, S. F., 1995. Antimicrobial effects of tea-tree oil and its major components on *Staphylococcus aureus*, *Staph. epidermidis* and *Propionibacterium acnes*. Lett. Appl. Microbiol. 21(4), 242-245.

Sahgal, G., Ramanathan, S., Sreenivasan Sasidharan, M., Mordi, N., Ismail, S., Mansor, S.M., 2010. Brine shrimp lethalityand acute oral toxicity studies on *Swietenia mahagoni* (Linn.) Jacq. seed methanolic extract. Phcog. Res. 2(4), 215.

Salari, M. H., Amine, G., Shirazi, M. H., Hafezi, R., Mohammadypour, M., 2006. Antibacterial effects of *Eucalyptus globulus* leaf extract on pathogenic bacteria isolated from specimens of patients with respiratory tract disorders. Clin. Microbiol. Infect. 12(2), 194-196.

Sarker, S.D., Nahar, L., 2018a. Evidence-based phytotherapy: what, why and how? Trends Phytochem. Res. 2(3), 125-126.

Sarker, S.D., Nahar, L., 2018b. Phytochemicals and phyto-extracts in cosmetics. Trends Phytochem. Res. 2(4), 185-186.

Sartorelli, P., Marquioreto, A.D., Amaral-Baroli, A., Lima, M.E.L., Moreno, P.R.H., 2007. Chemical composition and antimicrobial activity of the essential oils from two species of *Eucalyptus*. Phytother. Res. 21(3), 231-233.

Sherry, E., Boeck, H., Warnke, P.H., 2001. Topical application of a new formulation of *Eucalyptus* oil phytochemical clears methicillin-resistant *Staphylococcus aureus* infection. Am. J. Infect. Control. 29(5), 346.

Silva, J., Abebe, W., Sousa, S.M., Duarte, V.G., Machado, M.I.L., Matos, F.J.A., 2003. Analgesic and anti-inflammatory effects of essential oils of *Eucalyptus*. J. Ethnopharmacol. 89(2-3), 277-283.

Trivedi, N.A., Hotchandani, S.C., 2004. A study of the antimicrobial activity of oil of *Eucalyptus*. Indian J. Pharmacol. 36(2), 93.

Tzakou, O., Pitarokili, D., Chinou, I.B., Harvala, C., 2001. Composition and antimicrobial activity of the essential oil of *Salvia ringens*. Planta Med. 67(01), 81-83.

Vermani, K., Garg, S., 2002. Herbal medicines for sexually transmitted diseases and AIDS. J. Ethnopharmacol. 80(1), 49-66.

Wansi, J.D., Sewald, N., Nahar, L., Martin, C., Sarker, S.D., 2018. Bioactive essential oils from the Cameroonian rain forest: A review - Part I. Trends Phytochem. Res. 2(4), 187-234.

Wansi, J.D., Sewald, N., Nahar, L., Martin, C., Sarker, S.D., 2019. Bioactive essential oils from the Cameroonian rain forest: A review - Part II. Trends Phytochem. Res. 3(1), 3-52.

Warnke, P.H., Becker, S.T., Podschun, R., Sivananthan, S., Springer, I.N., Russo, P.A., Sherry, E., 2009. The battle against multi-resistant strains: renaissance of antimicrobial essential oils as a promising force to fight hospital-acquired infections. J. Craniomaxillofac. Surg. 37(7), 392-397.

Zlatković, B. K., Bogosavljević, S. S., Radivojević, A. R., Pavlović, M. A. 2014. Traditional use of the native medicinal plant resource of Mt. Rtanj (Eastern Serbia): Ethnobotanical evaluation and comparison. J. Ethnopharmacol. 151(1), 704-713.