ORIGINAL ARTICLE

Evaluation of antimicrobial and antiplatelet aggregation effects of *Solidago chilensis* Meyen

Liz Rafael, Neiva Teresinha, J. C. Moritz, I. G. Maria[1], M. Dalmarco Eduardo[2], S. Fröde Tânia
Departments of Clinical Analyses and [1]Pharmaceutical Sciences, Center of Health Sciences, Federal University of Santa Catarina, Campus Universitário - Trindade, 88040-970, Florianópolis, [2]Department of Pharmaceutical Sciences, Center of Health Sciences, University of Blumenau, Blumenau, SC, Brazil

Solidago species have been used in popular medicine for the treatment of several inflammatory conditions. The aim of this study is to evaluate both the antimicrobial and antiplatelet effects of *Solidago chilensis* Meyen rhizome aqueous extract and its derived fractions using *in-vitro* models. The antimicrobial analysis was performed against *Staphylococcus aureus* (ATCC 25922), *Escherichia coli* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853). The broth microdilution method was used to determine the minimal inhibitory concentration (MIC). The measurement of platelet aggregation was determined by turbidimetric methodology. Significant differences were determined by analysis of variance (ANOVA), Dunnett's or Student's t tests. Values of $P < 0.05$ were considered significant. The aqueous extract and its derived fractions prevented the growth of all the three tested microbial species. Furthermore, these extracts also significantly inhibited platelet aggregation (% of inhibition: AE: 45.0±4.0%, BuOH: 29.6±3.1% to 13.8±2.6%, and AR: 41.7±4.2%). *Solidago chilensis* Meyen rhizomes demonstrated important antimicrobial and antiplatelet aggregation activities, which may underlie their beneficial effect on bacterial infection and atherothrombotic diseases.

Key words: Antimicrobial effect, antiplatelet activity, *Solidago chilensis* Meyen

INTRODUCTION

Solidago species have been used in folk medicine for the treatment of various inflammatory and infectious processes.[1] Studies using *in-vitro* and *in-vivo* models have demonstrated that plants of this genus have important antimicrobial, analgesic, antineoplasic and antioxidant effects.[2-5]

In South America, the most abundant species is *Solidago chilensis* Meyen sinonimia *Solidago* microglossa (Asteraceae) and it is widely used in the popular medicine of several countries of this continent. In Brazilian popular medicine, it is recommended as a diuretic, analgesic and anti-inflammatory to treat burns and rheumatic disease, among other conditions.[6] There are few pharmacological studies demonstrating the biological activity of this species, although one study reports antimicrobial activity.[2]

Furthermore, in the case of *Solidago chilensis* Meyen, despite its wide utilization and presence in the Brazilian Pharmacopoeia, the pharmacological and chemical investigations are rather scarce.[7]

From the chemical point of view, flavonoids and their derived compounds have been isolated from the *Solidago* species.[8] Besides their anti-inflammatory effect, flavonoids have been demonstrated to have important antimicrobial and antiplatelet aggregation properties.[9-11] The principal compounds isolated from these species are: quercetin and quercitrin (3-rhamnosylquercetin).[12,13] The glycosylated flavonoid quercitrin has been reported as one of the main components in the aerial parts of *Solidago chilensis*.[14] Other compounds have been isolated from the *Solidago* species, such as, sesquiterpenes, labdane diterpenes, saponins, chlorogenic acid and caffeic acid.[13,15-18] Some of the mentioned compounds have antimicrobial and/or antiplatelet aggregation activities.[19-28]

In the present study we undertook to evaluate the potential antimicrobial effects against micro-organisms implicated in infectious diseases, as well as the platelet anti-aggregation activity of the aqueous extract and its derived fractions obtained from *Solidago chilensis* Meyen rhizomes.

MATERIALS AND METHODS

Plant Material

The rhizomes of *Solidago chilensis* Meyen were collected in Caibi, Santa Catarina State, Brazil. They were collected in March 2005, and were identified by the botanist Prof. Dr. Daniel Falkenberg of the Department of Botany at the Federal University of Santa Catarina,

Address for correspondence: Prof. T. S. Fröde, Department of Clinical Analyses, Center of Health Sciences, Federal University of Santa Catarina, Florianópolis, SC, Brazil. E-mail: saleh@ccs.ufsc.br.

Received: 02-09-2008; **Accepted:** 08-10-2008; **DOI:** 10.4103/0973-8258.49372

Florianópolis, SC, Brazil, where the voucher specimen was conserved under reference number FLOR34674.

Preparation of the Extract and its Derived Fractions, and Isolated Procedures for Compounds

Solidago chilensis Meyen rhizome was air-dried at room temperature for seven days. Subsequently, the powdered rhizome was filtered and concentrated with a rotaevaporator to obtain the aqueous extract (AE). AE was extracted using hot water at 90°C (plant : solvent, 1 : 10, w/v) under infusion for 10 minutes, yielding 12%. Thereafter, the extract was filtered and an aliquot was lyophilized (Edward® E-C Micromodulyo Freeze-Dryer, USA). Part of the aqueous extract of the rhizome was partitioned three times with 50 mL of n-butanol, resulting in the butanolic fraction (yield 12.9%) and aqueous residual fraction (yield 87.1%). These fractions were evaporated under reduced pressure at a temperature below 50°C, yielding dry residues, considered as the butanolic (BuOH) and aqueous residual (AR) fractions.

In accordance with results obtained in the *in-vivo* studies from the BuOH fraction of *Solidago chilensis*, some compounds were isolated.

The chemical composition of the BuOH fraction was analyzed by TLC. For TLC analysis, the following conditions were used: aluminum sheets coated with silica gel F254 (Merck), as adsorbent, toluene : chloroform : acetone (40 : 25 : 35 v/v/v) and ethyl acetate : isopropanol : H_2O : methanol : acetic acid (80 : 20 : 20 : 10 : 2 v/v/v/v/v) as the mobile phases and diphenylboryloxyethylamine (1%) in methanol (NP Reagent A) and anisaldehyde sulphuric acid as color reagents. The spots were observed under UV light at 254 and 366 nm. The caffeic and chlorogenic (5-o-caffeoylquinic) acids were used as authentic samples in the TLC analysis. Finally, to confirm the identification of these compounds HPLC analysis was carried out on Shimadzu (SCL-10A, LC-10AD model and SPD-10AV, UV detector) instrument. The presence of caffeic and chlorogenic acids in the BuOH fraction was compared with the reference substances.

Antimicrobial Assay

Micro-organisms and media

Tests were performed against the following micro-organisms: *Staphylococcus aureus* (ATCC 25922), *Escherichia coli* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853), acquired from the American Type Culture Collection (ATCC).

The identification of strains was confirmed by the use of biochemical profiles according to the recommendations of the Manual of Clinical Microbiology.[29] All organisms were maintained in brain-heart infusion (BHI medium) containing 30% (v/v) glycerol at 20°C. Before testing, the suspensions were transferred to trypticase soy broth, supplemented with 5% sheep blood (Difco) and aerobically grown overnight at 35°C. The inocula were prepared by adjusting the turbidity of the suspension to match the 0.5 McFarland standards on saline solution (0.9 %).

Minimal inhibitory concentration

The broth microdilution method was used to determine the minimal inhibitory concentration (MIC) of the *Solidago chilensis* rhizome aqueous extract and its derived fractions against the test micro-organisms as recommended by the National Committee for Clinical Laboratory Standards (NCCLS).[30] This test was performed on sterile 96-well microplates. The aqueous extract and its derived fractions were carefully prepared and transferred to each microplate well, in order to obtain a twofold serial dilution of the original extract (from 1 : 2 to 1 : 256 starting from the concentration of 50 mg/mL).

The inocula (10 µL), containing 5×10^5 CFU/mL of each micro-organism were added to each well. Bacterial solution was made in sterile water with absorbance from 0.08 to 0.10 within 620 nm (ELISA Plate Reader - Organon Tecknica, Roseland, New Jersey, USA). A number of wells were reserved in each plate as negative control (no inoculum added), positive control (no extract added) and reference drug control (inoculum with gentamicin from 100 to 0.1 µg/mL).[31] Plates were aerobically incubated at 35°C. After incubation for 18-24 hours, the bacterial growth was evaluated by addition of a methanol solution (5 mg/mL) of 2,3,5 triphenyltetrazolium chloride (TTC; Vetec), which was used to detect bacterial growth by a color change to red.

Antiplatelet Aggregation Assay

Isolation of platelets

The preparation of human platelets was performed as described previously.[32] In brief, nine parts of blood collected by venipuncture were drawn into one part of 3.8% trisodium citrate. Platelet-rich plasma (PRP) was prepared by centrifugation at 400 × g, for 6 minutes, at 22°C. Platelets were adjusted to 3.0×10^8 cells/mL with sterile saline.

Measurement of platelet aggregation

The platelet aggregation (PA) was determined by the turbidimetric method using a Chronolog aggregometer.[33] Aliquots of 400 µL of a human platelet suspension were transferred into a small cuvette and stirred at a constant speed of 180 × g at 37°C. The platelets were pre-incubated with the aqueous extract, different fractions of *Solidago chilensis* or vehicle (sterile saline) for 5 minutes at 37°C, before the addition of adenosine diphosphate (ADP-6 µM). The extent of aggregation (%) was recorded continually for 5 minutes after addition of the agonist.

Chemicals

The following drugs and reagents were used: glycerol and methanol (Synth, Diadema, SP, Brazil), trypticase soy broth (Oxoid, Basingstoke, UK), sheep blood (Newprov, Pinhais, PR, Brazil), adenosine diphosphate - ADP (Sigma, USA) and citrate (Fluka, Switzerland). Other reagents used were of analytical grade and were obtained from different commercial sources.

Data Analysis

Results are expressed as mean ± standard error of the mean (S.E.M.). Statistical evaluation by Student's t-test was performed when only two value sets were compared, and one-way ANOVA followed by Dunnett´s test was used when the data involved three or more groups. The computer software used was GraphPad Prism 3.0. Data were considered significant if the *P* value was $P < 0.05$.

RESULTS

Antimicrobial Effect

The aqueous extract (AE) of *Solidago chilensis* rhizome and its derived fractions demonstrated important antimicrobial activity against the following bacteria: *Pseudomonas aeruginosa* (ATCC 27853) with MIC values (AE: 3.1 mg/mL, BuOH: 12.5 mg/mL and AR: 6.2 mg/mL) [Table 1]. These extracts also inhibited the *Escherichia coli* (ATCC 25923) growth with an MIC value of 6.2 mg/mL for AE, BuOH and AR, and the Gram-positive bacterium *Staphylococcus aureus* (ATCC 25922) with MIC values (AE: 6.2 mg/mL, BuOH: 3.1 mg/mL and AR: 6.2 mg/mL). As expected, gentamicin (40-0.31 μg/mL) demonstrated an important antimicrobial activity with MIC values of 0.62, 1.25 and 0.62 μg/mL for *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*, respectively [Table 1].

Antiplatelet Effect

Regarding platelet aggregation induced by ADP, it was found that the aqueous extract (AE) of *Solidago chilensis* Meyen and its derived fractions was effective in inhibiting the platelet aggregation stimulated by ADP (6 μM) (% of inhibition AE: 400 μg/mL: 45.0±4.0%, $P = 0.00075$, BuOH: 50-400 μg/mL: 29.6±3.1 to 13.8±2.6%, $P = 0.0004$ and $P = 0.0002$, and AR: 400 μg/mL: 41.7±4.2%, $P = 0.00063$) [Tables 2-4].

DISCUSSION

Plants exhibit a wide range of biological effects. Furthermore, there are few reports in the literature elucidating the pharmacological activity of *Solidago chilensis* Meyen.

In this study we have demonstrated that *Solidago chilensis* Meyen has important an antimicrobial activity against both Gram-positive and Gram-negative micro-organisms that may be indicative of the presence of a broad spectrum of antibiotic compounds. In this study, the aqueous extract of *Solidago chilensis* Meyen was two-fold more effective in inhibiting the growth of *Pseudomonas aeruginosa* in comparison with the aqueous residual (AR) and four-fold in comparison with butanolic fraction (BuOH). However, the aqueous extract and its derived fractions (BuOH and AR) inhibited the growth of *Escherichia coli* at the same concentrations. Despite this, the butanolic fraction (BuOH) was two-fold more effective in inhibiting the growth of *Staphylococcus aureus* than AE and the aqueous residual fraction (AR).

Our results are in accordance with those of other studies that have demonstrated the antimicrobial effect of the *Solidago*

Table 1: Antimicrobial activitiy of *Solidago chilensis* Meyen and its derived fractions

Compounds	*P. aeruginosa* (ATCC 27853) (MIC – mg/mL)	*E. coli* (ATCC 25923) (MIC – mg/mL)	*S. aureus* (ATCC 25922) (MIC – mg/mL)
AE	3.1	6.2	6.2
BuOH	12.5	6.2	3.1
AR	6.2	6.2	6.2
Gentamicin[a]	0.62	1.25	0.62

The data represent the MIC values (mg/mL) of aqueous extract (AE), butanolic (BuOH) and aqueous residual (AR) fractions isolated from *Solidago chilensis* Meyen rhizomes; a = Reference drug (μg/mL) for NCCLS/CLSI strain

Table 2: Effect of aqueous extract of *Solidago chilensis* Meyen on the platelet aggregation induced by ADP-6 μM

	C	AE 400 μg/mL	AE 200 μg/mL	AE 50 μg/mL
Mean	69.7	45.0*	67.3	66.2
SEM	1.7	4.0	1.1	1.4
n	10	5	5	3

The values represent the percentage of inhibition of aqueous extract (AE) isolated from *Solidago chilensis* Meyen rhizomes; C = control of platelet aggregation induced by ADP (6 μM); *$P = 0.00075$

Table 3: Effect of butanolic fraction of *Solidago chilensis* Meyen on the platelet aggregation induced by ADP-6 μM

	C	BuOH 400 μg/mL	BuOH 200 μg/mL	BuOH 50 μg/mL
Mean	69.7	13.8**	17.6**	29.6*
SEM	1.7	2.6	1.4	3.1
n	10	5	5	5

The values represent the percentage of inhibition of butanolic fraction (BuOH) isolated from *Solidago chilensis* Meyen rhizomes. C = control of platelet aggregation induced by ADP (6 μM); *$P = 0.0004$; **$P = 0.0002$

Table 4: Effect of aqueous residual fraction of *Solidago chilensis* Meyen on the platelet aggregation induced by ADP-6 µM

	C	AR 400 µg/mL	AR 200 µg/mL	AR 50 µg/mL
Mean	69.7	41.7*	69.0	68.6
SEM	1.7	4.2	4.3	3.5
n	10	5	5	3

The values represent the percentage of inhibition of aqueous residual fraction (AR) isolated from *Solidago chilensis* Meyen rhizomes; C = control of platelet aggregation induced by ADP (6 µM); **P* = 0.00063

species.[1] Other studies have demonstrated that flavonoids, sesquiterpenes and labdane diterpenes and saponins have important antimicrobial activity.[10,21,24,27] There are rare studies that demonstrate the antimicrobial effect of chlorogenic acid and caffeic acid compounds, except those from Almeida *et al*. (2006) and Campos *et al*. (2003), who have shown that these phenolic compounds presented an important antimicrobial activity against Gram-negative and Gram-positive bacteria.[20,34] Campos *et al*. (2003), had also mentioned that the antimicrobial effect of chlorogenic acids may be due to their polar propenoic side chain.[34]

Our results also provided evidence that the aqueous extract of *Solidago chilensis* Meyen and its derived fractions (BuOH and AR) have an important antiplatelet property on account of the fact that they inhibit the ADP-induced aggregation platelets in human blood. These results are in accordance with those of the other studies which demonstrated that the flavonoid compounds isolated from many plants, including the *Solidago* species, inhibit platelet aggregation.[11] Nonetheless, it was shown that diterpenes have the antiplatelet activity of inhibiting cytosolic calcium mobilization in *in-vitro* studies.[25] Similar results demonstrated that diterpenoids isolated from Andrographis paniculata significantly inhibited thrombin-induced platelet aggregation in a dose- and time-dependent manner.[22] Other studies have also demonstrated that saponins are effective in inhibiting platelet aggregation induced by ADP or cyclopiazonic acid (CPA) by blocking the receptor-dependent Ca2+ channels, inhibiting the Ca2+ influx of human platelets, and/or inhibiting the production of leukotriene B4 (LTB4).[19]

In the case of *Solidago chilensis* Meyen, we have isolated some compounds identified as chlorogenic acid and caffeic acid from the BuOH fraction. Other *in-vitro* studies have shown that caffeic acid derivatives are significantly effective in inhibiting shear-platelet activation, besides inhibiting the platelet aggregation induced by collagen, arachidonic acid and ADP.[26,28] Moreover, studies have demonstrated that caffeic acid derivatives significantly and in a concentration-dependent manner, inhibit COX-1 activity of the lung homogenate of saline- or LPS-treated rats.[33] Moreover, it has also been shown that these compounds are effective in inhibiting COX-1 enzyme activity, which suppresses the expression of platelet activation.[35] Some of these antimicrobial and anti-aggregation platelet activities presented here may be attributed to these compounds, but further analysis needs to be carried out in order to clarify what other compounds are involved in these effects.

Our data support the interesting antimicrobial activity of *Solidago chilensis* Meyen aqueous extract and its derived fractions. The results may be indicative of the presence of a broad spectrum of antibiotic compounds in these fractions. Furthermore, the antiplatelet effect of *Solidago chilensis* has also been demonstrated.

Solidago chilensis Meyen may prove to be a valuable choice for studies targeted towards the development of new antimicrobial and antiplatelet agents.

ACKNOWLEDGMENT

The authors thank the University of Blumenau for their technical support.

REFERENCES

1. Melzig MF. Goldenrod: A classical exponent in the urological phytotherapy. Wien Med Wochenschr 2004;154:523-7.
2. Morel AF, Dias GO, Porto C, Simionatto E, Stuker CZ, Dalcol II. Antimicrobial activity of extractives of *Solidago* microglossa. Fitoterapia 2006;77:453-5.
3. Sampson JH, Phillipson JD, Bowery NG, O'neill MJ, Houston JG, Lewis JA. Ethnomedicinally selected plants as sources of potential analgesic compounds: Indication of *in-vitro* biological activity in receptor binding assays. Phytother Res 2000;14:24-9.
4. Gross SC, Goodarzi G, Watabe M, Bandyopadhyay S, Pai SK, Watabe K. Antineoplasic activity of *Solidago* virgaurea on prostatic tumor cells in an SCID mouse model. Nutr Cancer 2002;43:76-81.
5. McCune LM, Johns T. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. J Ethnopharmacol 2002;82:197-205.
6. Lorenzi H, Matos FJ. Plantas Medicinais no Brasil - Nativas e exóticas, Instituto Plantarum de Estudos da Flora. São Paulo, Brazil: 2002.
7. Brazilian Pharmacopoeia, Companhia Editora Nacional. São Paulo, Brazil: 1929.
8. Batyuk VS, Kovaleva SN. Flavonoids of *Solidago* canadensis and *Solidago* virgaurea. Khimiia Prirodnykh Soedinenii 1985;21:566-7.
9. Kim DW, Chi YS, Son KH, Chang HW, Kim JS, Kang SS, *et al*. Effects of sophoraflavanone G, a prenylated flavonoid from Sophora flavescens, on cyclooxygenase-2 and *in-vivo* inflammatory response. Arch Pharm Res 2002;25:329-35.
10. Mbaveng AT, Ngameni B, Kuete V, Simo IK, Ambassa P, Roy R, *et al*. Antimicrobial activity of the crude extracts and five flavonoids from the twigs of Dorstenia barteri (Moraceae). J Ethnopharmacol 2008;116:483-9.
11. Koleckar V, Brojerova E, Rehakova Z, Kubikova K, Cervenka F, Kuca K, *et al*. *In-vitro* antiplatelet activity of flavonoids from Leuzea carthamoides. Drug Chem Toxicol 2008;31:27-35.
12. Wu B, Takahashi T, Kashiwagi T, Tebayashi S, Kim CS. New

flavonoid glycosides from the leaves of *Solidago* altissima. Chem Pharm Bull (Tokyo) 2007;55:815-6.
13. Apáti P, Houghton PJ, Kéry A. HPLC investigation of antioxidant components in *Solidago* herba. Acta Pharm Hung 2004;74:223-31.
14. Gutierrez AB, Oberti JC, Juliani HR. Constituents of *Solidago chilensis* (Compositae). Anales. Asociación de Quimica del Argentina 1981;69:27-31.
15. Steliopoulos P, Wüst M, Adam KP, Mosandl A. Biosynthesis of the sesquiterpene germacrene D in *Solidago* canadensis: 13C and (2) H labeling studies. Phytochemistry 2002;60:13-20.
16. Bradette-Hébert ME, Legault J, Lavoie S, Pichette A. A new labdane diterpene from the flowers of *Solidago* canadensis. Chem Pharm Bull 2008;56:82-4.
17. Apáti P, Houghton PJ, Kite G, Steventon GB, Kéry A. *In-vitro* effect of flavonoids from *Solidago* canadensis extract on glutathione S-transferase. J Pharm Pharmacol 2006;58:251-6.
18. Bader G, Wray V, Hiller K. The main saponins from the aerial parts and the roots of *Solidago* virgaurea subsp. virgaurea. Planta Med 1995;61:158-61.
19. Zeng FR, Yin SM, Xie SF, Nie DN, Ma LP, Feng JH, *et al*. Effects of 2A-1-1 on the aggregation and Ca2+ influx of platelets. Zhonghua Xue Ye Xue Za Zhi 2004;25:544-7.
20. Almeida AA, Farah A, Silva DA, Nunan EA, Glória MB. Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria. J Agric Food Chem 2006;54:8738-43.
21. Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z, *et al*. Antimicrobial activity of saponins from Medicago sp.: Structure-activity relationship. Phytother Res 2006;20:454-7.
22. Thisoda P, Rangkadilok N, Pholphana N, Worasuttayangkurn L, Ruchirawat S, Satayavivad J. Inhibitory effect of Andrographis paniculata extract and its active diterpenoids on platelet aggregation. Eur J Pharmacol 2006;553:39-45.
23. Hsu HC, Yang WC, Tsai WJ, Chen CC, Huang HY, Tsai YC. Alpha-bulnesene: A novel PAF receptor antagonist isolated from Pogostemon cablin. Biochem Biophys Res Commun 2006;345:1033-8.
24. Kuzma Ł, Rózalski M, Walencka E, Rózalska B, Wysokińska H. Antimicrobial activity of diterpenoids from hairy roots of Salvia sclarea L.: Salvipisone as a potential anti-biofilm agent active against antibiotic resistant Staphylococci. Phytomedicine 2007;14:31-5.
25. Lee JJ, Jin YR, Lee JH, Yu JY, Han XH, Oh KW, *et al*. Antiplatelet activity of carnosic acid: A phenolic diterpene from Rosmarinus officinalis. Planta Med 2007;73:121-7.
26. Satake T, Kamiya K, An Y, Oishi Nee Taka T, Yamamoto J. The anti-thrombotic active constituents from Centella asiatica. Biol Pharm Bull 2007;30:935-40.
27. Djeddi S, Karioti A, Sokovic M, Koukoulitsa C, Skaltsa H. A novel sesquiterpene lactone from Centaurea pullata: Structure elucidation, antimicrobial activity, and prediction of pharmacokinetic properties. Bioorg Med Chem 2008;16:3725-31.
28. Natella F, Nardini M, Belelli F, Pignatelli P, Di Santo S, Ghiselli A, *et al*. Effect of coffee drinking on platelets: inhibition of aggregation and phenols incorporation. Br J Nutr 2008;28:1-7.
29. Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RA. Manual of Clinical Microbiology. 8th ed. Washington, DC: ASM Press; 2003.
30. National Committee for Clinical Laboratory Standards (CLSI), Performance standards for antimicrobial susceptibility testing, Fifteenth Informational Supplement M100-S15, Wayne, PA: 2005.
31. Drummond AJ, Waigh RD. Recent Research Developments in Phytochemistry 2000;4:143-52.
32. Born GV, Cross MJ. Aggregation of blood platelets. J Physiol 1964;168:178-95.
33. Rossi A, Longo R, Russo A, Borrelli F, Sautebin L. The role of the phenethyl ester of caffeic acid (CAPE) in the inhibition of rat lung cyclooxygenase activity by propolis. Fitoterapia 2002;73:30-7.
34. Campos FM, Couto JA, Hogg TA. Influence of phenolic acids on growth and inactivation of Oenococcus oeni and Lactobacillus hilgardii. J Appl Microbiol 2003;94:167-74.
35. Park JB. Caffedymine from cocoa has COX inhibitory activity suppressing the expression of a platelet activation marker, P-selectin. J Agric Food Chem 2007;55:2171-5.

Source of Support: Nil, **Conflict of Interest:** None declared.

Author Help: Reference checking facility

The manuscript system (www.journalonweb.com) allows the authors to check and verify the accuracy and style of references. The tool checks the references with PubMed as per a predefined style. Authors are encouraged to use this facility, before submitting articles to the journal.

- The style as well as bibliographic elements should be 100% accurate, to help get the references verified from the system. Even a single spelling error or addition of issue number/month of publication will lead to an error when verifying the reference.
- Example of a correct style

  Sheahan P, O'leary G, Lee G, Fitzgibbon J. Cystic cervical metastases: Incidence and diagnosis using fine needle aspiration biopsy. Otolaryngol Head Neck Surg 2002;127:294-8.
- Only the references from journals indexed in PubMed will be checked.
- Enter each reference in new line, without a serial number.
- Add up to a maximum of 15 references at a time.
- If the reference is correct for its bibliographic elements and punctuations, it will be shown as CORRECT and a link to the correct article in PubMed will be given.
- If any of the bibliographic elements are missing, incorrect or extra (such as issue number), it will be shown as INCORRECT and link to possible articles in PubMed will be given.