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Full Length Research Paper

Antimicrobial effects of four medicinal plants on dental plaque

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Dental caries is a common disease in human population which has a multifactorial etiology. A major cause of the disease is believed to be commensal bacteria which exist in dental plaque, particularly oral *Streptococci*. Our study is aimed at investigating the effects of aqueous, alcoholic and etheric, extracts as well as essence of four medicinal plants (*Thymus vulgaris* L., *Melissa officinalis* L., *Rhus corriaria* L. and *Magnolia grandiflora* L.) on *Streptococcus mutans* L. (ATCC-1 27607) and *Streptococcus sanguis* L. (PTCC-1449). None of the aqueous extracts had an effect on either *S. mutans* or *S. sanguis*. However, ethanol extract of *M. officinalis* had a minimal inhibitory concentration (MIC) of 310 µg/ml on both pathogens. *R. corriaria* had a MIC and a minimal bactericidal concentration (MBC) of 250 µg/ml on *S. mutans* and *S. sanguis*. Plant essence demonstrated superior effect than aqueous and alcoholic extracts. *M. grandiflora* had a MIC of 1/5200 ml and MBC of 1/3200 ml on *S. mutans*. The MIC and MBC was 1/5200 ml and 1/2400 ml respectively on *S. sanguis*. *T. vulgaris* had a MIC of 1/2000 ml and a MBC of 1/1200 ml on *S. mutans*. The plant essence inhibited *S. sanguis* up to 1/400 ml but no MB effect on *S. sanguis*. Essence of *M. officinalis* had a MI effect on both *S. mutans* and *S. sanguis* up to 1/1200 dilution but no MB effect on the pathogens.

Key words: aqueous extract, alcoholic extract, dental plaque.

INTRODUCTION

Dental caries is a multifactorial human disease that has widely affected many populations all over the world. Bacterial plaque plays the primary role in the pathogenesis of the disease. Dental plaque is a general term for the diverse microbial community (predominantly bacteria) found on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin. Plaque develops naturally on teeth, and forms part of the defence systems of the host by helping to prevent colonisation of enamel by exogenous (and often pathogenic) microorganisms (colonisation resistance). Plaque is an example of a biofilm; current researches are showing that the properties of bacteria associated with a surface in a biofilm can be markedly different than those of the same cells growing in liquid broth (planktonic cells).

Plaque is found preferentially at protected and stagnant surfaces, and these are at the greatest risk of disease (Scheie, 1994).

The attachment, growth, removal and reattachment of bacteria to the tooth surface is a continuous and dynamic process. However, several distinct processes can be recognized:

Absorption of salivary proteins and glycoproteins, together with some bacterial molecules, to the tooth surface to form a conditioning film (the acquired pellicle). Long-range (>50nm), non-specific interaction of microbial cell surfaces with the acquired pellicle via van der Waals attractive forces.

Shorter-range (10 – 20 nm) interactions, in which the interplay of van der Waals attraction forces and electrostatic repulsion produces a weak area of attraction that can result in reversible adhesion to the surface.

Irreversible adhesion can occur if specific inter-molecular interactions take place between adhesins on the cell surface and receptors in the acquired pellicle.

Secondary or late-colonisers attach to primary coloni-

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nisers (coaggregation), also by specific inter-molecular interactions.

Cell division of the attached cells to produce confluent growth, and a bioflim (Freedman, 1974; Houte, 1982).

The present study is aimed at investigating the antimicrobial effects of four medicinal herbs namely, *T. vulgaris* L, *M. officinalis* L., *R. corriara* L., *M. grandiflora* L., on two inhabitants of dental bacterial plaque, *S. mutans* L. (ATCC-1 27607) and *S. sanguis* L. (PTCC-1449). This is with a view to advancing knowledge on the causes of caries, preventing the occurrence and curbing the effects.

M. grandiflora L. (Family: Magnoliaceae) is a popular beautiful decorative tree also known as balm and lemon balm. It is as tall as 8 to 20 m tall. It has large, leathery, shiny leaves which are green on the adaxial surface and light brown on the abaxial surface (Vázquez, 1984). Various magnolias are distinguished by their many interesting biological features. The genus Magnolia consists of about 120 - 130 species and in the Tertiary period Magnolias were common in Europe (Azuma et al., 2001; Kim et al., 2001; Hunt, 1998). Originally from Mexico (Vázquez, 1984), the majority of *Magnolia* (120 – 130) species grow in the temperate and tropical zones of southeastern Asia, while others are found in the New World. *M. grandiflora*, is utilized in Mexican traditional medicine (Bastidas et. al., 1998). It is claimed to have antibacterial, antiviral properties (it is effective against herpes simplex), and it is also used as a mild sedative or calming agent. At least one study has found it to be effective at reducing stress (Kennedy et al., 2004). Its antibacterial properties have also been demonstrated scientifically, although they are markedly weaker than those from a number of other plants studied (Nascimento et al., 2002). Principally, the plant contains phenolic compounds and terpenoids. Three phenolic constituents (Magnolol, honokiol, and 3.5'-diallyl-2'-hydroxy-4-methoxybiphenyl) exhibited significant activity against gram-positive and acid-fast bacteria and fungi (Clark et al., 1981; Chang et al., 1998; Ho et al., 2001).

Melissa officinalis L. (family: Lamiaceae/labiatae) is 30 to 100 cm tall. It grows in southern and central Europe, Spain, and Iran. The leaves are oval or heart-shaped and indented. They often blossom in May and June with white flowers (Mackenzie, 2002). The leaves of *M. officinalis* contain a bitter material, Tanon, Caffor, various sugars, resin components, pectic components, 0.1 – 0.25% (0.75%) essence. Several therapeutic effects such as energizer, anticonvulsant, and tranquilizer have been attributed to the plant particularly when proper dosage of essence is taken. Others are digestive and antiflatulent, indigestion, abdominal cramps, unilateral headache, palpitation, insomnia, tinnitus, hysteria, iron-deficiency anemia in young females, nausea and vomiting during pregnancy, asthma and bronchitis, paralysis, psychogenic dental pain, irregular menstruation (Wink, 2004). In some parts of Iran, it is added to food and salad. The leaves are used to relive burning sensation and pain after insects bite (Adinee et al., 2008; Duke, 1985).

R. coriaria L. (Anacardiaceae) commonly known as sumac (or sumach), occurs in the wild from the Canary Island over the Mediterranean coastline to Iran and Afghanistan. It is native to the Mediterranean and southeast Anatolian region of Turkey. The name is derived from “sumaga”, meaning red in Syriac. The name sumac is given also to the commercial preparation of the dried and ground leaves of the Sicilian or tenners sumac of Southern Europe, long used in making leather. The spice, produced by grinding the dried fruit with salt, is used as a condiment and sprinkled over kebabs and grilled meat as well as over the salad (Dogan and Akgul, 2005). *R. coriaria* L. is a 1-3 m high shrub or small tree. The leaves are imparipinnate with 9 - 15 leaflets. The inflorescence is a compact and erect panicle, with small and greenish white flowers and one-seeded drupe (Güvenç, 1998). Tannins are common constituent of the plant and make 13 - 27% by weight of dried leaf. The stem contains 11% tannic acid and 3.4% tannins. The fruit has an alkaline malate. It has been used to control diarrhoea, hemorrhage, fever, vaginal discharge, as a mouth rinse to treat pharyngitis and stomatitis (Grieve, 1984; Chopra et al., 1986).

Thymus (Lamiaceae) is distributed in Eurasian and the Mediterranean region. The Iberian Peninsula and northwest Africa are considered to be the center of diversity. *T. vulgaris* (common thyme) is native to southern Europe, (Spain to Italy). It is also cultivated in mild-temperature and subtropical climates, which include southern and central Europe (Grieve, 2006; Peter, 2006). Common thyme (*T. vulgaris*) is a perennial subshrub, 10 - 30 cm in height with slender, wiry and spreading branches. The leaves are small, narrow and elliptic. It blossoms in June and July (Peter, 2006; Woodville, 2008). Thyme has two main classes of secondary products, the volatile essentials oil and non-volatile polyphenols. The essential oil, thymol – a phenolic monoterpene, is responsible for the typical spicy smell of thyme (Gruenwald et al., 2000, Peter, 2006). It is stored in glandular peltate trichomes situated on both surfaces of the leaves. Dried plant material contains 1 - 2.5% of essentials oil). (Gruenwald et al., 2000). Common thyme has a long history of folk use for a wide range of ailments (Westwood, 1993). In particular, thyme is valued for its antiseptic and antioxidant properties, it is an excellent tonic and is used in treating respiratory diseases and a variety of other ailments (Chevallier, 1996). The flowering tops are anthelmintic, strongly antiseptic, antispasmodic, carminative, deodorant, diaphoretic, disinfectant, expectorant, sedative and tonic (Grieve, 1984; Westwood, 1993; Chiej, 1984). The plant is used internally in the treatment of dry coughs, whooping cough, bronchitis, bronchial catarrh, asthma, laryngitis, indigestion, gastritis and diarrhoea and enuresis in children. It should not be prescribed for pregnant women. Externally, it is used in the treatment of tonsillitis, gum diseases, rheumatism, arthritis and fungal infections (Bown, 1995). Thymol, an antiseptic, is the main active ingredient of mouthwash (Pierce, 1999). Thyme is active against periodontopathic bacteria including *Actinobacillus*, *Capnocytophaga, Fusobacterium, Eikenella* and *Bacterioides* species, and, hence, may be used for plaque control (Duke and Ayensu, 1985). It is also effective against the fungus that infects toenails (Ramsewak et al., 2003). *Tymol* showed antiparasitic properties against *Leishmania*, inhibiting its mitochondrial DNA polymerase (Khan and Nolan, 1995).

MATERIALS AND METHODS

Preparation of aqueous extracts

150 ml of distilled water is added to 15 g of ground dried plant, heated below the boiling point and stirred for 2 ½ - 3 h. The extract was filtered and lyophilized.

Preparation of alcoholic extract

The experiment follows the procedure for aqueous extraction but ethanol was used as solvent.

Preparation of ether extract

Ether extraction is as carried out above.

Preparation of aqueous and methanol extract

100 ml of distilled water is added to 15 g of ground dried plant, heated below the boiling point and stirred intermittently for 1½ h. 50 ml of ethanol is added and the solution is stirred for 1 h. The extract is filtered and lyophilized in a glass container.

Preparation of aqueous-ether extract

Aqueous ether extraction follows the same procedure as for the aqueous methanol preparation.

Preparation of essence

The plant is air dried under room temperature below 35°C. 50 g of ground is heated in distilled water in a soxhlet apparatus. The heating produced steam which in turn carries essence and other volatile agents. These were cooled and condensed to obtain the essence. The low-weight essence was separated from the extract by filtering. It was stored in coloured and sterile containers and sealed with aluminum foil.

The bacterial species employed in the study

Streptococcus mutans (ATCC-1 27607) and *S. sanguis* (PTCC-1449) were provided by Faculty of Paramedics, Iran University of Medical Sciences and Iranian Center for Science and Industry respectively. They were cultured in BHI (Brain Heart Infusion), TSB (Trypticase Soy Broth) and CDM (Chemically Defined Medium). The TSB medium was selected for the experiment.

MIC and MBC

Using the bacterial broth dilution, minimum concentrations of plant extracts capable of inhibiting bacterial growth was determined ("Minimum Inhibitory Concentration"). Samples from bacteria free cultures were transferred to control media with no extracts and incubated in optimum conditions. The number of colonies was counted. If they were less than 1/1000 colonies in the control medium, the extract concentration is termed "Minimal Bacteriocidal Concentration" (MBC).

RESULTS

Methanolic extract of *T. vulgaris* and aqueous extracts of the plants showed no effect either on *S. mutans* or *S. sanguis* (Tables 1 and 2). However, the MIC for alcoholic extract of *M. officinalis* was 310 µg/ml (Table 3). Alcoholic extract of *R. coriaria* had MIC and MBC of 250 µg/ml on *S. mutans* and 250 µg/ml on *S. sanguis*. In general, essence demonstrated a superior effect than aqueous and alcoholic extracts. Essence of *Magnolia grandiflora* showed MIC of 1/5200 ml and MBC of 1/3200 ml on *S. mutans* and MIC of 1/5200 ml and MBC of 1/2400 ml on *S. sanguis*. The essence of *T. vulgaris* showed MIC of 1/2000 ml and MBC of 1/1200 ml on *S. mutans* but demonstrated no MIC or MBC above 1/200 ml and 1/400 ml on *S. sanguis* respectively. Essence of *M. officinalis* showed MIC of 1/1200 ml against *S. mutants* and *S. sanguis*. There was no appreciable MBC against the two pathogens (Figures 1 - 4).

DISCUSSION

Herbal medicine dates back to 5000 years ago in China and up to 60,000 years in Iran. For instance*, M. officinalis* has been used in traditional Chinese medicine over centuries for treating neurological disorders such as dementia, neurosis and Parkinson's disease and also gastrointestinal and cardiovascular diseases. Watanabe et al. (1973, 1975) investigated the pharmacological effects of extract of the dermis of Magnolia and suggested antibacterial effects against diplococci*, streptococci, staphylococci, bacillus, Pseudomonas, shigella and Salmonella* (Watanabe et al., 1973; Watanabe et al., 1975). Namba et al. (1982) studied the anticariogenic effect of ether and methanolic extracts of *M. officinalis* and *Magnolia obovata* and their chemical constituents, magnolol and honokiol, on *S. mutans*, the major bacterial species causing dental caries in humans and animals. The chemicals had an MIC of 6.5 and 12.5 µg/ml respectively on S. *mutans.* Protective effect of newly-produced glucans or glycosyltransferase on the cell surface may account for increased MIC value. Magnolol and hankiol possess bactericidal effect (MBC: 50-75 µg/ml) on various strains of *S. mutans* within 5 min. Magnolol and Hankiol are exclusively found *in M. obovata* var. *yanagidan*a, *M. officinalis,* and *M. grandiflora.* Our study showed that the essence of the three species had antimicrobial properties. Essence of the plant may possess a significant part of the effective substance. Interestingly, alcoholic was more potent than aqueous extracts indicating that active constituents dissolved more in alcoholic solvents and the resultant sec-

Table 1. Effects of Methanol extract of *Thymus vulgaris* on Streptococcal growth.

Bacterial strain	Concentration (µg/ml)						
	0	100	200	250	310	420	500
S. mutants	+	+	+	+	+	+	+
S. sanguis	+	+	+	+	+	+	+

+ =Growth

Table 2. Effects of aqueous plant extracts on *Streptococcal* growth.

Bacterial strain	Plant	Concentration (µg/ml)						
		0	100	200	250	310	420	500
S. mutans	*T. vulgaris*	+	+	+	+	+	+	+
	M. officinalis	+	+	+	+	+	+	+
	R. coriaria	+	+	+	+	+	+	+
	M. grandiflora	+	+	+	+	+	+	+
S. sanguis	*T. vulgaris*	+	+	+	+	+	+	+
	M. officinalis	+	+	+	+	+	+	+
	R. coriaria	+	+	+	+	+	+	+
	M. grandiflora	+	+	+	+	+	+	+

+ = Growth

Table 3. Effects of ethanolic plant on streptococcal growth.

Strain	Plant	Concentration(µg/ml)						
		0	100	200	250	310	420	500
S. mutans	*T. vulgaris*	+	+	+	+	+	+	+
	M. officinalis	+	+	+	+	-	-	+
	R. coriaria	+	+	+	-	-	-	-
S. sanguis	*T. vulgaris*	+	+	+	+	+	+	+
	M. officinalis	+	+	+	+	-	-	-
	R. coriaria	+	+	+	-	-	-	-

+= Growth
- =No growth

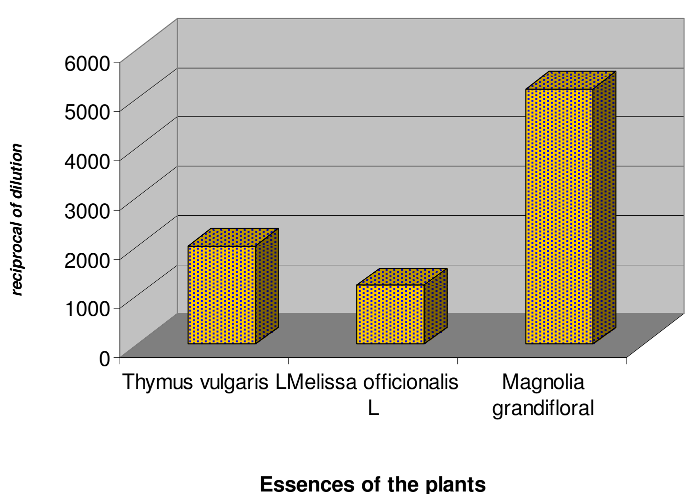

Figure 1. Comparative MICs of plant essence on *Streptococcus mutants.*

ondary compounds may account for the greater antimicrobial effects (Namba et al., 1982).

Phenol ring is a common part of the molecular structure of alkaloids existing in these plants. *T. vulgaris* possesses thymolocarvacrol, an isothymol, which is basically a phenol ring. Magnolol and hankiol have two phenol rings in their chemical structures which have been found to exert bactericidal effects against *S. mutans* (Namba et al., 1982; Watanabe et al., 1973). There is great deal of Tannins in *R. corriaria* which hydrolyse to phenols. Hydrolysis of tannins yields in polyhydraulic phenols such as galic acid which is converted to pyrogalol. Protocatechuic acid is converted to catechol, ellagic acid and other phenols. Phenolic compounds are also present in the essence of *Melissa officinalis.* Watanabe et al. (1975) found that these compounds exert antiviral properties. A phenolic ring exists in R chain of Penicillin G which is a potent antibacterial against oral streptococci. It can be inferred

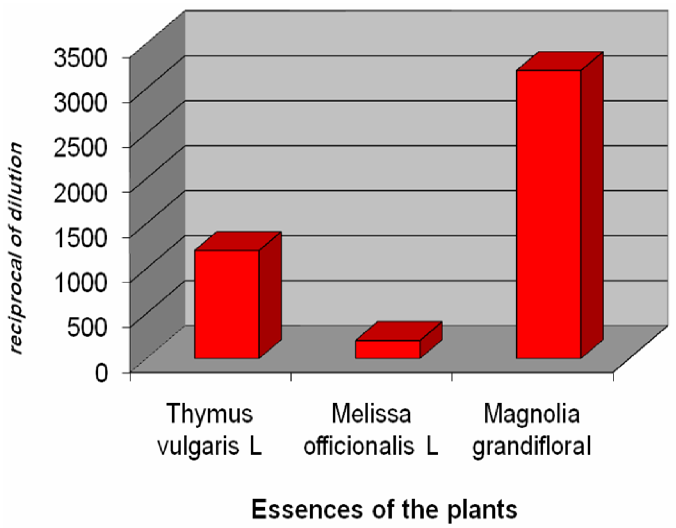

Figure 2. Comparative MBCs of plant essences on *Streptococcus mutants*.

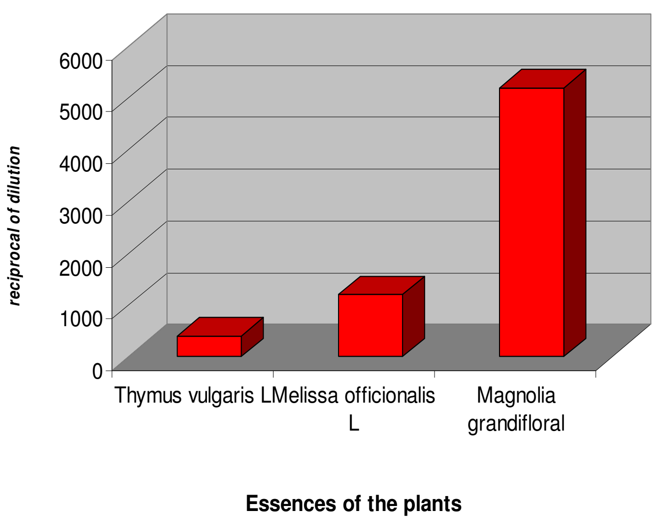

Figure 3. Comparative MICs of plant essences on *Streptococcus sanguis.*

that phenolic compounds may be responsible for antibacterial property of essence and alcoholic extracts. The essence of *M. grandiflora* had the greatest antibacterial effects with pleasant frangrance over the other extracts. However, alcololic extract of *R. coriaria* demonstrated the highest antibacterial effects. Its effective substance had been found to exert bactericidal effects on *S. mutans* (MBC: 50 - 75 µg/ml) within 5 min of contact (Namba et al., 1982; Watanabe et al., 1973; Watanabe et al., 1975). This essence may stimulate increased salivary secretion with resultant high antimicrobial effects which inhibit bacterial plaque formation and, as a result, minimize or prevent dental caries and periodontal diseases. It is recommended that essence of *M. grandiflora* added to toothpastes as earlier suggested by Watanabe et al. (1983).

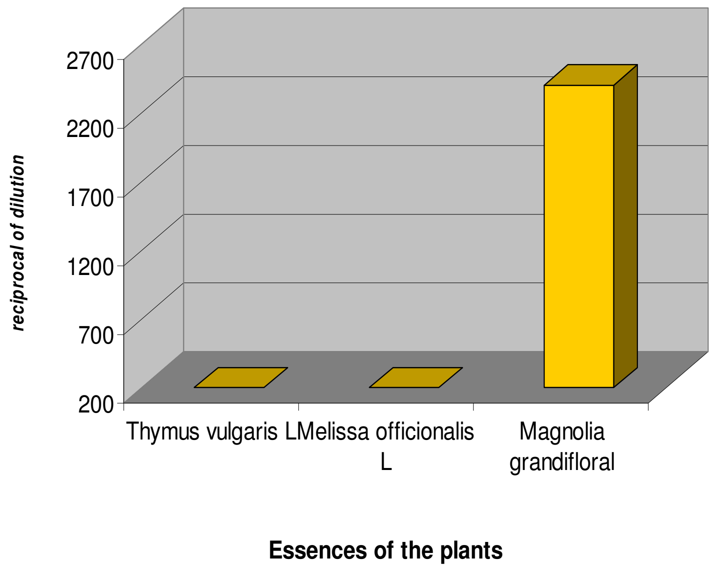

Figure 4. Comparative MBCs of plant essences on *Streptococcus sanguis*

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