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In vitro antimicrobial activity of *Ruta chalepensis* methanol extracts against the cariogenic *Streptococcus mutans*

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Medicinal plants have been used for centuries and have become part of complementary medicine worldwide because of their potential health benefits. Since dental caries is one of the most common oral diseases and is considered a major public health problem, the present study evaluated *in vitro* antimicrobial potential of methanol extracts of *Ruta chalepensis* against the major etiologic agent of dental caries, *Streptococcus mutans*. The antimicrobial effect of *R. chalepensis* was evaluated in liquid medium by the dimethylthiazol-diphenyltetrazolium bromide (MTT) reduction colorimetric assay and in solid medium by the determination of colony forming units (CFU). We found that the minimum inhibitory concentration (MIC) was 250 µg/mL (p <0.05) in liquid medium and 3.9 µg/mL (p <0.05) in solid medium.

Key words: Antimicrobial agents, plant extracts, *Ruta chalepensis*, dental caries, *Streptococcus mutans.*

INTRODUCTION

Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide. The importance of plants to modern medicine is recognized; for a long time, natural remedies and medicinal plants were the main or even the only resource for the physicians. For all cultures and in all times, medicinal plants have been used as the basis of their own medicine (Nuñez, 1982). Among the many diseases afflicting the world's population, infections, both bacterial and fungal diseases with inflammatory processes, which in some cases incapacitate the sufferer, represent a major group (Drusano, 2004). There are several plants of the Mexican medicinal plants that exhibit antimicrobial activities and are used in treating various human diseases such as burns, diabetes, anti-inflamatory, skin diseases and hypertension (Pushpam, 2004).

Oral diseases are still a major health problem worldwide (Petersen et al., 2005). Problems such as oropharyngeal cancer and soft tissue injuries are considered as oral health problems, however, dental caries and periodontal disease are considered the most important ones (Petersen, 2003). Dental caries is a transmissible infectious disease that remains as a major public health problem in many developing countries and disadvantaged populations of developed countries (Mattos et al., 1998).

Streptococcus mutans is considered one of the main

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etiological agents of dental caries. The World Health Organization (WHO) estimates that five billion people worldwide suffer from tooth decay, which affects 60 to 90% of the school population and the vast majority of adults in developed countries. In Mexico it is estimated that 44% of the population have cavities or oral diseases that affect their health (Petersen, 2003).

In the present study, leaf methanol extracts of *Ruta chalepensis*, a plant of the Rutaceae family, was tested on *St. mutans* growth, the major etiologic agent of dental caries, which might be important for the development of alternative treatments in dental health.

MATERIALS AND METHODS

Preparation of *Ruta chalepensis* leaf methanol extract

R. chalepensis leaves were collected in the city of Aramberri in the State of Nuevo Leon, Mexico (Latitude: 24° 06´ 05´´ N, Longitude: 99° 51´19´´ W), they were rinsed to remove traces of dust and insects material, and allowed to dry at 37°C for five days; then the plant material was pulverized and stored in 50-mL Falcon tubes. Five grams of pulverized material were transferred to a 10 x 10 cm gauze and a pouch was formed, securing it with a wire to the rim of a beaker where 80 ml of methanol were placed, which were in contact with the pulverized leaves in the gauze. The methanol extraction was facilitated by stirring with a magnetic bar in a magnetic stirrer (Laboratory Stirrer PC-410, Corning, NY), and allowed to mix for 24 h at room temperature. One milliliter of the extract was distributed in Eppendorf tubes, previously weighed, and then they were dried in a vacuum concentrator (CentriVap Desiccator Labconco) for 4 h. Extracts were dissolved in culture medium and adjusted to experimental concentrations.

Effect of *R. chalepensis* methanol extract on *S. mutans* growth

Fifty microliters of 1 x 10^3 *S. mutans* bacteria/ml suspensions were plated in brain heart infusion broth (BHI) medium (Remel, Lenexa, KS), in flat-bottomed 96-well plates (Corning Incorporated, Corning, NY), in the presence or absence of serial dilutions (1:2) of 50 µl of *R. chalepensis* methanol extract), antibiotic control (1 µg/ml tetracycline), plant extract free-methanol vehicle control and culture medium (vehicle control was similarly processed as with plant methanol extractions, but without plant material). Plates were then incubated for 6 h at 37°C, after which the tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, St. Louis, MO) was added to all wells at a final concentration of 0.5 mg/ml in saline solution, and plates were incubated for 4 additional hours. At the end of the incubation period, 50 µl of extraction buffer [this buffer was prepared by dissolving 20% (wt/vol) sodium dodecyl sulfate (SDS) at 37°C in a solution of 50% each N,N-dimethylformamide (DMF) and demineralized water, and the pH was adjusted to 4.7 (SDS and DMF were purchased from Sigma-Aldrich)] were added to all wells and plates were incubated for 16 h at 37°C (Yamato IC600 incubator); optical densities resulting from dissolved formazan crystals were then read in a microplate reader (Beckman Coulter, Inc., Fullerton, CA) at 570 nm (Gomez-Flores et al., 1995). In regard to CFU determination, 50 µl of 1 x 10^3 *S. mutans* bacteria/ml suspensions were plated in BHI broth medium, in flat-bottomed 96-well plates (Corning Incorporated), in the presence or absence of serial dilutions (1:2) of the *R. chalepensis* methanol extract (50 µl), antibiotic control (3 mg/ml tetracycline), and vehicle controls (methanol and culture medium); the vehicle controls were similarly processed as with plant methanol extractions, but without plant material, similarly as mentioned above. Then, 1:10,000 dilutions were prepared from the wells and 100 µl were plated on BHI agar plates (Becton Dickinson, Mexico, D.F.) using sterile bent glass rods. Agar plates were then incubated at 37°C for 24 h and colonies were counted in a colony counter (ULB-100, Lightbox 37864-2000, Scienceware BEL-ART products, Pequannock, NJ) (Kansal et al., 1997).

RESULTS AND DISCUSSION

Inhibition of *Streptococcus mutans* growth by *Ruta chalepensis* methanol extracts

R. chalepensis methanol extract showed MICs of 250 µg/ml and 3.9 µg/ml, and induced a maximum of 63 and 94% growth inhibition against *S. mutans*, as measured by the MTT reduction (Figure 1a) and CFU methods, respectively (Figure 1b), whereas the vehicle control and medium alone (both free from plant extract) did not alter bacterial growth (data not shown). Medicinal plants have become part of alternative medicine worldwide because of their potential health benefits. These plants can be consumed or directly applied to treat infections (Rojas et al., 2006). Compounds synthesized by plants have a wide therapeutic potential due to their chemical constituents, for which the evaluation of their biological activity is important to develop new and alternative products with pharmacological potential and to validate treatments traditionally used by the Mexican population and other people from developing countries (Rodriguez-Fragoso, 2008).

Because of the increasing resistance of many pathogens to common therapeutic agents used today, such as antibiotics and antiviral agents, there is a renewed interest in the discovery of new compounds to treat systemic and oral diseases (Chinedum, 2005; Moreillon, 2000; Russell, 2000). Plant antimicrobials are not commonly used in a health program because of their low activity, unless their MICs are in the range of 0.1 to 1 mg/ml (Drusano, 2004); thus, the results of the present study may be an indication of an important antibiotic activity of *R. chalepensis* extracts.

Dental caries, periodontal disease, and tooth loss affect most of the population and can alter overall health. The value of medicinal plants to treat cariogenic bacteria is well known (Ramakrishna et al., 2011) and antimicrobial phytochemicals capable to treat oral diseases have been reported by many. In this regard, allicin from garlic has been shown to have antimicrobial activity against oral bacteria such as *Streptococcus mutans, Streptococcus sobrinus, Actinomyces oris, Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum* (Bachrach et al., 2011). In addition, compounds such as oleanolic acid, oleanolic aldehyde, linoleic acid, linolenic acid, betulin, betulinic acid, 5-(hydroxymethyl)-2-furfural, rutin, beta-sitosterol, and beta-sitosterol glucoside from raisins were shown to suppress *in vitro* adherence of *S. mutans* biofilm (Wu, 2009). Furthermore, *Dryopteris*

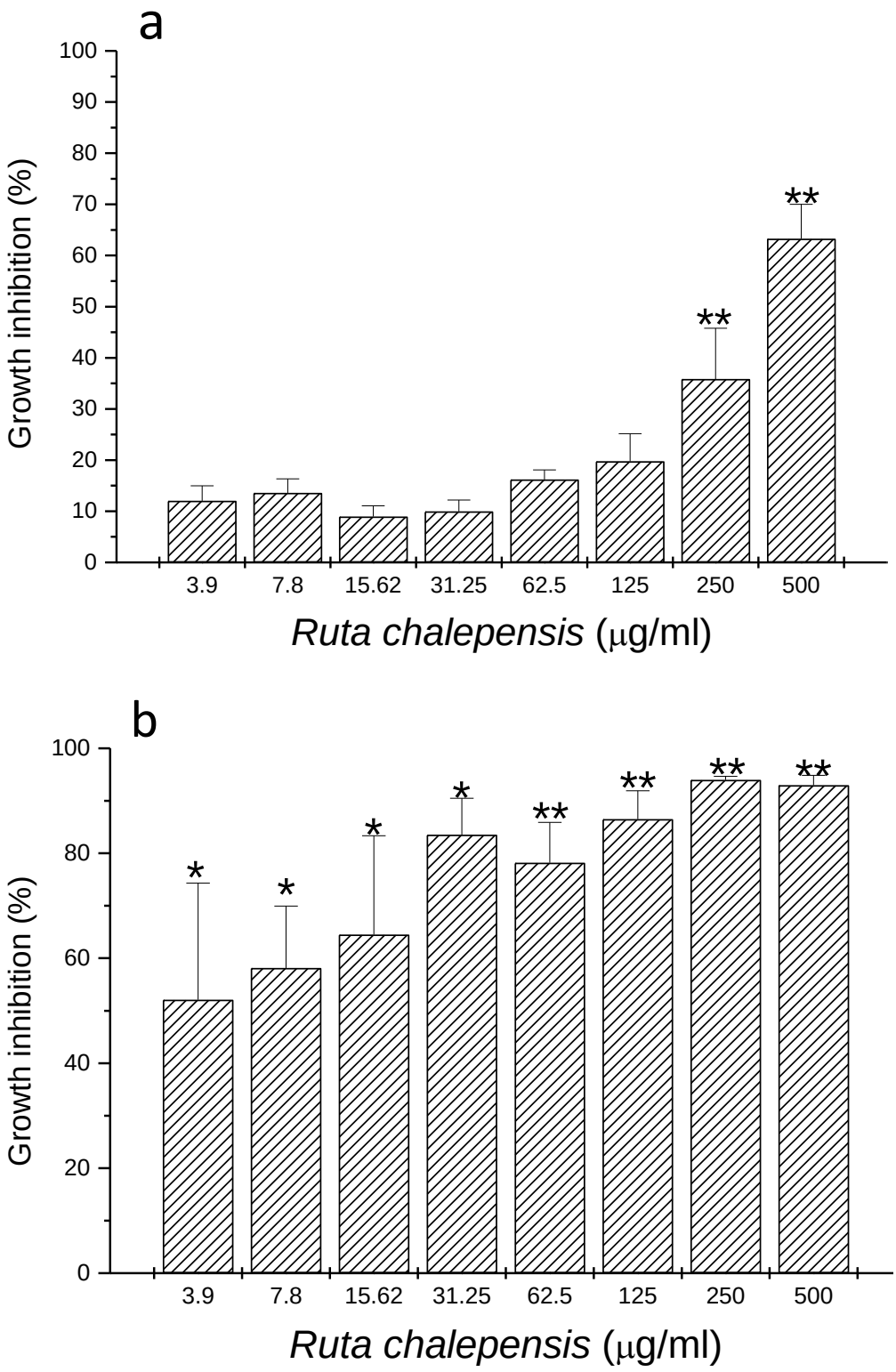

Figure 1. Antimicrobial effect of *R. chalepensis* leaves methanol extract on *S. mutans* (ATCC UA130 serotype *c*) growth. *S. mutans* culture suspensions ($1x10^3$ bacteria/ml) were incubated in the presence or absence of various concentrations of *R. chalepensis* methanol extract, after which growth was measured by the MTT reduction (*a*) and CFU (*b*) methods. Data represent means ± SEM of triplicate determinations from three independent experiments. $^{**}p < 0.01$, $^{*}p < 0.05$ when compared with *R. chalepensis* extract-untreated control. Optical density at 570 nm for untreated cells was 0.59 ± 0.05 for the MTT reduction technique, whereas CFU control value for untreated cells was $5.8 \times 10^8 \pm 68 \times 10^6$.

crassirhizoma and *Aloe vera* extracts were reported to have bactericidal and bacteriostatic activity against *S. mutans* (Ban et al., 2012; Fani and Kohanteb, 2012),

To our knowledge, this is the first report showing that *R. chalepensis* methanol extracts inhibit *S. mutans* growth *in vitro*. There are still a number of plant compounds that remain to be evaluated at the molecular, cellular and physiological levels for their potential to treat human diseases.

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