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Antimicrobial and Antiinflammatory Potential of the Swedish Herbs Extract

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Research Article

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ABSTRACT

Oral conditions that produce the greatest damage on individuals are cavities and periodontal disease, hence non-expensive and effective solutions are immediately required, particularly for communities with no access to dental services. The antimicrobial and anti-inflammatory potential of the Swedish bitter herbal extract was evaluated, using pure microbial cultures and clinical samples of 29 patients. It was observed that the extract caused significant (p<0.05) *in vitro* growth inhibition of up to 29%, 17%, 15%, and 50% against *Prevotella intermedia, Bacteroides forsythus, Porphyromonas gingivalis* and *Streptococcus intermedius,* respectively. In addition, the extract significantly (p<0.05) inhibited oral flora growth in patient samples showing MICs of < 7.8 μg/ml in 21% of the patients, 15.6μg/ml in 17% of the patients, 31.2 μg/ml in 10% of the patients, 62.5 μg/ml in 17% of the patients, 125 μg/ml in 3% of the patients, and 250 μg/ml in 7% of the patients, and induced a maximum of 75% growth inhibition, as measured by the MTT reduction assay. The extract was also observed to significantly suppress production of the inflammatory marker nitric oxide by LPS-treated murine peritoneal macrophages. The Swedish herbal extract may be considered in the clinics to

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prevent or treat bacterial oral infections and at the same time reducing inflammation.

Keywords: Bacteria; inflammation; medicinal plants; oral infection; Swedish herbal extract;

1. INTRODUCTION

A number of traditional medicine products contain plant compounds. To date, complementary medicine continues to be essential to solve people´s health problems regardless of gender, marital status, education, socioeconomic status, place of residence and religious affinity (Dev, 2010; Ben-Arye et al., 2009).

Oral pathologies such as cavities and periodontal disease are among the most prevalent worldwide and are the cause of increased tooth decay (Petersen et al., 2005). Unattended oral disease may progress to oral partial or permanent disability, which currently represents 40% of the causes that require medical attention that is commonly offered privately, making it inaccessible to most of the population (Petersen et al., 2005).

Oral diseases impact people´s quality of life and may lead to systemic and threatening diseases. The relationship between the high incidence of oral diseases and microorganisms is well known and because of the increased bacterial resistance to antibiotics, toxic and harmful effects of some common antibacterial agents, and economical issues, there is a continuous need for alternative therapies that are affordable, not toxic and effective, such as plants (Palombo, 2009; Rishton, 2008).

In the present study, the antimicrobial and anti-inflammatory effect of the Swedish herbal extract were evaluated. This extract, also known as *Swedish Bitters,* is a traditional herbal ancient tonic rediscovered in the 18th Century by Dr. Claus Samst and Dr. Urban Hjärne. It is claimed to cure many illnesses, including gastrointestinal and inflammatory disorders, but without any scientific validation. It is made from the combination of *Aloe vera* (aloe), *Commiphora myrrha* (myrrh), *Sassafras albidum* (saffron), *Senna* sp. (senna), *Cinnamomum camphora* (camphor), *Rheum rhabarbarum* (rhubarb roots), *Tamaris gallica* (manna), *Theriac venezian* (theriac venezian), *Carlinina acaulis* (carline thistle roots), and *Angelica archangelica* (angelica roots). Significant *in vitro* (against *Prevotella intermedia, Bacteroides forsythus, Porphyromonas gingivalis* and *Streptococcus intermedius*) and oral bacteria (from 29 patients) growth inhibition of the Swedish herbs extract was observed. In addition, it was shown that this extract inhibited nitric oxide production by macrophages, thus suggesting the anti-inflammatory potential of the extract.

2. MATERIALS AND METHODS

2.1 Reagents and Culture Media

RPMI 1640 and AIM-V media were obtained from Life Technologies (Grand Island, NY). Fetal bovine serum (FBS), lypopolysaccharide (LPS) from *Escherichia coli* 0111:B4 strain, sodium dodecyl sulfate (SDS), N, N-dimethylformamide (DMF), and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO). The Swedish bitter herbal extract was obtained from “La Botica del Señor” (Ciudad de

México, D.F.). Tryptic soya (TS) broth was purchased from Difco Laboratories (Detroit, Mich). Extraction buffer was prepared by dissolving 20% (wt/vol) SDS at 37°C in a solution of 50% each DMF and demineralized water, and the pH was adjusted to 4.7.

2.2 Bacterial Strains and Culture

The bacterial strains *Porphyromonas gingivalis* (ATCC 33277)*, Prevotella intermedia* (ATCC 25611), *Bacteroides forsythus* (ATCC 43037), and *Streptococcus intermedius* (ATCC 27335) were provided by Dr. Stanley Holt, Department of Microbiology and Periodontics, Texas Health Science Center, University of Texas at San Antonio. They were incubated in trypticasein-soy agar supplemented with 5% lamb blood at 37°C in an oxygen-free atmosphere, and stored in the fridge until use. They were preserved by freezing suspensions at -70°C in skimmed milk supplemented with a cryoprotectant. For the experiments, bacterial suspensions (pure cultures and patient samples) were inoculated in TS broth and incubated for 7 days at 37°C, after which they were placed in the fridge. For activation, 100 µl of the suspension were placed in 5 ml of TS broth and were incubated at 37°C for 24 h.

2.3 Preparation of the Swedish Herbal Extract

The extract was first paper-filtered (Whatman International Ltd., Maidstone, England) and then lyophilized (Labconco Corporation, Kansas City, MO). A 10-ml stock solution at 1 mg/ml was then prepared in TS broth (for bacterium cultures) or complete AIM-V medium (for macrophage cultures), and sterilized by filtering through a 0.22 µ–membrane (Millipore, Bedford, MA). Dilutions were made at appropriate concentrations in these media.

2.4 Selection of Patient Parameters

The individuals participating in the study group (29 patients) were selected using the following inclusion criteria: males or females, first-visit patients or further visit patients not having received any treatment for cavities or periodontal disease, cavities and periodontal disease patients, and patients who have approved the present study protocol. Samples were aseptically collected from the subgingival crevices using a periodontal probe introduced into the periodontal pocket, and from grooves and fissures of the chewing sides of patient teeth with cavities.

2.5 Antimicrobial Activity of the Swedish Herbal Extract

We selected *Prevotella intermedia, Bacteroides forsythus, Porphyromonas gingivalis* and *Streptococcus intermedius* species because they are clinically relevant, particularly in oral pathologies. We determined the percentage of microbial growth inhibition by the extract in liquid medium by a colorimetric technique (Gomez-Flores et al., 2005, 1997), against pure culture bacteria and patients-recovered bacterial samples. After bacterial activation in TS medium, bacterial stock suspensions were prepared at a concentration of 1 x 10^3 bacteria/ml (Gomez-Flores et al., 2005, 1997). Next, 50 µl of the microbial suspensions were plated in TS broth in flat-bottomed 96-well plates (Corning Incorporated, Corning, NY) in the presence or absence of serial dilutions (1:2) of the Swedish herbal extract (50 µl), antibiotic control (3 mg/ml tetracycline), and vehicle control (culture medium). Plates were then incubated for 6h at 37°C, after which MTT was added to all wells at a final concentration of 0.5 mg/ml, and plates were incubated for 4 additional hours; we have selected this colorimetric technique, since it has been shown to be reliable, reproducible, highly sensitive, and convenient for

rapid routine susceptibility testing of bacteria (Gomez-Flores et al., 1995). At the end of the incubation period, 50μl of extraction buffer were added to all wells and plates were incubated overnight at 37°C. Optical densities resulting from dissolved formazan crystals were then read in a microplate reader (Beckman Coulter, Inc., Fullerton, CA) at 570 nm.

2.6 Animals

Six to eight-week old Balb/c female mice were purchased from Harlan Mexico S.A. de C.V. (Mexico, D.F.). They were kept in a pathogen- and stress-free environment at 24^0 C, under a light-dark cycle (light phase, 06:00-18:00 h), and given water and food *ad libitum*. Animals were euthanized by asphyxiation in a 100% CO_2 chamber.

2.7 Macrophage Cultures

Peritoneal macrophages were prepared by washing the peritoneal cavity with cold RPMI 1640 medium, and the resulting cell suspension was washed twice in this medium. One hundred-microliter cell suspensions at 1.7 x 10^6 cells/ml in complete RPMI 1640 medium were then plated in flat-bottomed 96-well plates (Becton Dickinson) for 2 h at 37°C. Non-adherent cells were removed, and adherent cells (about 70% of the input cells or about 1 x 10^6 cells/ml) were then used for determining nitric oxide production. The final adherent cell monolayer consisted of 95-99% macrophages as judged by Giemsa's stain procedures.

2.8 Nitrite Determination

Accumulation of nitrite in the supernatants of macrophage cultures was used as an indicator of nitric oxide production by LPS-activated cells. Peritoneal macrophages were incubated for 72 h in 200 □l AIM-V medium (since serum has been reported to activate macrophages (Chen et al., 1994), the culture medium was changed at this step to the serum-free medium AIM-V, which has been observed to support cell culture (Kaldjian et al., 1992), in the presence or absence of various concentrations of the Swedish herbs extract + LPS (20 ng/ml) in triplicates, in a total volume of 200 μl AIM-V medium. After incubation, supernatants were obtained and nitrite levels were determined with the Griess reagent (Gomez-Flores et al., 1997). Optical densities at 540 nm were then determined in a microplate reader (Beckman Coulter, Inc.) at 570 nm. Macrophage viability was determined by the MTT reduction assay as previously described (Gomez-Flores et al., 2005, 1997).

The percentage of viability was calculated as follows:

$$\% \text{ viability} = \frac{A_{570} \text{ in extract-treated cells}}{A_{570} \text{ in untreated cells}} \times 100$$

2.9 Statistical Analysis

The results were expressed as mean ± SD of triplicate determinations from three independent experiments. Statistical significance was assessed by the Student *t* test and one-way analysis of variance.

3. RESULTS

3.1 *In Vitro* Antimicrobial Activity of the Swedish Herbs Extract

The Swedish extract showed MICs of < 7.8μg/ml, 31.2μg/ml, 250μg/ml and < 7.8μg/ml, and induced a maximum of 29%, 15%, 15% and 50% growth inhibition against *Prevotella intermedia, Bacteroides forsythus, Porphyromonas gingivalis* and *Streptococcus intermedius*, respectively (Fig. 1a), whereas tetracycline (3 mg/ml) control caused 77%, 76%, 83% and 88% growth inhibition, respectively (Fig. 1b).

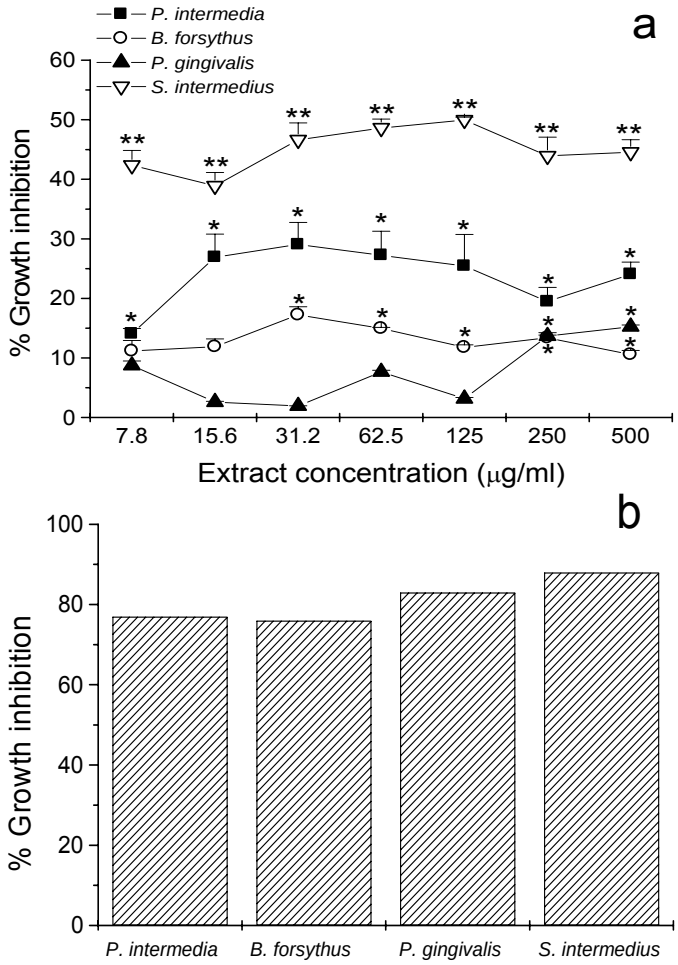

Fig. 1. ***In vitro* antimicrobial effect of the Swedish herbal extract.**
*Prevotella intermedia, Bacteroides forsythus, Porphyromonas gingivalis and Streptococcus intermedius suspensions were incubated in the presence or absence of various concentrations of the Swedish herbal extract (a) and tetracycline (3 mg/ml) (b), after which growth was measured colorimetrically, as explained in the text. Data represent means ± SD of triplicate determinations from three independent experiments. **p < 0.01, *p < 0.05 compared with extract-untreated control (culture medium). Optical density at 570 nm for untreated cells was 0.53 ± 0.06.*

3.2 Effect of the Swedish Herbs Extract on Patients Oral Flora

The extract showed an MIC of < 7.8μg/ml in patients 3, 6, 7, 8, 10, and 21 (21% of the patients), and induced a maximum of 38%, 29%, 56%, 65%, 53% and 21% growth inhibition, respectively (Table 1).

Table 1. Percent microbial growth inhibition of the Swedish herbal extract on patient samples

	Extract concentration (μg/ml)						
Patient	**7.8**	**15.6**	**31.2**	**62.5**	**125**	**250**	**500**
1	0[a]	0	0	0	0	0	0
2	0	0	0	0	0	4.6±0.6*	9±0.8*
3	38±3**	25±4**	35±4**	38±6**	38±3**	35±4**	38±3**
4	15±2	13±2	15±2*	31±3**	30±3**	31±2**	41±3**
5	9±4	37±15**	41±8**	54±9**	53±9**	53±5**	61±29**
6	16±2*	31±3*	26±3*	20±9*	29±3*	26±3*	28±5*
7	27±7*	34±10**	45±8**	49±18**	55±4**	54±3**	56±5**
8	20±7**	34±13**	62±8**	62±9**	64±5**	63±6**	65±5**
9	12±4	12±6	8±2*	19±3*	19±4*	23±5*	24±3*
10	26±12**	28±12**	35±15**	45±16**	50±9**	51±8**	53±4**
11	24±17	26±14*	42±26*	50±26**	54±28**	63±17**	64±12**
12	6±3	27±5*	30±7**	33±7**	32±5**	33±4**	35±5**
13	0	0	18±8*	34±20*	51±14**	57±8**	57±13**
14	1±0.5	7±3	6±2	32±10**	45±9**	55±6**	56±4**
15	5±2	22±11*	27±8**	43±9**	46±11**	51±4**	53±4**
16	0	0	8±3	21±9*	44±14**	53±14**	54±7**
17	0	0	14±7	34±10*	42±10*	54±9**	56±11**
18	0	0	6±3	31±10**	36±10*	44±8*	44±5*
19	0	24±12*	23±16*	27±10*	48±23*	56±11**	61±4**
20	8±5	3±2	26±17	32±15*	38±24**	55±27**	75±11**
21	16±3*	20±5*	16±5*	20±6*	16±3*	21±2**	17±2**
22	0	0	0	0	0	19±3*	19±4*
23	4±0.4	8±0.9	1±0.1	0	0	3±0.4	1±0.5
24	1±0.2	0	8±0.6	2±0.1	0.6±0.03	0.9±0.1	0
25	3±0.4	8±1	12±1	11±1	6±0.8	5±0.6	12±2
26	0	0	0	0	0	0	3±0.4
27	0	0.6±0.1	0.6±0.1	13±2	22±7*	31±6**	39±7**
28	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0

[a]*Values represent percent growth inhibition ± SD. *, p<0.01;**, p<0.05 compared with untreated control.*

The extract also showed an MIC of 15.6μg/ml in patients 5, 11, 12, 15, and 19 (17% of the patients), and induced a maximum of 61%, 64%, 35%, 53%, and 61% growth inhibition,

respectively (Table 1). Furthermore, the herbal extract showed an MIC of 31.2μg/ml in patients 4, 9, and 13 (10% of the patients), and induced a maximum of 41%, 24%, and 57% growth inhibition, respectively (Table 1).

The extract also caused an MIC of 62.5μg/ml in patients 14, 16, 17, 18, and 20 (17% of the patients), and induced a maximum of 56%, 54%, 56%, 44%, and 75% growth inhibition, respectively (Table 1). In addition, the herbs extract showed MICs of 125μg/ml in patient 27 (3% of the patients) and 250μg/ml in patients 2 and 22 (7% of the patients), and induced a maximum of 39%, 9% and 19% growth inhibition, respectively (Table 1). Overall, the extract inhibited bacterial growth from 86% of the patient samples (Table 1).

3.3 Effect of the Swedish Herbal Extract on Nitric Oxide Production

Nitrite levels in supernatants of LPS-stimulated murine macrophage cultures treated with the extract were significantly lower than the untreated controls. At concentrations ranging from 3.9 to 500 μg/ml, the Swedish herbal extract caused, respectively, significant ($p < 0.05$) 14% to 100% in nitrite levels, as compared with untreated control (Fig. 2). MTT reduction by macrophages treated with the extract significantly ($p<0.05$) increased (% viability) (Fig. 2).

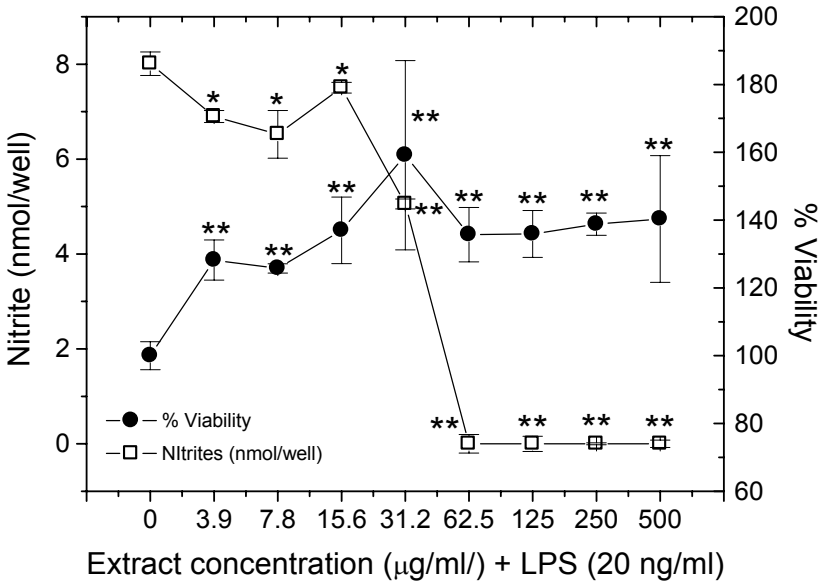

Fig. 2. Effect of the Swedish herbal extract on the production of nitric oxide by murine macrophages

*LPS-stimulated murine peritoneal macrophages were treated with the Swedish herbal extract (7.8-500 μg/ml) for 72 h, after which supernatants were collected to measure nitrite levels, as explained in the text. Data represent means ± SD of triplicate determinations from three independent experiments. **p < 0.01, *p < 0.05 compared with LPS-treated control.*

4. DISCUSSION

Alternative medicine is commonly included in therapeutic and diagnostic disciplines outside the conventional health system (Eisenberg et al., 1993). Oral diseases, particularly cavities

and periodontal diseases continue to be a major health problem worldwide (Petersen et al., 2005). Despite the progress in their prevention and treatment, their prevalence is considerably high in children and adults (Petersen et al., 2005); in developing countries, access to dental healthcare is restricted and expensive and thus it is limited to emergency dental care procedures (Petersen et al., 2005). The relationship between microorganisms, including species of *Streptococcus, Prevotella,* and *Porphyromonas* and dental diseases is well recognized (Jenkinson and Lamont, 2005; Loesche, 2007; Tichy and Novak, 1998). In periodontal diseases, areas around the gingival crevice can be infected causing inflammation (Loesche, 2007).

The need for affordable, effective, and non toxic alternatives has lead to the search for compounds from natural sources such as plants (Prabu et al., 2006), which may overcome the high incidence of oral diseases, the toxicity of current treatments, the microbial resistance to antibiotics, and the economical impact of conventional therapies, particularly in developing countries (Badria and Zidan, 2004; Tichy and Novak, 1998; Bidault et al., 2007; Knoll-Köhler and Stiebel, 2002; Lachenmeier, 2008; McCullough and Farah, 2008; Neumegen et al., 2005; Rodrigues et al., 2009).

The list of what is considered complementary medicine constantly changes, because once it is established that a particular therapy is effective and safe, it must be incorporated into conventional health treatment (Sirois and Gick, 2002); however, it is important that the same rigorous scientific evaluation used to assess conventional approaches be used to evaluate alternative medicine therapies (Prabu et al., 2006).

There are numerous scientific findings that confirm the enormous healing potential of plants that intervene in biological responses, such as wound repairing, immune response enhancing, and potentiating anticancer and antimicrobial activities (Palombo, 2009). In this rergard, garlic allicin has been reported to possess antibiotic activity against *Streptococcus mutans, S. sobrinus, Actinomyces oris, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum,* and *Porphyromonas gingivalis* (Bachrach et al., 2011). In addition, cinnamon bark oil, Papua-mace extracts and clove bud oil have been effectively used against oral bacteria (Saeki et al., 1989). Numerous studies on the use of plant extracts and active compounds as antimicrobial agents can found elsewhere (Palombo, 2009; Wilmes et al*.,* 2011).

In this regard, the present study showed a significant antibiotic effect of the Swedish herbs extract against pure cultures of *Prevotella intermedia, Bacteroides forsythus, Porphyromonas gingivalis* and *Streptococcus intermedius* and against oral flora from patients. Most known studies on the effect of plant extracts against disease-causing oral bacteria have been performed with pure cultures, using a variety of methods in solid and liquid media (Palombo, 2009). In addition, we showed that the extract showed significant concentration-dependent inhibition on nitric oxide production by LPS-activated macrophages, which suggests an anti-inflammatory potential of the extract. Inducible nitric oxide is synthesized by macrophages and mediates a variety of biological functions including antimicrobial action (Moncada et al., 1991, MacMicking et al., 1997). Nitric oxide may play a role in the pathogenesis of a variety of inflammatory diseases; thus, the amount of nitric oxide production, may correlate with the degree of inflammation. Sesquiterpenes (Reddy et al*.,* 2006), flavonoids (Paoletti et al*.,* 2009), polyacetylenes (Wang et al*.,* 2000), and lignans (Kim et al*.,* 2010) have been demonstrated to inhibit nitric oxide production by LPS-activated macrophages. Interestingly, the Swedish herbal extract was shown to stimulate MTT

reduction by macrophages, which may be an indication of reactive nitrogen intermediates-independent activation (Hyo-Jin, 2008), that requires further investigation.

5. CONCLUSION

Taken together, the results of the present study showed that the Swedish herbs extract possessed the following activities:

- Antimicrobial *in vitro* effect against *Prevotella intermedia, Bacteroides forsythus, Porphyromonas gingivalis* and *Streptococcus intermedius*
- Antimicrobial effect against patients oral flora, and
- Inhibition of nitric oxide production by LPS-activated macrophages.

The extract is then a promising candidate for the isolation and characterization of active compounds, which may have therapeutic potential for the prevention and treatment of oral infections and inflammation.

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CONFLICT OF INTEREST

Authors do not perceive or have any current conflicts of interest.

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