TOXICITY OF ESSENTIAL OILS - A BRIEF OVERVIEW OF BIOASSAYS

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Since the ancient times, people have used essential oils as a cure because they have noticed their beneficial effects on the human mind and body. What they did not know was how these essential oils actually affected the human body, as well as exactly what component or more of them were responsible for the activity of a particular oil. Therefore, a lot of attention has recently been paid to the detailed identification of the constituents of essential oil and determination of the biological activity of the essential oil itself, as well as of those identified constituents. The aim of this paper is to systematize the most used, most accessible and easily feasible techniques for determining the biological activity of essential oils. For this purpose, the following tests are mentioned in this paper: fumigation, insecticidal, pediculi-cidal, nematocidal, larvicidal, ovicidal, cytotoxic and antinociception bioassay.

Keywords: Essential oil, Aromatherapy, Biological activity, Toxicity bioassays

Introduction

Essential oils (EOs) are specific, most common liquid products of the plant tissue. These are natural, multi-component mixtures formed mainly of terpenes in addition to some other non-terpene components, such as phenylpropanoids [1]. The essential oils are mainly the products of higher plants. These plants are distributed in over fifty families and the most used are aromatic plants of the family Asteraceae, Lamiaceae, Apiaceae, Rutaceae, Myrtaceae and Lauraceae [2]. These compounds from EOs are characterized by a strong odor and they are formed by aromatic plants as secondary metabolites. They can be synthesized by all plant organs (flowers, leaves, stems, seeds, fruits, roots, wood) and can be stored in secretory cells, cavities, canals, epidermal cells or glandular trichomes [3]. The essential oils localized in different parts of the same plant can be of a similar composition but can also be significantly different. Many factors such as locality of the plants, climate factors, genetic variation, plant variety, plant nutrition and fertilizers, stress during growth and others, determine the yield and the composition of essential oils [4,5].

Aromatherapy was once considered to be an auxiliary medical treatment, but it became widely accepted and appreciated and is being offered to a greater extent to hospital patients as part of their therapy [6]. The term "aromatherapy" was invented by the French chemist Gattefossé to emphasize the significant antibacterial activity of the essential oils and their good permeability to the skin during the usage [7]. This therapy is a physical way of curing a person's body, mind and soul [8]. The manufacturers of medicinal products, cosmetics and perfumes increasingly recognize the value of the essential oils in improving the quality and attractiveness of their products. At the same time, home usage of essential

oils is on the rise because people discover the therapeutic benefits and unique aesthetic pleasure in essential oils. In order to confirm and explain the potential of essential oils for healing, current scientific research on the chemical and medical uses of certain essential oils must be taken into consideration.

Essential oils are aromatic, volatile liquids obtained from plant material through steam distillation or hydrodistillation. Essential oils can be defined as products or mixtures of fragrant substances. These fragrant substances are pure chemical compounds that are volatile under normal conditions.

Four methods of obtaining essential oils are common:

1. Hydrodistillation
2. Cold pressing
3. Extraction with organic solvents
4. Supercritical fluid extraction

Essential oils are mainly obtained by hydrodistillation (the plant material is heated in two to three times its weight of water and essential oil is collected along with steam, then separate post condensation). A technique for the separation of essential oils using microwave radiation was developed in order to obtain a good essential oil yield and reduce the extraction time [9-11].

Chemical analysis of essential oils is commonly performed using gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). Compounds of essential oils that would not separate easily by GC and the molecules structurally similar (like stereoisomers) are analyzed by ^{13}C NMR [12].

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Biological effects and therapeutic properties of essential oils

Essential oils and fragrance compounds have been well-known for their therapeutic properties since the ancient times [13]. They have been widely used for fungicidal, insecticidal, virucidal, bactericidal, antiparasitical, medicinal and cosmetic applications since the Middle Ages. Today, essential oils are especially used in cosmetic, pharmaceutical, sanitary, food and agricultural industries [3,14,15]. In addition to the great variety, it is possible to extract several activities that are characteristic for numerous essential oils, such as antimicrobial activity, and those essential oils that possess antimicrobial properties are used as disinfectants and antiseptics [16,17]. Monoterpenes from EOs are effective in treating early and advanced cancers [18]. In pharmacology, essential oils are used as antispasmodics [12], antiphlogistics [15], for strengthening the organism and improving appetite [19], for stomach problems [20], for burns, wounds and skin inflammation [21] and against jaundice [22]. Aromatic substances are often used to treat coughs and unspecific irritations of the respiratory tract [23].

Bearing in mind all the pharmacological effects of essential oils so far, it is clear why they are increasingly applied in everyday life. It is important to know what effects essential oil could have on different types of organisms, as well as how it is all related to human health. This paper is primarily aimed at summarizing the bioactivity methods of toxicity of essential oils and investigating essential oils from different aromatic plants as potential biological and pharmacological resources.

Fumigant toxicity

Fumigants played a major role in pest insect elimination from stored products and the use of fumigants was the most economical tool for managing stored-grain insect pests. Fumigants should be biologically active, nonflammable, non-corrosive, sufficiently volatile to be removed by aeration and not absorbed by grain [24]. Methyl bromide and phosphine are the two common worldwide fumigants used for stored-product protection. However, they have their own deficiencies, and it is important to test new products that could be used for these purposes. The use of essential oils as fumigants is especially emphasized since the 1980s. It has been proven that essential oils of many plants can be used as fumigants, particularly plant species belonging to the families Anacardiaceae, Apiaceae (Umbelliferae), Araceae, Asteraceae (Compositae), Brassicaceae (Cruciferae), Chemopodiaceae, Cupressaceae, Gramineae, Lamiaceae (Labiatae), Lauraceae, Liliaceae, Myrtaceae, Pinaceae, Rutaceae and Zingiberaceae [25]. The active compounds of essential oils showing insect toxicity were monoterpenoids, alkaloids, cyanohydrins and cyanates, sulphur compounds and other like salicylates and benzene derivatives. Most of the active components were secondary metabolites located in plants as a chemical defense against different pest organisms.

Fumigation bioassay described by Lee et al. [26]: Fumigation test was performed with 50 adults of stored-product insects (some of them are shown in Table 1.) in 250 ml conical containers sealed with glass stopper containing a filter

paper as a septum to deposit chemicals. Each flask had its volume measured by weighing the amount of water it could contain. Essential oil (10, 25, 50 and 100 μ l) was injected into the conical flasks using a gas syringe. Flasks used as control do not contain oil. During exposures, cultures were kept at 25 ± 0.5 °C. In all measurements minimum four different concentrations of EO were used. The concentrations of atmospheric/respiratory gases in each flask were also measured and were found to be $1.06\pm0.02\%$ CO₂ and $22.25\pm0.52\%$ O₂. CO₂ and O₂ were measured using a Fisher Gas Partition. After 24 h insects were moved into clean vials and mortality was determined. The LD₅₀ and LD₉₅ values were calculated by Probit analysis [27]. Control mortality was accounted by Abbott's formula [28].

Table 1. Fumigant toxicity of compounds of essential oils against stored-product insects.

Compound of essential oil	Stored-product insect	Reference
1,8-Cineole	<i>Rhyzopertha dominica</i>	[29]
	<i>Sitophilus oryzae</i>	[24]
	<i>Oryzaephilus surinamensis</i>	[29]
	<i>Tribolium castaneum</i>	[30]
p-Cymene	<i>Tribolium castaneum</i>	[26]
Menthone	<i>Tribolium castaneum</i>	[26]
α -Pinene	<i>Acanthoscelides obtectus</i>	[31]
Benzaldehyde	<i>Sitophilus oryzae</i>	[24]
Linalool	<i>Oryzaephilus surinamensis</i>	[29]
Carvacrol	<i>Oryzaephilus surinamensis</i>	[29]
Terpinen 4-ol	<i>Rhyzopertha dominica</i>	[29]

Based on the results from Table 1., it could be concluded that different components of the essential oils may show fumigant toxicity against different stored-product insects.

Insecticidal activity

Essential oils from plants can be an alternative source of insect control agents, since they represent a rich source of bioactive components that are biodegradable into nontoxic products and potentially convenient for use for various purposes [32]. EOs can be used as insecticide and insect reproduction retardant [33]. Many terpenoids from essential oils are found to possess repellent, attractant, ovipositional stimulant activities, feeding deteriorate and alighting behavior against various insect species [34]. These plant derivatives are medically safe for use, which is very important for human health. For this reason, essential oils and their constituents are increasingly being tested as a potential sources of insect control agents. Lamiaceae family is well-known for its insecticidal activity, especially genera *Micromeria*, *Mentha*, *Monarda*, *Ocimum*, *Origanum*, *Pycnanthemum*, *Satureja*, *Teucrium* and *Thymus* [35]. Myrtaceae family is also showing enormous potential as a natural insecticide, with main genera: *Eucalyptus*, *Eugenia*, *Leptospermum*, *Melaleuca*, *Myrtus*, *Pimenta*, *Psidium* and *Syzygium* [32].

Insecticidal bioassay against housefly, *Musca domestica* L. by Singh and Singh [33]: In this paper two methods for insecticidal toxicity were described:

Repellent property described by Singh et al. [36]: The housefly adults anaesthetized with diethyl ether were placed in dishes (1.5 cm) and directly kept under potter's precision laboratory spray tower. After spraying 1 ml of stock solution of each essential oil at 700 g/cm² air pressure, the dishes were closed with their lids. Acetone was used as the negative control. Malathion and pyrethrum were used as the positive control. The knockdown (KD) effect was noted after 2 h and 5 h of test application. The observed mean KD per cent was transformed into corrected KD per cent [28].

Insecticidal property (direct toxicity) described by Singh and Jain [37]: The housefly adults were anaesthetized by diethyl ether and each test animal was treated by applying 1 µl per insect stock solution of each essential oil on their thoracic part by Arnold Hand microapplicator. The treated flies were put in clean and dry beakers with the help of cheese cloth and rubber band. The soaked cotton swabs in the sugar solution of 20% were provided as food for each treated lot of the flies. Acetone was used as the negative control, while malathion and pyrethrum were used as the positive control. The observation on KD was recorded after 2 h and mortality after 5 h and 24 h of the test application. The observed data were subjected to corrected per cent KD/mortality [28] and the results of some samples calculated in this way were shown in Table 2.

Table 2. Essential oils tested for repellent and insecticidal properties against houseflies (*Musca domestica* L.).

Sample	Corrected % repellency		Corrected % mortality		
	After 2 h	After 5 h	After 2 h	After 5 h	After 24 h
Acetone	/	/	/	/	/
Malathion	100.00	100.00	100.00	100.00	100.00
Pyrethrum	100.00	100.00	100.00	100.00	100.00
<i>Thymus serpyllum</i> L.	100.00	100.00	16.28	16.28	40.47
<i>Illicium verum</i> Hook f.	100.00	100.00	2.22	4.44	2.33
<i>Myristica fragrans</i> Houtt.	100.00	100.00	/	/	/
<i>Ocimum gratissimum</i> L.	93.33	100.00	6.67	8.89	13.96
<i>Acorus calamus</i> L.	80.95	82.92	60.46	44.19	38.09

Table 2. was shown that, unlike commercially used malathion and pyrethrum which have 100% of both repellency and mortality, these essential oils have high repellency (approximately 100%) while their mortality is significantly lower.

Pediculicidal activity

Pediculus humanus capitis De Geer (the human head louse) is an ectoparasite, constrained to the scalp and hair of humans. It is represented worldwide, especially common among schoolchildren in both developed and developing countries [38]. *Pediculus humanus capitis* infections cause skin irritation, sleep loss and pruritus, as well as periodical secondary bacterial infection from scraping. Although the symptoms are relatively light, infestation by *P. humanus capitis* has resulted in various mental, social and economic problems [39]. As essential oils are an alternative source of materials for insect control, much effort has been focused on their potential use

as commercial head lice control agents [40].

Pediculicidal bioassay described by Yang et al. [40]: A filter paper contact bioassay was used to evaluate the toxicity of the essential oils and insecticides to female *P. humanus capitis*. In a preliminary experiment with essential oils, as well as δ -phenothrin and pyrethrum, 0.25 mg/cm² was a suitable starting dose for a primary screening. If an essential oil gave similar or better activity than δ -phenothrin or pyrethrum, further bioassays were implemented.

Essential oils (0.0625, 0.1250, and 0.2500 mg/cm²) were applied to filter papers in 80 µl of acetone. After drying in a fume hood for 2 minutes, filter paper was placed on the bottom of a petri dish. The amount of 20 *P. humanus capitis* females (7-9 days old), given a human blood meal 4 h before the bioassay, were placed on each petri dish, containing a few strands of human hair and the dish covered with a lid (method A). In a separate experiment, vapor phase toxicity of the test essential oils against female *P. humanus capitis* was investigated according to the method of Yang et al. [41]. The difference between the previously described (method A) and this test (method B) is only in that the petri dish is not covered in this case. Control filter papers received 80 µl of acetone. Treated and control (solvent only) females were held at 31±1 °C and 65±5% RH in darkness. Mortalities were determined every 5 minutes for 5 h. Females were considered dead if they exhibited lethargic response or no movement and dead individuals never recovered. δ -Phenothrin and pyrethrum were used as standards for comparison in toxicity tests. The LT₅₀ values (median lethal time values) were calculated by probit analysis [42].

Table 3. Pediculicidal activity of four selected essential oils and standards against female *P. humanus capitis* at 0.25 mg/cm².

Sample	LT ₅₀ , min	
	Method A	Method B
δ -Phenothrin	>300.0	>300.0
Pyrethrum	>300.0	>300.0
Eucalyptus oil	6.2	>300.0
Rosemary oil	12.8	>300.0
Marjoram oil	12.6	>300.0
Pennyroyal oil	7.5	>300.0

Eucalyptus, marjoram, pennyroyal and rosemary oils were highly effective and more toxic than either δ -phenothrin or pyrethrum. Also, median lethal time values (LT₅₀) were significantly higher when method B was used (not covered petri dish) for all essential oils (Table 3.).

Nematicidal activity

Plant parasitic nematodes are the most harmful group of plant pathogens worldwide and they can be ectoparasites or endoparasites. *Meloidogyne* spp. (the root-knot nematodes) are one of the most economically damaging genera of plantparasitic nematodes on horticultural and field crops. These nematodes move through the plant, causing an extensive damage as they move and feed. As previously said, essential oils are widely used as fungicides and insecticides. However, only a small number

of essential oils are used as nematicides, especially EOs of the aromatic plants of the genera *Artemisia*, *Cymbopogon*, *Lavandula*, *Mentha*, *Oreganum*, *Ocimum*, *Rosmarinus*, *Thymus* and aromatic trees of the genera *Citrus*, *Eucalyptus*, *Eugenia* and *Melaleuca* which have been traditionally used for protection of stored goods [43].

Nematicidal bioassay described by Pandey et al. [44]: Bioassay studies were made by collecting the egg masses of root-knot nematode *Meloidogyne incognita* Chitwood from the stock culture maintained on tomato in a glasshouse. These egg masses were kept on wire gauze supported with tissue papers in a funnel containing water at 28 °C.

Stock solutions of the tested essential oils (concentrations 2000, 1000, 500 and 250 ppm) were prepared by dissolving 10 mg of test essential oils in 0.5 ml dimethylsulphoxide (DMSO) and filled to 5 ml with 0.5% Tween-80 and distilled water. These solutions were further diluted to obtain required concentrations. Nematode larval suspension (1 ml) containing approximately 200 nematodes was poured into the test vials with 1 ml tested solutions (concentrations of 1000, 500, 250 and 125 ppm) and vials were kept at 27 °C in an incubator. After 24 h, the immobilized nematodes were transported into tap water and kept for another 24 h to check the mobility. Negative control was 0.5 ml DMSO, 0.5% Tween-80 and distilled water mixture. There were five replications for each treatment. The percentage mortality of the second stage juveniles was recorded by counting them under a microscope.

Table 4. Nematicidal activity of different essential oils against *Meloidogyne incognita*.

Sample	Mortality % after 24 h at different concentrations (ppm)			
	125	250	500	1000
<i>Ocimum basilicum</i>	70	100	100	100
<i>Eucalyptus citriodora</i>	25	100	100	100
<i>Eucalyptus hybrida</i>	22	66	100	100
<i>Cymbopogon martinii</i>	15	32	40	100
<i>Mentha arvensis</i>	3	20	40	68
<i>Pelargonium graveolens</i>	5	7	35	60
<i>Mentha spicata</i>	4	23	30	50
<i>Mentha piperita</i>	6	15	25	35

Based on the presented results from Table 4., it can be concluded that the mortality of the second stage juveniles depends on the solution concentration of the tested essential oils.

Larvicidal activity

Due to their composition, essential oils could be used as suitable active substances for new botanical larvicides and thereby enable the provision of the expected replacement for some of the risky synthetic substances developing in the last few decades [45]. These botanical pesticides are often effective, ecological, easily biodegradable and inexpensive [46]. Mosquitoes are important source of many human and veterinary parasites and pathogens. Vector diseases (malaria, filariasis, leishmaniasis, dengue and Chagas disease) cause extensive

morbidity and mortality and represent major problem in the countries of endemic disease [47]. The ideal method for controlling mosquito infestation would be the prevention of mosquito breeding by using larvicides. Many plants produce secondary metabolites that inhibit the growth of mosquitos by affecting mortality of their larvae [48].

Larvicidal bioassay described by Dharmagadda et al. [46]: Fourth instar larvae (the fourth larval stage in the life cycle of mosquito, after which the larva finally becomes a pupa) were exposed to sublethal concentrations (6.25, 12.50, 25.00, 50.00, 100.00 and 150.00 ppm) of the essential oils in distilled water for 24 h according to standard World Health Organization (WHO) procedure [49]. As the essential oil does not dissolve in water, ethanol was used for dissolving. The tested sample was prepared by adding 1 ml of the suitable solution of essential oil in ethanol and mixed with a certain amount of water to make up 250 ml of the test solution. The prepared solution was further stirred for 30 seconds with a glass rod. After 15 minutes, 25 larvae taken on a strainer with fine mesh were transferred to the test medium by tapping. Two replicates, with 25 larvae in each, were taken for each mosquito species in each solution and the control of ethanol–water mixture. Commercial malathion was used as the positive control. The larvae were fed with dry yeast powder by sprinkling on the water surface. The dead larvae were counted after every hour and the mortality percentage is reported from the average for the two replicates taken together. The deviation between the replicates is $\pm 10\%$. Observations were also made on the larval behavior, morphological changes and adult emergence. Probit analysis was guided on the mortality data collected after 24 h exposure to different concentrations of oil using statistical package Minitab [50] to establish the lethal concentration for 50% and 90% of mortality (LC_{50} and LC_{90}).

Table 5. Larvicidal effect of *T. patula* essential oil against larvae of *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* after 24 h.

Mosquito species	LC_{50}	LC_{90}
<i>Aedes aegypti</i>	13.57	37.91
<i>Culex quinquefasciatus</i>	22.33	71.89
<i>Anopheles stephensi</i>	12.08	57.62

This study shows that *Tagetes patula* oil has huge larvicidal potential and compared suitably with commercial malathion used as a control (Table 5.).

Ovicidal activity

The essential oils and their monoterpenoids are especially important as insecticides that can be used to destroy pests even before their development, because they exhibit ovicidal activity. This particularly reduces their adverse effects on living organisms and can be useful in protection of stored products. Some of the EOs and their constituents are widely used as flavoring agents in foods and beverages. Natural products are an excellent

alternative to synthetics, as the means to reduce negative impacts on human health, and furthermore they are environmentally friendly [51].

Ovicidal bioassay described by Rice and Coats [52]: *Musca domestica* eggs were treated with monoterpenoids to determine the ovicidal properties of each compound. Each monoterpenoid (15 mg) was diluted in 2 ml of acetone to make a concentrated solution and 0.6 ml of that solution was added to 5.4 ml of distilled water. Each tested solution (1.5 ml) was dispensed into a treatment container containing about 50 eggs (not older than 12 h). The containers were gently shaken, sealed with paraffin film and placed in an incubator (25 ± 2 °C with a photoperiod of 12:12 [L:D] h) for the duration of the test. Corn oil was used as the negative control, and pyrethrin was a positive standard for the comparison. Each test was replicated at least four times and completed when the control eggs had hatched (minimum 4 days). The number of hatched larvae and the total number of eggs were counted. The percentage of inhibition of hatch was determined using the formula by Sharma and Saxena [53].

Table 6. Ovicidal activity of monoterpenoids from essential oils against *Musca domestica*.

Monoterpenoids	% inhibition of egg hatch
Menthone	100
Carvacrol	99
Geraniol	99
Terpineol	99
Cinnamaldehyde	97
Citral	96
Perillaldehyde	89
Pyrethrin	100

This study shows which monoterpenoids in general had huge ovicidal potential compared suitably with commercial pyrethrin used as a negative control (Table 6.).

Antinociceptive activity

Nociception is the response of sensory nervous system to some harmful or potentially harmful stimulus. Nociceptors are potentially damaging mechanical, thermal and chemical stimuli. Nowadays, researchers are working on finding natural substances that will prevent this effect. One of these natural sources are essential oils and their components [54,55].

Acetic acid-induced writhing test described by Koster [56]: The number of writhes per mouse was counted during a period of 10 minutes, starting 10 minutes after the intraperitoneal (i.p.) injection of 0.6% acetic acid (10 ml/kg). Carrier (2% Tween 80 in saline, 10 ml/kg), essential oil (100, 200 and 400 mg/kg) or its major components (400 mg/kg) were given orally 45 minutes before the acetic acid injection.

Formalin test described by Hunskaar and Hole [57]: Mice were injected 20 ml of 1% formalin (in 0.9% saline) into the sub plantar space of the right hind paw, and the duration of paw licking was determined 0–5 minutes (first phase) and 20–25 minutes (second phase) after formalin. The essential oil suspended in 2% Tween 80

was administered orally (100, 200 and 400 mg/kg) 45 minutes before the formalin injection at the dose of 10 ml/kg. Control animals received an equal volume of the carrier (2% Tween 80 in saline). To investigate the possible mechanisms involved in the antinociceptive effect of the essential oil, animals received caffeine (20 mg/kg, intraperitoneal) or naloxone (1 mg/kg, sub cutaneous) 15 minutes before the oral administration of the essential oil (400 mg/kg). The effect of major components of the plant essential oil were also tested (400 mg/kg) to verify whether these constituents were responsible for the antinociceptive activity of the essential oil.

Hot-plate test described by Eddy and Leimbach [58]: A hot-plate test is the test for efficacy of the plant essential oil against thermal nociception. Mice were preselected by placing them individually on the hot plate maintained at 51 ± 0.5 °C, and animals that showed a reaction time (latency for jumping or licking of the hind paw) greater than 20 seconds were discarded. The reaction time was measured before and 30, 60 and 90 minutes after oral administration of the essential oil (100, 200 and 400 mg/kg), its major components (400 mg/kg) or carrier (2% Tween 80 in saline). A cut-off time of 45 seconds was used to prevent damage to the limbs.

Cytotoxic activity

Anti-tumor activity and possible application of medicinal herbs for the prevention of cancer is of particular importance to human existence. The essential oils and their components have been honored for their pharmaceutical properties. One of these properties is also cytotoxic, which is in the development phase.

Cytotoxicity assay described by Chou and Talalay [59]: The cytotoxic effect of carvacrol was assessed in human cervical cancer HeLa and SiHa cells by the MTT assay (colorimetric assay for measuring cell metabolic activity). Cells were seeded at the number of 2×10^4 per well onto 96-well plates (200 µl/well), allowed to attach and grow for 24 h, and subsequently exposed to different concentrations of carvacrol (25–500 mg/l) for 48 h. At the end of the treatment, the medium was removed, and the cells were incubated with 20 µl of MTT (5 mg/ml in PBS) in fresh medium for 4 h at 37 °C. After 4 h, formazan crystals (formed by mitochondrial reduction of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide, MTT) were solubilized in DMSO (150 µl/well) and the absorbance was read at 570 nm after incubation of 10 minutes on the iMark Microplate Reader. Percent inhibition of cytotoxicity was calculated as a fraction of control (without carvacrol) and the cytotoxicity of carvacrol was expressed as the half maximal inhibitory concentration, IC_{50} . Quercetin was used as positive control.

Carvacrol is a potential anti-cancer component with an IC_{50} of 50 mg/l at 48 h inducing the growth inhibition in both (HeLa and SiHa) human cervical cancer cells [60].

Conclusion

The present review outlines different bioassays and methods which describe significant and valuable properties of essential oils. Some of the most important activities listed here are antinociceptive, cytotoxic, fumigant, insecticidal, pediculicidal, ovicidal, larvicidal and nematocidal. It is also well-known that essential oils possess antimicrobial and antioxidant activities. Because essential oils have been used since the ancient times, there are a lot of literature data about their uses, as well as their components that are responsible for all beneficial properties. Nowadays EOs are increasingly being tested as potential biologically active agents because they are much better to use than other commercial products. Some of the reasons are that they are non-toxic for human health, environmentally friendly, easily biodegradable, not too expensive and have many other favorable properties.

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Izvod**TOKSIČNOST ESENCIJALNIH ULJA - KRATAK PREGLED BIOESEJA**

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Od davnina su ljudi koristili etarska ulja kao lek, jer su primetili njihovo blagotvorno delovanje na ljudski um i telo. Ono što nisu znali bilo je to kako zapravo ta etarska ulja deluju na ljudski organizam, kao i to koja je tačno komponenta ili više njih odgovorna za aktivnost određenog ulja. Zato se u skorije vreme dosta pažnje posvećuje što detaljnijoj identifikaciji konstituenata nekog etarskog ulja i određivanju biološke aktivnosti samog ulja, kao i aktivnosti tih identifikovanih konstituenata. Cilj ovog rada je da se sistematizuju najčešće korišćene, najdostupnije i u lako izvodljive tehnike za određivanje biološke aktivnosti etarskih ulja. U tu svrhu su u ovom radu pomenuti sledeći testovi: fumigantni, insekticidni, pedikulicidni, nematocidni, larvicidni, ovicidni, citotoksični i antinociceptivni biološki test.

KLjučne reči: Etarsko ulje, Aromaterapija, Biološka aktivnost, Biološki testovi toksičnosti