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Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for *Striga* suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

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Review

## Medicinal importance of genus *Origanum*: A review

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**Genus *Origanum* is important medicinally as it has antimicrobial, antifungal, antioxidant, antibacterial, antithrombin, antimutagenic, angiogenic, antiparasitic and antihyperglycaemic activities. Phytochemical investigations of the species of this genus have resulted in the extraction of a number of important bioactive compounds. This emphasizes on the need of extensive study for reporting the additional information on the medicinal importance of other unattended species of genus *Origanum*.**

**Key words:** Lamiaceae, secondary metabolites, *Origanum*, carvacrol.

### INTRODUCTION

Primary and secondary metabolites produced by plants, encompass a wide array of functions. Secondary metabolites have been subsequently exploited by humans for their beneficial role. At the same time, essential oils and their components are also being exploited for potential multipurpose functional use (Sawamura, 2000; Ormancy et al., 2001; Gianni et al., 2005). Thus, medicinal and aromatic plants are valued for their biological activities which can be justified from the fact that about 80% of the local population still depend on these plants for primary health care. The formation and accumulation of essential oil in plants has been reviewed by many workers (Guenther, 1972; Corteau, 1986; Fischer, 1991). The compounds from the plant based essential oil are useful as an alternative therapy, either directly or as models for new synthetic products (Houghton, 2000).

*Origanum* is an important multipurpose medicinal plant which belongs to the family Lamiaceae, tribe Mentheae and comprises of 42 species and 18 hybrids widely distributed in Eurasia and North Africa (letsvaart, 1980; Duman et al., 1988). It is native to the mountainous parts of Mediterranean region of Europe and Asia. Following letsvaart (1980) classification, the genus *Origanum* has been divided into 10 sections. These include the following.

#### ***Amaracus* (Gleditsch) Benth**

*Amaracus* (Gleditsch) Benth consists of seven species, all restricted in the east Mediterranean region. The species are mainly characterized by their usually purple bracts, 1 or 2-lipped sepals without teeth, and saccate corollas: *Origanum boissieri* letsvaart, *Origanum calcaratum* Jussieu, *Origanum cordifolium* (Montbret et Aucher ex Benth) Vogel, *Origanum dictamnus* L., *Origanum saccatum* Davis, *Origanum solymicum* Davis and *Origanum symes* Carlstrom.

#### ***Anatolicon* Benth**

*Anatolicon* Benth comprises of eight species, presenting a very restricted distribution in Greece, Asia Minor, Lebanon and Libya. The plants have strongly bilabiate 5-toothed sepals: *Origanum akhdarensis* letsvaart et Boulos, *Origanum cyrenaicum* Beguinot et Vaccari, *Origanum hypericifolium* Schwarz et Davis, *Origanum libanoticum* Boissier, *Origanum scabrum* Boissier et Heldreich, *Origanum sipyleum* L., *Origanum vetteri* Briquet et Barbey and *Origanum pampaninii* (Brullo et Furnari) letsvaart.

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### **Brevifilamentum** letswaart

*Brevifilamentum* letswaart comprises of six species which are steno-endemics mainly in the eastern part of Turkey. These species are characterized by bilabiate sepals and strongly unequal length of stamens; upper two stamens are very short and enclosed in the corolla: *Origanum acutidens* (Handel-Mazzetti) letswaart, *Origanum bargyli* Mouterde, *Origanum brevidens* (Bornmuller) Dinsmore, *Origanum haussknechtii* Boissier, *Origanum leptocladum* Boissier and *Origanum rotundifolium* Boissier.

### **Longitubus** letswaart

*Longitubus* letswaart consists of a single species found in a few places in the Amanus Mountains. It is mainly characterized by the slightly bilabiate calyx, the lips of the corolla are nearly at right angles to the tube and has the presence of very short staminal filaments: *Origanum amanum* Post.

### **Chilocalyx (Briquet)** letswaart

*Chilocalyx* (Briquet) letswaart includes four species which are steno-endemics of South Anatolia or of the island of Crete. The plants are slightly bilabiate, conspicuously pilose in throat sepals: *Origanum bigleri* Davis, *Origanum micranthum* Vogel, *Origanum microphyllum* (Bentham) Vogel and *Origanum minutiflorum* Schwarz et Davis.

### **Majorana (Miller) Bentham**

*Majorana* (Miller) Bentham consists of three species. The species are characterized by 1-lipped sepals and green bracts. Out of these *Origanum syriacum* is further subdivided into three geographically distinct varieties; these are recognised mainly from differences in their indumentum and leaf shape: *Origanum majorana* L., *Origanum onites* L., *O. syriacum* L. Var. *syriacum*, Var. *bevanii* (Holmes) letswaart and Var. *sinaicum* (Boissier) letswaart.

### **Campanulaticalyx** letswaart

*Campanulaticalyx* letswaart consists of six local endemic species. The sepals of the plants have 5 sub equal teeth and are campanulate even when bearing fruits: *Origanum dayi* Post, *Origanum isthmicum* Danin, *Origanum ramonense* Danin, *Origanum petraeum* Danin, *Origanum punonense* Danin and *Origanum jordanicum* Danin and Kunne.

### **Elongatispica** letswaart

*Elongatispica* letswaart comprises of three steno-

endemic species of North Africa, which are characterized by loose and tenuous spikes and tubular calyces with 5 equal teeth: *Origanum elongatum* (Bunnet) Emberger et Maire, *Origanum floribundum* L. Munby and *Origanum grosii* Pau et Font Quer ex letswaart.

### **Origanum**

*Origanum* is a monospecific section consisting of the species *O. vulgare*, widely distributed in North Africa and in temperate and arid zones of Eurasia (Baser et al., 2010). Introduced by humans, this species has also been encountered in North America (letswaart, 1980). The plants of *O. vulgare* have dense spikes, and tubular 5-toothed calyces, never becoming turbinate in fruit: *O. vulgare* L.

Six subspecies have been recognised within *O. vulgare* L. based on differences in indumentum, number of sessile glands on leaves, bracts and calyces, and in size and colour of bracts and flowers: subsp. *vulgare*, subsp. *glandulosum* (Desfontaines) letswaart, subsp. *gracile* (Koch) letswaart, subsp. *hirtum* (Link) letswaart, subsp. *viridulum* (Martrin-Donos) Nyman and subsp. *virens* (Hoffmannsegg and Link) letswaart.

### **Prolaticorolla** letswaart

*Prolaticorolla* letswaart comprises of three species endemic to eastern or western parts of the Mediterranean. These species are characterized by dense spikes and tubular calyces becoming turbinate in fruiting: *Origanum compactum* Bentham, *Origanum ehrenbergii* Boissier and *Origanum laevigatum* Boissier.

### **ORIGANUM SPECIES**

*Origanum* spp. have been used for thousands of years as spice and in ethnomedicine (Fleisher and Fleisher, 1988). It has antifungal, antimicrobial, insecticidal and anti-oxidant activities (Kokkini, 1997; Baydar et al., 2004; Kulisic et al., 2004; Bakkali et al., 2008). Antispasmodic, antitumoral, antifungal and analgesic activities of *Origanum* spp. have been reported (Elgayar et al., 2001; Puertas et al., 2002; Sokovic et al., 2002; Sari et al., 2006). Baser (2002) and Dundar et al. (2008) reported that *Origanum* has been used as expectorant, antiparasitic, antihelminthic and for gastrointestinal complaints in Turkish folk medicine. *Origanum* spp. are also used as a carminative, diaphoretic, stimulant and tonic (Hummer et al., 1999). Silva et al. (2012) have suggested that carvacrol present in the essential oil of *Origanum* probably interferes in the release and/or synthesis of inflammatory mediators, such as the prostanoids and thus favour the healing process for gastric ulcers. Further as a

folk remedy, it is used against colic, cough, toothaches and irregular menstrual cycles (Force et al., 2000; Kintzios, 2002a). *Origanum* spp. are also used as powerful disinfectant, flavouring agent in perfumes and in scenting soaps (Guenther, 1949; Chiez, 1984; Kotb, 1985). As a culinary herb, it is used in flavouring food products and alcoholic beverages (Aligiannis et al., 2001; Bendahou et al., 2008; Sivropoulou et al., 1996). Oregano has a promising potential for preventing diabetes complications in the long term treatments and has an anti-inflammatory efficacy as depicted by inhibiting soybean lipoxygenase (Koukoulitsa et al., 2006). The secondary metabolites of this plant have been well studied in terms of polyphenolic compounds and essential oils. Consequently, more than one hundred nonvolatile compounds have already been identified in this plant which includes flavonoids, depsides and organosides (Nakatani et al., 1987; Lin et al., 2003; Koukoulitsa et al., 2006; Liang et al., 2010; Skaltsa et al., 2010). The species of this plant which have been subjected to chemical profiling as discussed subsequently.

### ***Origanum vulgare* (L.)**

*O. vulgare* (L.) is a medicinal and perennial plant, locally known as Jungali Tulsi or Oregano or Himalayan marjoram. It is widely distributed in Mediterranean areas and Northern Africa (Ietswaaart, 1980; Kokkini, 1997). This is the only species of genus *Origanum* which is found in India. It is found in temperate Himalayas from Kashmir to Sikkim at an altitude of 1500 to 3600 m. It is particularly grown in Simla Hills, Gilgit, Nilgris and in the Kashmir valley.

Vokou et al. (1993), Kokkini et al. (1997), D'antuonu et al. (2000) and Skoula (2002) reported carvacrol and thymol as dominant components of its essential oil. Andreas et al. (2013) found carvacrol as dominant component in the essential oil of *O. vulgare* ssp. *hirtum*. Lagouri et al. (1993), Aeschbach et al. (1994), and Yanishlieva et al. (1999) revealed that antioxidant effect of this plant is as a result of carvacrol and thymol. Mastelic et al. (2008) reported its antimicrobial and antioxidant properties and in addition revealed carvacrol's antiproliferative activity on tumor cells of HeLa. Essential oil of this plant possesses a variety of biological activities, namely, antiradical (Cervato et al., 2000; Ahmad et al., 2010a, b; 2011a, b), antifungal (Cleff et al., 2010; Farag et al., 1989; Curtis et al., 1996; Sahin et al., 2004), antihyperglycaemic (Lemhadri et al., 2004), antibacterial (Dorman and Deans, 2000; Govaris et al., 2001; Harpaz et al., 2003; Burt and Reinders, 2003; Burt, 2004), and antithrombin (Goun et al., 2002). Antioxidant (Lamaison et al., 1991; Lagoun and Boskou, 1996; Nakatani, 2000; Vichi et al., 2001; Stashenko et al., 2002) function of this species could become helpful agent in treatment of cancer, heart disease and high blood pressure. Cervato

et al. (2000) reported that antioxidant activities of *O. vulgare* leaves can inhibit all places of lipid peroxidative processes.

### ***O. onites***

*O. onites* (L.) is distributed in Western and Southern Turkey. Baser et al. (2010) have reported that it grows naturally in Aegean and Mediterranean regions of Turkey. It is a perennial species with woody stem and is commonly known as 'Turkish oregano'. An essential oil reported from this plant contains carvacrol as a major component and has the potential to be utilized at reasonable concentrations to control tick infestations (Sevki et al., 2008). Diversified effects such as anti-spasmodic (Daferera et al., 2000, 2003), antibacterial (Burt, 2004), and antifungal (Ultee et al., 1997) have been attributed to this plant by modern pharmacological study. It is also used in flavouring Turkish delight candy (Facciola et al., 1998) and is also used in tea, salads and meat dishes (Small et al., 1997). Consumption of *O. onites* distillate has beneficial effects on lipid profiles, antioxidant status and endothelial functions in patients with mild hyperlipidaemia (Ozdemir et al., 2008).

### ***O. syriacum***

*O. syriacum* commonly known as 'Syrian marjoram' is an aromatic, herbaceous and perennial plant growing wild in the Sinai desert of Egypt (Tackholm, 1974). Biblical authorities consider this plant to be referred in the Bible as hyssop, particularly in the old Testament pages (Moldenke et al., 1952). It is a very popular culinary herb that has been used through ages in traditional medicine mainly in Lebanon and Arab world. It has antiseptic properties and has the ability to relieve stomach and intestinal pain. It is also used to treat heart problems, cough, toothaches (Gardner et al., 1989), cold, anxiety and wounds (Chandler et al., 2004). Kamela et al. (2001) and Baser et al. (2003) isolated thymol and carvacrol as a major constituent of its essential oil. Carvacrol is the signature chemical largely responsible for its sharp, pungent oregano flavor (Tucker et al., 1992). The volatile phenolic oil has been reported to be among the top 10 essential oils (Letchamo et al., 1995), showing antibacterial, antimycotic, antioxidative, natural food preservative and mammalian age delaying properties (Jackson and Hay, 1994; Letchamo et al., 1995).

### ***O. majorana* (L.)**

*O. majorana* (L.) is an endemic medicinal plant of Cyprus and is commonly known as 'Sampsishia'. Johannes et al. (2002) reported sabinene linalyl acetate and Cis-sabinene

hydrate from the essential oil of this plant species. It is used against common cold, as spasmolytic and as an antirheumatic. Dried leaves and flowering tips of this species are used in formulation of vermouths and bitters. The essential oil is used for flavouring sauces, condiments and other products (de Vincenzi et al., 1997). In India, it is used as diuretic, antiasthmatic and an antiparalytic drug (Yadava and Khare, 1995). This was a common salad herb in the 16th century (Picton et al., 2000). It is also used in herbal vinegars and tea can be made from its leaves (Facciola et al., 1998). Furthermore, it has been used to treat cancer as well (Johnson et al., 2002; Leung et al., 2003). Stefanakis et al. (2013) have assayed the essential oil extracted from *O. majorana* L., *O. onites* L. and *O. vulgare* L. ssp. *hirtum* as potential antibacterial agents for disinfection of rotifers (*Brachionus plicatilis*). Abdel Massih et al. (2010) suggested that Marjoram extracts exhibit antiproliferative effect and have high antioxidant activity as well.

### ***O. microphyllum***

*O. microphyllum* is a medicinal plant species of genus *Origanum*, endemic to Cretan and commonly known as 'Cretean marjoram' (Karousou, 1995). Aligannis et al. (2001) and Gotsiou et al. (2002) isolated carvacrol, terpineol-4, linalool, sabinene,  $\alpha$ -terpinene,  $\gamma$ -terpinene, cis-sabinene hydrate and trans-sabinene hydrate from the essential oil of this species. This small leaved oregano has a strong spicy flavor (Small et al., 1997).

### ***O. hypericifolium***

*O. hypericifolium* is an endemic species of Turkey. It is used as a condiment, for flavouring meat and as herbal tea for treatment of common cold and stomach complaints (Baser et al., 1994). The essential oil of pre flowering stage extracted from this plant species is carvacrol rich.

### ***O. dictamnus***

*O. dictamnus* commonly known as 'Dittany of Crete' is native to the Island of Crete, Greece. It is used in traditional medicine in Greece and all over Europe (Christos et al., 2010). It possesses numerous medicinal uses like antibacterial (Aureli et al., 1992; Biondi et al., 1993; Vokou et al., 1993) and antifungal (Arras and Picci, 1984; Collin et al., 1989; Paster et al., 1993). It is also used in cooking as well. As a culinary and medicinal herb, it is used as a tonic and digestive aid (Simon et al., 1984; Bown et al., 2001) for treating kidney and liver problems, obesity and headaches (Skoula et al., 1997). Its flowers and bracts are used to make tea and the plant when combined with garlic, thyme, salt and pepper is used in a Saxon fish sauce (Jones et al., 1973). Harvala

and Skaltsa (1986) and Harvala et al. (1987) reported that the leaves of this plant contain flavonoids and flavonoid glycosides some of which have spasmolytic activity. The essential oil has carvacrol,  $\alpha$ -terpinene, p-cymene, caryophyllene, borneol, terpin-1-en-4-ol and carvacrol methyl ether as predominant compounds. Various studies, concerning *O. dictamnus*, have shown that their oils possess strong antimicrobial activity; this activity could be attributed to their high percentage of phenolic compounds and specifically, carvacrol, thymol, p-cymene and their precursor c-terpinene (Sivropoulou et al., 1996). The variety of non-polar components such as fatty acids, lipids, sterols and essential oil has been identified from *O. dictamnus* (Revinthi-Moraiti et al., 1985; Komaitis et al., 1988).

### ***Origanum dubium***

*O. dubium* an endemic Mediterranean shrub is widely spread in Cyprus, Greece and in Southern Turkey. It is commonly called 'Rigani'. An infusion of its leaves, flowering stems and flowers is used as a digestive aid while its essential oil when applied externally acts as an antirheumatic (Arnold et al., 1993). Carvacrol is the major component of its essential oil. The essential oil shows antimicrobial activity and their potential antioxidant activity was investigated and found to be significant in scavenging  $O_2$  (Karioti et al., 2006). Further, *O. dubium* shows inhibition of soybean lipoxygenase LOX and has high inhibitory activity (Karioti et al., 2006).

### ***O. sipyleum* (L.)**

*O. sipyleum* (L.) commonly called 'Showy Pink Oregano' is a polymorphic species of eastern Mediterranean area and is native to Western Anatolia, Turkey. It is used as a spice and against cough. It is also beneficial for the treatment of various respiratory and gastrointestinal disorders. The essential oil of this species is rich in  $\alpha$ -terpinene and monoterpenes (Baser et al., 1992).

### ***O. compactum***

*O. compactum* is found in Morocco, South-West Spain and North Africa (Tutin et al., 1972). It is commonly called 'Compact Oregano'. It produces one of the most powerful antimicrobial essential oil. It has antibacterial (Bouhdid, 2009), antioxidant (Bouhdid, 2008) and antifungal (Bouchra, 2003) properties. It demonstrates cytotoxic activity by oxidative stress as seen by mitochondrial damage (Bakkali, 2005). It has carvacrol, p-cymene and  $\gamma$ -terpinene as major components of its essential oil.

### ***O. floribundum***

*O. floribundum* is recorded in Algerian site. It is used to

stimulate the appetite of cattle, sheep and horses. Furthermore, it is also used against diarrhoea and other digestive disorders (Houmani et al., 2002). Carvacrol, linalool, p-cymene and  $\gamma$ -terpinene were isolated by Baser et al. (2000) from the essential oil of this species.

### *O. acutidens*

*O. acutidens* is an endemic, herbaceous, and perennial plant growing mainly in calcareous and non calcareous rocks, slopes, and screes in the Central Anatolia region of Turkey (Davis, 1982). The major components of *O. acutidens* oil are carvacrol, p-cymene, borneol,  $\gamma$ -terpinene,  $\beta$ -caryophyllene and linalool. Sokmen et al. (2004) has suggested that its essential oil has antagonistic activity against food-borne pathogenic bacteria and hence could be used in the food and pharmaceutical industries, and as an alternative to common synthetic antimicrobial products.

## DISCUSSION

The aim of the present review is to present comprehensive information about the medicinal importance of genus *Origanum*. Although, there are 42 species of genus *Origanum*, only about less than 50% species have been subjected to chemical profiling. Other species have not been broadly subjected to chemical characterization and biological studies as evident from perusal of the literature. Current studies have shown that the essential oil, as well as their active principles possess several pharmacological properties like antimutagenic, angiogenic, antiparasitic, antiplatelet, antielastase, antihepatotoxic ones (Baser, 2008). The species studied by various workers indicate that the genus *Origanum* is a potent source for isolation of a variety of bioactive molecules like terpenes, phenols, flavonoids, etc. Thereby, this genus has important biological activities and acts against different types of diseases and is being used for culinary and economic uses. Furthermore it is also used as a feed additive, in honeybee breeding and in treatment of gastrointestinal ailments (Baser, 2008).

The recent scientific data and the rich historical evidence of its medicinal uses could support further research as well as its use as a safe herbal medicinal product. The antimicrobial activity can promote the use of the aforementioned natural products as potent preservative and conservation agents, not only in the food industry after testing the toxic and irritating effects on humans, but also in cosmetics and medical preparations. The present literature study further reveals that the biological properties of the investigated genus are not due only to their essential oils and their main compounds such as carvacrol and thymol, but also to the other polar constituents acting synergistically or possessing different biological activities.

## Conclusion

The synthesis of medicinally important phytochemicals by the species of genus *Origanum* has been established beyond doubt. The plant species are being used both in allopathic and traditional system of medicine as a remedial measure for number of ailments. Since, only limited species are being subjected to phytochemical investigation, there is a need to broaden this study for further phytochemical and pharmacological studies for the rest of the species as well, which may prove of vital importance and could lead to new therapeutic products. Furthermore, since the plant extracts of the genus *Origanum* and its essential oils are used as dietary supplements or for medicinal purposes, it has become crucial to screen them for ensuring authenticity and product quality as toxic adulterants may prove to be life threatening.

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Full Length Research Paper

## Antimicrobial effect of Microdacyn 60, OxOral, and sodium hypochlorite 5.25% in anaerobic bacteria

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Irrigants have traditionally been used in the root canal. Sodium hypochlorite (NaOCl) is the most commonly used solution, because of its advantages; however, it is known to be toxic to periradicular tissues and can cause necrosis of support tissues and edema. The objective of this study is to compare the antimicrobial effect of Microdacyn 60<sup>®</sup>, OxOral<sup>®</sup>, and NaOCl 5.25% against *Streptococcus sobrinus*, *Porphyromona gingivalis*, *Streptococcus intermedius*, *Tanarella forsyntensis*, and *Enterococcus faecalis*. Thirty-three extracted uniradicular teeth were inoculated with 10 µl of a mixture of anaerobic bacteria. After seven days, the samples were irrigated with the solutions and samples were taken. These were placed in an Eppendorf tube and incubated at 37°C. After seven days, a sample was taken for a bacterial count. Polymerase chain reaction (PCR) was performed to identify the bacteria present in the samples. Student's *t* test and analysis of variance were used with a 95% confidence interval. NaOCl showed complete bacteria elimination with no statistically significant difference with OxOral. The saline and Microdacyn 60 groups showed greater bacterial growth than OxOral. In the Microdacyn 60 group, *E. faecalis* was the most resistant microorganism. NaOCl 5.25% had a greater antibacterial effect, followed by OxOral, and lastly by Microdacyn 60.

**Key words:** Irrigants, sodium hypochlorite (NaOCl), *Enterococcus faecalis*, Microdacyn 60, OxOral, irrigation.

### INTRODUCTION

Successful treatment of the root canal system depends on the method and quality of instrumentation, irrigation, disinfection, and three-dimensional obturation of the root canal space (Himel and DiFore, 2009; Canalda-Sahli, 2006). For this, different types of handpieces, machining, and irrigation solutions have been used to obtain a clean space, shaped to receive a filling (Sen et al., 1995). Basrani and Cañete (1988) define endodontic irrigation as the introduction of one or more solutions in the pulp chamber and the root canals with subsequent aspiration.

Furthermore, it is an essential complement to instrumentation; therefore, it must be used before, during, and after it. Sodium hypochlorite (NaOCl) is the most commonly used irrigating solution in current practice because of its effectiveness in eliminating vital and non-vital tissue and its broad effect against bacteria, spores, fungi and viruses (including HIV, rotavirus, HSV-1 and Hepatitis A and B) (Siqueira et al., 2000). However, NaOCl has toxic effects on vital tissues, which can result in haemolysis, skin ulceration, and necrosis (Hülsmann and Hahn, 2000).

It does not remove the smear layer since it only acts on the organic matter in the pulp and predentine (Di Lenarda et al., 2000). In the past 20 years, super oxidized solutions (SOSS) have shown to be potent antimicrobial agents and disinfectants through oxidative damage (Huang et al., 2008). Electrolyzed water (EW) contains a mixture of inorganic oxidants such as hypochlorous acid (HClO), hypochlorous acidic ion (ClO<sup>-</sup>), chlorine (Cl<sub>2</sub>), hydroxide (OH), and ozone (O<sub>3</sub>), which are effective for inactivating a variety of microorganisms (Chittoria, 2007). This product is prepared with purified water plus a saturated solution of sodium chloride (NaCl). SOSS have been used mainly as disinfectants in endoscopic equipment and minimally invasive instruments as well as for washing and disinfecting hemodialysis equipment. Different types of electrolyzed solutions have been the subject of many studies and reviews as a method of decontaminating vegetables, killing *Escherichia coli*, *Listeria*, *Salmonella*, *Bacillus anthracis*, and many other infectious strains (Horiba et al., 1999; Guentzel et al., 2008). Their mechanism of action is based on the effect that Na<sup>+</sup>, Cl<sup>-</sup>, and O<sub>2</sub> ions have on the bacterial wall, where they cause protein denaturation, and fragmentation of carbohydrates and lipids, in addition to viral alteration of capsids, DNases, and RNases (Tang et al., 2011). They have been approved by the Food and Drug Administration (FDA) to debride traumatic wounds and burns, to lubricate and moisten wounds and burns, and to disinfect medical equipment (Gutierrez, 2006). OxOral<sup>®</sup> is a pH neutral SOSS 0.005% (50 ppm) of active Cl, formulated for dilution at 1:5. It is effective against viruses, fungi, and Gram-positive and Gram-negative bacteria (PLM México, 2012). Microcyn 60<sup>®</sup> (60 ppm) is an electrolyzed solution manufactured in Mexico for the care of skin wounds. It does not cause irritation, or sensitize, and requires no rinsing. It is an antiseptic and disinfectant with a neutral pH and a broad spectrum against microorganisms. Its bactericidal effect takes place in 30 s and disinfection occurs in 15 min with a long shelf life (>12 months). It is a hypotonic solution with an osmolarity of 13 mOsm/kg (Chittoria et al., 2007; Landa-Solis et al., 2005; Yahagi et al., 2000).

The aim of this study is to compare the antimicrobial effect of two electrolyzed water solutions, Microdacyn 60 and OxOral, and NaOCl 5.25% against *Streptococcus sobrinus*, *Porphyromona gingivalis*, *Streptococcus intermedius*, *Tanarella forsyntensis*, and *Enterococcus faecalis*.

## MATERIALS AND METHODS

Five anaerobic bacteria strains from the laboratory of the School of Dentistry of the Universidad Autonoma de Nuevo Leon that were present in necrotic tissue were selected: *S. sobrinus* (ATCC 27607), *P. gingivalis* (ATCC 33277), *S. intermedius* (ATCC 27335), *T. forsyntensis* (ATCC43037) and *E. faecalis* (ATCC 11420). Uniradicular teeth with a single straight canal, complete root formation, free of fractures, and free of caries in their root portion with a closed

apex were also selected.

### Preparation of teeth

A total of 33 extracted human single root teeth were selected. The crowns were sectioned at the cemento-enamel junction and the working length was determined with #15 K-type Maillefer file subtracting a millimeter from its outlet, flush with the apical foramen. The teeth were instrumented to a #40 apical diameter with ProTaper Universal files (Dentsply International, York, PA) and using irrigation with NaOCl 5.25%. After instrumentation to maintain the canal permeable, root canals were dried with Hygenic #40 Sterile Absorbent Paper Points (Hygenic Corporation, Akron, Ohio) and canals were filled with 17% ethylenediaminetetraacetic acid (EDTA) for 5 min after which the solution was removed with a NaOCl 5.25% wash and again dried with paper points. The outer surface of the roots was covered with a layer of clear lacquer, which also sealed the apical foramen to avoid external contamination.

At the end of this process, teeth were placed in a rack made of heavy silicone (Speedex Trial<sup>™</sup>, Coltene whaledente, Altstaetten, Switzerland). The teeth were autoclaved for 30 min at 121°C and under 15 pounds of pressure. After this, a sample of the specimens was taken with a #40 Hygenic sterile paper point (Coltene) and placed in an Eppendorf tube with trypticase soy broth for incubation for 24 h. At the end of this time, if there was no turbidity, this showed that there were no bacteria. If turbidity was present, the presence of bacteria needed to be confirmed by seeding this broth in a Petri dish with 5% sheep blood agar and incubating it for 24 h. Subsequently, the test specimen was resterilized and the procedure repeated.

### Preparation of strains

Inside an anaerobic chamber, 100 µl of each bacterium was obtained with an Eppendorf pipette and inoculated individually in Eppendorf tubes with trypticase soy broth. The tubes were placed in an incubator at 37°C for 7 days to activate them. At the end of this time, 100 µl from each tube was inoculated and plated on 5% sheep blood agar plates individually, sealing them with tape and placing them in airtight bags. The dishes were taken to the incubator for 7 more days. After this time, Gram staining of the bacterial colonies was performed to observe their morphology with an optical microscope at 100x, and the viability of each bacterial strain was confirmed.

To confirm optimal growth of the strains, 1000 µl were taken from each tube containing the reactive bacteria and this was placed in a single test tube containing 5000 µl of trypticase soy broth to mix the five bacterial strains, obtaining a total volume of 10,000 µl, simulating the clinical conditions of the oral environment, living as an ecological community and not isolated.

### Inoculation of specimens and placement of solutions

The teeth were divided into three experimental and one control groups (10 teeth in each experimental group and 3 teeth in the control group). These were placed in individual silicone racks for easy handling inside the anaerobic chamber.

After sterilization of dental specimens was proven and the bacteria mixture cultured, starting with a bacterial concentration of 0.5×10<sup>8</sup> CFU/ml, 10 µl of this mixture was used to fill the root canal using an Eppendorf micropipette, slowly withdrawing it from the canal and later sealing it with sterile tape. Each group was placed in airtight sealed bags and subsequently incubated for 7 days. After bacterial incubation, we proceeded to irrigate the samples with different solutions under aseptic conditions and in strict

anaerobiosis: Group 1, sterile saline/NaCl (n = 3); Group 2, Microdacyn 60 (n = 10); Group 3, OxOral (n = 10); and Group 4, Sodium Hypochlorite/NaOCl 5.25% (n = 10). The samples were irrigated with 5 ml of each solution for 5 min with a 5 ml syringe and a NaviTip ISO 30 (Ultradent) hypodermic needle, and afterwards rinsed with sterile saline solution. The same procedure was performed for each group.

### Sampling

Five minutes after irrigating each specimen, they were dried with sterile paper points and a sample was collected from each with a paper point previously dampened with sterile saline. Each sample was introduced into an Eppendorf tube with 1,000  $\mu$ l of trypticase soy broth. Subsequently, the tubes were placed in a Vortex Maxi-Mix Thermolyne M16700 (Thermo, Fisher Scientific Inc., Asheville, NC) for homogenization, and again in the anaerobic chamber; 100  $\mu$ l was drawn from each tube and its contents were seeded in Petri dishes with 5% sheep blood agar and incubated at 37°C for seven days.

### Bacterial count

After seven days, a sample was taken from each of the dishes to make a  $10^{-3}$  dilution in sterile bidistilled water, since this dilution proved to be the best to carry out a viable cell count. From the dilution, 10  $\mu$ l was obtained and placed in a Neubauer chamber and a bacterial cell count was performed under an optical microscope to determine the number of cells per milliliter. Mean colony-forming unit (CFU) counts were log transformed for performing calculations and statistical analyses.

### Statistical analysis

To compare results between irrigation solutions and each irrigation solution, analysis of variance (ANOVA) was used. Tukey honestly significant difference (HSD) test was also applied to specifically determine significant differences between irrigants. The tests were performed considering an alpha of 0.05 and a confidence interval (CI) of 95%.

## RESULTS

The mean number of bacteria present in the different solutions was the following: the three elements irrigated with NaCl (control group) had an average of  $3.52 \pm 0.16$  CFU/ml (95% CI: 3.12 to 3.92). The 10 elements irrigated with Microdacyn 60 had a mean of  $2.57 \pm 1.58$  CFU/ml (95% CI: 1.44 to 3.69), while the 10 elements irrigated with OxOral had a mean of  $0.26 \pm 0.46$  CFU/ml (95% CI: 0.08 to 0.59). The 10 elements irrigated with NaOCl showed no bacterial growth (Figure 1). Values for NaCl were in the range of 3.40 to 3.70 CFU/ml. The sample irrigated with Microdacyn 60 had values between 0.90 and 5.85 CFU/ml, while the sample irrigated with OxOral was 0.00 to 1.40 CFU/ml. The best antimicrobial effect was observed with NaOCl, followed by OxOral. There was no statistically significant difference between OxOral and NaOCl ( $P = .924$ ) or between NaCl and Microdacyn 60 ( $P = .408$ ). A statistically significant difference was found between the groups NaCl and Microdacyn 60 ver-

sus OxOral and NaOCl ( $P < .01$ ).

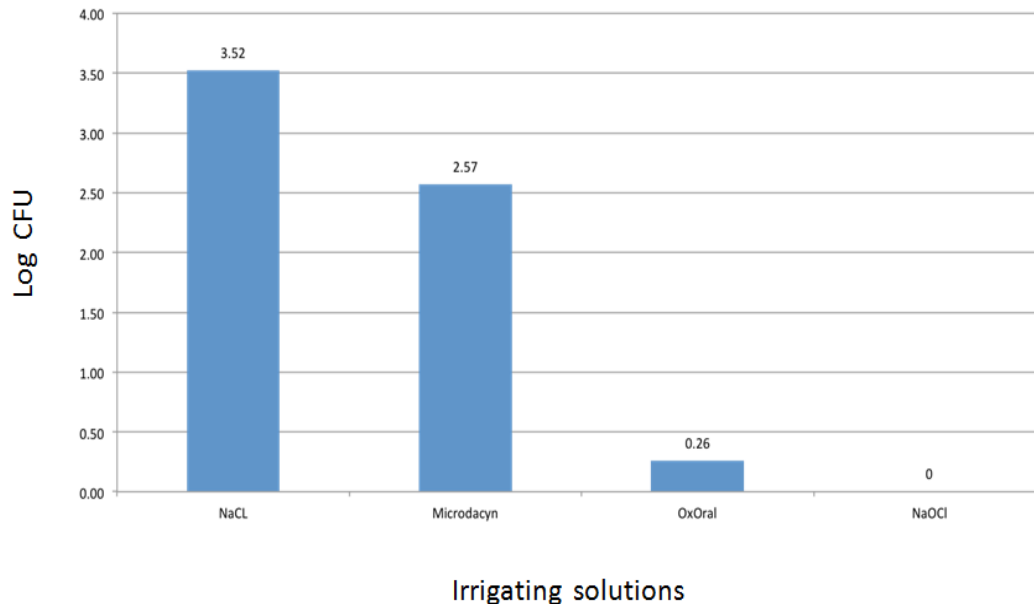
## DISCUSSION

The results show a greater antibacterial effect with NaOCl, followed by OxOral, with a statistically significant difference between the control group (NaCl) plus Microdacyn 60 and OxOral and NaOCl. These results are similar to those by Prabhakar et al. (2010) who analyzed the antimicrobial efficacy of Triphala, green tea polyphenols, MTAD, and NaOCl 5% against *E. faecalis* biofilm formation on tooth substrate. NaOCl 5% showed complete bacterial inhibition against *E. faecalis* biofilm formation.

Retamozo et al. (2010) investigated the concentration and irrigation time of NaOCl for disinfecting dentin canals colonized by *E. faecalis*. Their findings were that irrigation with NaOCl 5.25% for 40 min was the most effective, while irrigation with lower concentrations and lower exposure times to NaOCl were not effective against *E. faecalis*. They concluded that high concentrations and long exposure to NaOCl are needed for removal of *E. faecalis* in contaminated dentin. These results agree with those of the present study, although the exposure time in our study was 5 min; however, one of the drawbacks of our study was that NaOCl was not neutralized with sodium thiosulfate. Prabhakar et al. (2010) used human single premolars and NaOCl 5% without neutralization by sodium thiosulfate and found maximum antibacterial activity against *E. faecalis* biofilm with an exposure time of 10 min.

Root canal irrigation is an important part of the preparation procedure. NaOCl and  $H_2O_2$  are the irrigants most frequently used in the debridement and disinfection of the root canal. In the clinical setting, irrigant exposure time should be short to avoid patient discomfort and allow better endodontic conditions for the clinician. In a review of complications of NaOCl during root canal irrigation, Hulsman and Hahn (2000) reported that NaOCl has toxic effects on vital tissues, resulting in haemolysis, skin ulceration and necrosis. It can also damage clothing and the eye, and produce tissue necrosis when NaOCl is injected beyond the apical foramen when wide apical foramina is present or the apical constriction has been destroyed during root canal preparation.

Electrolyzed water (superoxidized solution) has been used to disinfect produce because of its strong bactericidal effect (Guentzel et al., 2008; Huang et al., 2008). The active substances in SOSS are  $Cl_2$ , HClO, and  $ClO^-$  (Tang et al., 2011; Huang et al., 2008; Landa-Solis et al., 2005). It has been used in dentistry for disinfection of dental instruments, and root canal irrigation (Horiba et al., 1999). Horiba et al. (1999) also studied the bacteriostatic/bactericidal effect of electrolyzed neutral water against 15 bacteria strains obtained from infected root canals. They found that electrolyzed neutral water had a bactericidal or growth-inhibitory effect against



**Figure 1.** Effect of NaCl, superoxidized solutions (Microdacyn 60, OxOral), and NaOCl 5.25%, on mean bacterial growth.

the bacteria studied.

With regard to biofilm, Ozaki et al. (2012) studied biofilm removal and antimicrobial effects of microbubbled tap water and electrolyzed water with *Candida albicans* and *Streptococcus mutans*. They found that electrolyzed water in the microbubbled state was effective against both planktonic and biofilm microorganisms. This is evidence that superoxidized solutions could be effective in removing biofilm. Studies with microorganisms present in the oral cavity and dental materials need to be carried out.

Our results found a growth-inhibitory effect with the two electrolyzed solutions studied (Microdacyn 60 and OxOral). OxOral showed a greater effect than Microdacyn 60 and an effect similar to NaOCl, although this similarity was not statistically significant. The difference in effectiveness between Microdacyn 60 and OxOral could be because one comes in a closed container and the other in an open container, but this requires further study.

Superoxidized solutions represent a possible alternative to NaOCl for irrigating root canals; however, these formulas need to be tested clinically in order to determine their antibacterial effect in root canal procedures when confronted with the diversity of organisms that populate necrotic root canals.

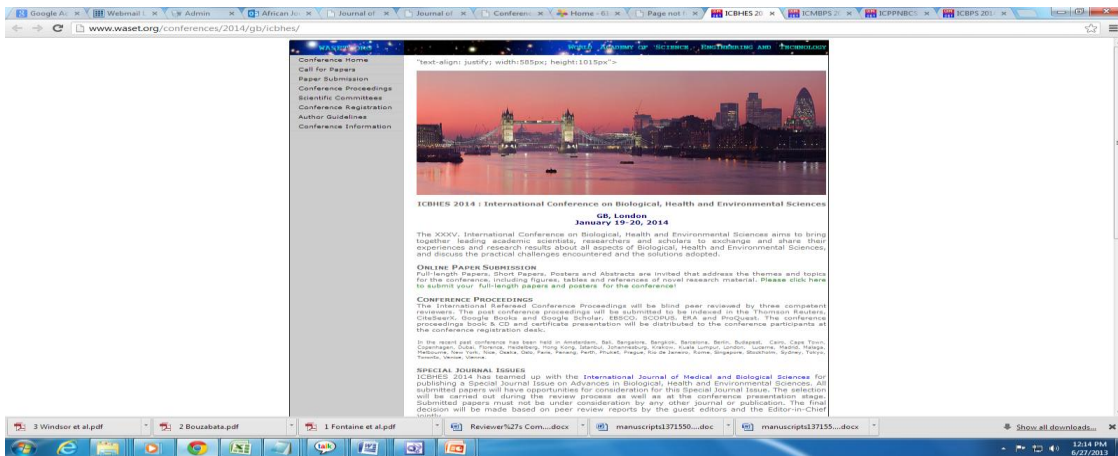
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