Brazilian Journal of Microbiology (2006) 37:75-77
ISSN 1517-8382

EVALUATION OF EFFICACY OF PRESERVATIVES ASSOCIATED WITH *ACHILLEA MILLEFOLIUM* L. EXTRACT AGAINST *BACILLUS SUBTILIS*

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Submitted: August 12, 2004; Approved: February 08, 2006

SHORT COMMUNICATION

ABSTRACT

The antimicrobial efficacy of three preservatives used in cosmetic formulations was evaluated. Phenova® and imidazolidinyl urea inhibited the growth of *Bacillus subtilis* when added to leaf extract of *Achillea millefolium* L., whereas 0.2% Nipagin®/ Nipasol® in propylene glycol did not.

Key words: *Achillea millefolium* L., preservatives, antibacterial activity, *Bacillus subtilis*

The antiseptics used in the formulation of toiletries should be distinguished from preservatives, the former being intend to render the product active against microorganisms present on the skin, scalp or in the mouth, whereas the function of preservatives, often the same antibacterial agent, is to maintain the product in a satisfactory condition during its shelf life and use (12).

Achillea millefolium L., belonging to the Asteraceae family (formerly Compositae), is native to Europe, North America, Southern Australia, Asia and widely present in the Brazilian flora. It is also known by names such as "mil-em rama, milefolio, erva-do-carpinteiro (1); Achillee millefeuille, millefoli herba, milfoil and yarrow" (14). *A. millefolium* L, a dark green perennial with tough stems, 30 – 90 cm high with abundant, long, pinnate leaves and pink or white flowers, has been used, mainly in Herbal and Homeopathic medicine, for a wide variety of purposes: diaphoretic, anti-swelling, antitumoral, antibacterial, anti-hypertensive and cicatrizing (3,14). However, the study of these properties is not sufficient and conclusive yet (6).

Achillea millefolium L. contains essential oils such as borneol, chamazulene, alfa- and beta-pinene, trans-nerolidol, cineol, camphor, beta-caryophyllene, guaiol, proazulen, eucalyptol (5,10). It also has flavonoids like apigenin, luteolin and rutin; amino-acids; sugars; tannins; mucilage; resins; alkaloids; hydroxyl coumarins; salicylic and caffeic acids; phytosterol and beta-sitosterol; besides sesquiterpenic lactones (8).

The objective of this work was to analyze the efficacy of antimicrobial activity of preservatives associated to *A. millefolium* L extract in the inactivation of *Bacillus subtilis*, in a attempt to determine which preservative, among those commonly used in cosmetic preparations, can ensure the absence of *B. subtilis*, since the latter is a common contaminant in plant extracts, to which no attention has been given until now.

A. millefolium was collected in the Medicinal and Toxic Plant Garden, FCF-UNESP, Araraquara Campus. A voucher specimen of the plant (HRCB 35292) was deposited in the herbarium of the Instituto de Biociências, UNESP, Rio Claro Campus. The leaves of *A. millefolium* L. were air dried at 40ºC for 7 days under natural convection and homogenized in a blender. 10 g of dry powdered leaves were extracted in a volume of ethanol:water

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(7:3, v/v) to prepare 100 mL of ethanolic extract. A glycolic extract was prepared by diluting 8% of the ethanolic leaf extract in propylene glycol and it was shown in microbiological tests to be free of microorganisms. Half of the glycolic extract was divided into three aliquots, to which were added, respectively: Phenova® (phenoxyethanol plus parabens), imidazolidinyl urea, and Nipagin®/Nipasol® (methyl and propylparabens) (13) in propylene glycol solution, to achieve 0.2% of each one as the final concentration in the growth test (4).

The inoculum prepared to contaminate the extract was adjusted to the 0.5 Mc Farland scale (1-3 x 10^8 CFU/mL) in brain and heart infusion broth (BHI) with *Bacillus subtilis* ATCC 9372 previously grown at 37ºC for 24h. Part of each the three test solutions and the *A. millefolium* L. leaf extract, was immediately contaminated with 10^7 CFU/mL of *B. subtilis*. The *A. millefolium* glycolic extracts (100 µL), contaminated or not, were diluted in 1 mL of BHI broth to perform the antibacterial tests. In parallel, the following were incubated at 37ºC for 24h: microorganism-free *A. millefolium* L. glycolic and *B. subtilis* contaminated *A. millefolium* L. glycolic extract, with or without the preservatives being tested, propylene glycol, which was the solvent for the preservative, and BHI as a culture medium control. Bacterial growth was observed by the presence of turbidity, its absence showing a bacteriostatic effect. Next, a sample of each tube was removed and plated on Müeller-Hinton agar, which was incubated at 37ºC for 24h to observe bacterial growth as a test for bactericidal properties.

In Table 1 we can see that the previously contaminated extracts showed no microbial growth when Phenova® and imidazolidinyl urea were used as preservatives. The preservative imidazolidinyl urea has good bacteriostatic activity with at a concentration of 0.2%, a bactericidal effect only against gram-positive bacteria, such as *B. subtilis*, and in concentrations ranging from 0.2 – 0.5%, there is no antifungal activity (7,9,11). However, the *B. subtilis*-contaminated *A. millefolium* L. extract, in the presence of 0.2% Nipagin®/ Nipasol®, allowed microbial growth. These preservatives are frequently used in cosmetic at a concentration of 0.4% for the single ester; 0.8% for a mixture of esters and 0.18% methyl plus 0.02% propylparabens ester (7,9). No growth was obtained with microorganism-free extract in the presence of any preservatives or none.

Table 1. Evaluation of preservatives associated with *Achillea millefolium* L. extract against *Bacillus subtilis.*

Preservatives 0.2%	Without contamination	With contamination
Extract	/-/	+
Control Preservative 1	/-/	/-/
Control Preservative 2	/-/	/-/
Control Preservative 3	/-/	+
Propyleneglycol	/-/	+
Medium Control	/-/	+

a) (+) Bacterial growth on plate; b) / - / No bacterial growth; c) Preservative 1: Phenova®; d) Preservative 2: Imidazolidinyl urea; e) Preservative 3: Nipagin®/ Nipasol®.

Under the conditions used in this analysis, we conclude that 0.2% Nipagin®/Nipasol® mixture was not effective, during the 24h contact against this bacterium. However, under different assay conditions, these preservatives might show better activity against *B. subtilis.* These results are rather significant since *B. subtilis* may be present as a contaminant in plant extracts, in which it does not reveal itself by any modification in physico-chemical and organoleptic properties.

RESUMO

Avaliação da eficácia de conservantes associados a extrato de *Achillea millefolium* L. contra *Bacillus subtilis*

A eficácia antimicrobiana de conservantes empregados em formulações cosméticas foi avaliada usando Phenova® e Imidazolinidil uréia que inibiram o crescimento de *Bacillus subtilis* no extrato de *Achillea millefolium* L. e Nipagin®/ Nipasol® 0,2% em propilenoglicol não apresentaram efeito microbicida.

Palavras-chave: *Achillea millefolium* L., conservantes, atividade antibacteriana, *Bacillus subtilis*

REFERENCES

1. Balbach, A. As plantas curam, Vida Plena, Itaquaquecetuba, 1995, p.218.
2. Barbosa, A.D.; Valente, P.H.M. Atividade antimicrobiana de extratos fluidos de plantas medicinais brasileiras. LECTA – USF, 12, 153-163, 1994.
3. Isaac, V.L.B.; Donizetti-Netto, E.; Bosetto, J.C.; Santos, L.E.; Barbosa, R.G.; Couto, L.B.; Corrêa, M.A.; Moreira, R.R.D. Atividade antiinflamatória tópica de cremes contendo oleo essencial de *Achillea millefolium* L. (Asteraceae), in press.
4. Farmacopéia Brasileira. Atheneu, São Paulo, 1988, V.5.1.1 p.
5. Haggag, M.Y. Thin layer and gas chromatographic studies on the essential oil from *Achillea millefolium*. *Plant Med.*, 27, 361-366, 1975.
6. Jorge, L.I.F.; Pregnolatto, B.P.; Chicourel, E.L.; Zamariolli, L.A.; Graciano, R.A.S. Anatomy and evaluation of the antimicrobian activity *in vitro* of yarrow (*Achillea millefolium* L), *Rev. Ciênc. Farm.*, 20, 49-58, 1999.
7. Kabara, J.J. Cosmetic and Drug Preservation. Principles and Practice, Marcel Dekker, New York, 1984, p.599-683.
8. Lorenzi, H.; Matos, F.J.A. Plantas medicinais no Brasil. Nativas e Exóticas, Instituto Plantarum de Estudos da Flora Ltda, Nova Odessa, São Paulo, Brazil, 2002, p.129.

9. Moral, J. Cosmetic Microbiology: New Ingredients, New Preservation Strategies. *Cosm. Toil*., 107, 65-72, 1992.
10. Orav, A.; Kailas, T.; Ivask, K. Composition of the essential oil from *Achillea millefolium* L. from Estonia. *J. Essen. Oil Res*., 13, 290-294, 2001.
11. Orth, D.S.; Eck, K.S.D. Preservative efficacy testing of aqueous cosmetics and drugs without counting colonies. *Cosm. Toil.*, 116 (4), 41-48, 2001.
12. Orth, D.S.; Kabara, J.J. Preservative-free and self-preserving cosmetics and drugs. *Cosm. Toil*., 113, 51-58, 1998.
13. Pinto, T.J.A.; Kaneko, T.M.; Ohara, M.T. Controle Biológico de Qualidade de Produtos Farmacêuticos, Correlatos e Cosméticos, Atheneu, São Paulo, 2000, p.309.
14. Sweetman, S.C. (Ed). 33rd. Martindale: The complete drug reference. Pharmaceutical Press, London, 2002, p.1572.