Journal of Food Technology 3 (1): 30-34, 2005

Antimicrobial Activity of Phenolic Compound Extracts of Various Onions (*Allium cepa* L.) Cultivars and Garlic (*Allium sativum* L.)

[1]Noureddine Benkeblia, [2]Said Dahmouni, [1]Shuichi Onodera and [1]Norio Shiomi
[1]Department of Food and Nutrition Sciences, Graduate School of Dairy Science Research, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido 069-8501, Japan. [2]Department of Biology, Faculty of Science and Engineering Sciences, Mostaganem University, P.O. Box 300, 27000 Mostaganem, Algeria

Abstract: Antimicrobial activity of different concentrations (50, 100, 200, 300 and 500 mL L^{-1}) of phenolic compound extracts (PCEs) of three type of onions (green, yellow and red) and garlic against two bacteria; *Staphylococcus aureus*, *Salmomella* Enteritidis, and three fungi; *Aspergillus niger*, *Penicillium cyclopium* and *Fusarium oxysporum* was investigated. The PCEs of these *Allium* plants (garlic and onions) exhibited marked antibacterial activity, with garlic showing the highest inhibition and green onion the lowest. Comparatively, 50 and 100 mL L^{-1} concentrations of onions extracts were less inhibitory than 200, 300 and 500 mL L^{-1} concentrations. However, with garlic extract, high inhibitory activity was observed for all tested concentrations. *S. aureus* showed less sensitivity towards EO extracts inhibition, however *S.* Enteritidis was strongly inhibited by red onion and garlic extracts. The fungus *F. oxysporum* showed the highest sensitivity towards PCEs, whereas *A. niger* and *P. cyclopium* were significantly inhibited particularly at high concentrations. Conclusively, where food preservation is desired, onions and garlic PCEs extracts could be suitable for incorporating in various food products as natural antimicrobial additives.

Key words: Phenolic extracts; inhibition; *Allium cepa*; *Allium sativum*; bacteria; fungi

Introduction

At the present time, the *Allium* family has over 500 members, each differing in appearance, color and taste, but close in biochemical, phytochemical and neutraceutical content. *Alliums* were revered to possess anti-bacterial and anti-fungal activities, and contain numerous phenolic compounds which arouse great interests (Griffiths *et al.*, 2002; Rivlin, 2001). Onions and garlic are composed mainly of water (85 - 90 g 100 g^{-1} and 60 - 70 g 100 g^{-1} fresh weight respectively) and ones of the most significant components are the phenolic-containing compounds. Garlic and especially onions, which are a major source of phenolic compounds, has been reported to contain a wide range flavonols, quercetins and kaempferol (Bilyk *et al.*, 1984; Price and Rhodes, 1997). However, total phenolic levels in onions and garlic vary considerably and depend mainly on cultivars (Bilyk *et al.*, 1984; Price and Rhodes, 1997). In onion tissues, total phenolics vary from 200 to 2025 mg kg^{-1} fresh weigh (Benkeblia, 2000; Bravo, 1999; Kaur and Kapoor, 2002) while in garlic total phenolics 1450 mg kg^{-1} fresh weight (Kaur and Kapoor, 2002).
Plant phenolics, besides their physiological roles, have been shown to have biological activities, and recognition of the antimicrobial activities of many phenolic compounds has realigned thinking towards the technological benefits provided by many of these compounds (Thomàs-Barberàn *et al.*, 1990; Weidenbörner *et al.*, 1990). Recent investigations have also demonstrated an inhibitory effect by aqueous extracts on numerous bacterial and fungal species (Hsieh *et al.*, 2001; Sivam *et al.*, 1997; Ward *et al.*, 2002). During the fifty last years, protection of food from spoilers and pathogens aroused great interest, and was achieved by various physical and chemical methods. Among these numerous and abundant naturally occurring compounds, phenolic compounds have been considered as natural preservatives or food additives, and can be used as additional methods of controlling pathogens (Naidu, 2000). The aim of this investigation was to study the effect of phenolic compound extracts of various onion types and garlic on two major bacterial pathogens, and three fungal species usually causing rotting of *Allium* crops during their storage.

Materials and Methods

Onions and Garlic: Three type of onions (*Allium cepa*) – green onion (var. Blanc), yellow (var. Jaune d'Espagne) and red (var. Rouge Amposta), and garlic (*Allium sativum*) (var. Cristo) were selected for this investigation. Onions and garlic samples, free of any pre harvest chemical treatment (organic products), were freshly harvested, sorted for uniformity and absence of defects and stored at 4 °C prior analyses.

Microbial Strains : The microorganisms, maintained on Nutrient Agar (Merck, Darmstadt, Germany), were supplied by the microbiology laboratory of the university. The bacteria were selected because they are frequently reported

in food spoilage, while the selected fungi are commonly encountered in onions and responsible of bulb diseases. Two species of bacteria; *Staphylococcus aureus* (ATCC 11522) and *Salmonella* Enteritidis (ATCC 13076) and three species of fungi *Aspergillus niger* (ATCC 10575), *Penicillum cyclopium* (ATTC 26165) and *Fusarium oxysporum* (ATCC 11850) were used in this study.

Phenolic compounds extraction : Samples (100 g) were chopped in small pieces, homogenized in 100 mL of distilled water-EtOH (Merck, Darmstadt, Germany) solution (80:20, v/v) using a domestic blender (model MX-X61-W, National, Japan) during 1 min at medium speed, then homogenate was macerated during 15 min at 4°C. After homogenization, the mixture was pressed through two layers of cheesecloth, and the residue was extracted a second time in same conditions and the filtrate were combined. The total filtrate was concentrated on a rotary evaporator at 45°C. Then, to obtain a final yield of extraction (ratio final volume of extract/weight of fresh plant) of 1, the volume of PCEs were adjusted to 100 mL with sterile distilled water, thus obtaining the crude PC extracts used for antimicrobial tests. Each PC extraction was running in duplicate.

Preparation of inoculum : Bacteria inocula were prepared by growing cells in Brain Heart Infusion broth (Merck) for 24 h at 37°C. These cell suspensions were diluted with peptone water (Merck) to provide initial cell counts of about 10^5-10^6 CFU mL^{-1}. An aliquot of 1 mL is used for antimicrobial test. Fungi were cultured on YGCA medium (Merck), and a mycelia mass of 5 mm of diameter was used for antifungal test.

Antibacterial activity test : The antibacterial activity of the extracts was carried out by disc diffusion test. The concentrations tested were 50, 100, 200, 300 and 500 mL L^{-1}. Appropriate volume of PC extracts were added to sterile water (vol/vol) to obtain desired concentrations cited above. The Petri dishes containing Potato Dextrose Agar (PDA) medium (Merck) were used for antibacterial test. An aliquot of 1 mL was evenly spread on agar using a glass rod spreader. The Petri dishes were left at room temperature for 1 h to allow agar surface to dry. Sterile filters paper (Wathman No. 1, diameter 5 mm) were impregnated with PCEs of different concentrations and placed on the culture medium (PDA). For control, discs were impregnated with sterile water. After 30 min, plates were turned upside down and incubated at 37°C for 48 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its antimicrobial activity. Five discs per plate and three plates were used, and each test was run in duplicate.

Antifungal activity test : The antifungal activity was carried out in vitro, in Petri dish containing Yeast Glucose Chloramphenicol Agar (YGCA, Merck) as described by Lattenzio *et al.* (1994). The concentrations tested were the same as described above, but PCEs were added to YGCA medium. For control tests, sterile water (50, 100, 200, 300 and 500 mL L^{-1}, vol/vol) was added to YGCA medium. Then the fungi were inoculated immediately after preparation of the Petri dishes by placing in the center of each plate a 5 mm diameter of the mycelial mass of the cultivated test fungi, cut with a sterile cork borer from the periphery of growing cultures on YGCA plates prepared as described above. The Petri dishes were incubated in dark at 21°C and the diameter of the mycelial growth was measured. The incubation was stopped when the mycelial mass of control Petri dishes had almost filled the Petri dish (ca. 11-13 days). Diameter of the growth mass was determined by averaging the radial growth of the mycelial mass in two orthogonal directions. Three Petri dishes were used per test and each test was rune in duplicate.

Statistical analysis : All experiments were conducted in triplicate and tests were duplicated (two extractions). Experiment was conducted twice, and data were averaged and analyzed statistically by determination of least significant difference (LSD at $p < 0.05$) using GraphPad Prism 4 statistical software (GraphPad, San Diego, CA, USA).

Results

Antibacterial activity of PCEs : Onions and garlic PCEs exhibited different inhibition levels against *S. aureus* and *S.* Enteritidis as shown in Table I and II. In the dose response study, the inhibition zone increased with increasing concentration of extracts. Low concentrations (50 and 100 mL L^{-1}) inhibited moderately the development of bacteria; however *S.* Enteritidis was more sensitive than *S aureus*. At high concentrations (200, 300 and 500 mL L^{-1}), PCEs exhibited marked inhibition activity against bacteria, and inhibition of PCEs of garlic was strongest than those of onions PCEs. Comparatively, *S. aureus* was less sensitive to the inhibitory activity of the onions and garlic extracts than *S.* Enteritidis which was more inhibited at same concentrations of PCEs.

Antifungal activity of PCEs : Antifungal activity of PCEs on *Aspergillus niger*, *Penicillum cyclopium* and *Fusarium oxysporum* are shown in Fig. 1, 2 and 3. In dose response study, *A. niger* was less inhibited by low concentrations (50 mL L^{-1}) of PCEs of green and yellow onions, however higher concentrations exhibited marked

Table 1: Antibacterial activity of phenolic compound extracts (PCEs) of Allium plants against Staphylococcus aureus after 48 h.

	PCEs concentration (mL L^{-1})				
	50	100	200	300	500
Green onion	6.8 ±0.2	7.2 ± 0.7	7.5 ± 1.1	7.6 ± 0.5	9.3 ± 0.5
Yellow onion	6.5 ± 0.7	7.4 ± 0.3	7.5 ± 1.4	8.3 ± 1.8	9.2 ± 0.4
Red onion	7.1 ± 0.3	7.4 ± 0.2	9.0 ± 0.5	10.0 ± 0.3	10.1 ± 0.7
Garlic	8.3 ± 0.4	9.7 ± 0.8	10.0 ± 0.9	10.1 ± 0.7	10.2 ± 0.2

Zone of inhibition is expressed in mm.

Table 2: Antibacterial activity of essential oil extracts of *Allium* plants against *Salmonella* Enteritidis after 48 h.

	PCEs concentration (mL L^{-1})				
	50	100	200	300	500
Green onion	6.5 ± 0.7	6.9 ± 0.1	7.2 ± 0.7	7.3 ± 1.7	8.3 ± 0.9
Yellow onion	7.4 ± 0.2	8.3 ± 0.7	8.9 ± 0.4	9.2 ± 0.5	9.4 ± 0.4
Red onion	8.0 ± 1.9	8.3 ± 0.7	9.3 ± 0.2	10.0 ± 0.2	10.4 ± 0.4
Garlic	10.1 ± 0.5	10.3 ± 0.1	10.4 ± 0.5	15.3 ± 1.0	1.55 ±0.2

Zone of inhibition is expressed in mm

inhibition (Fig. 1). On the other hand, all concentrations of red and garlic PCEs showed strong inhibitory effect against *A. niger* and developments were close despite low concentrations. Statistically, no significant difference was observed between control and concentrations 50, 100 and mL L^{-1} of PCEs of green and yellow onions. Nevertheless, concentrations 300 and 500 mL L^{-1} of green and yellow onions, and all concentrations of red onion and garlic were significantly different. The sensitivity of *P. cyclopium* to PCEs of onions and garlic was globally higher than these of *A. niger*. However, PCE of red onion showed less inhibitory effect against *P. cyclopium* at low concentrations (50, 100 and 200 mL L^{-1}) (Fig. 2). On the other hand, similar and strong inhibitory effect of PCE of garlic on *P cyclopium* was observed and development was very low compared to control or other PCEs. Statistical analysis showed that 50, 100 and 200 mL L^{-1} concentrations of green and yellow onions were not significantly different. On the other hand, concentrations 300 and 500 mL L^{-1} of PCEs of onions, and concentrations 100, 200, 300 and 500 mL L^{-1} of PCE of garlic were significantly different. *Fusarium oxysporum* showed the highest sensitivity to PCEs which inhibited markedly its development (Fig. 3). On the other hand, similar strong inhibitory activity of PCE of garlic was noted. Statistical analysis showed significant difference between control and all concentrations of PCEs of onions and garlic.

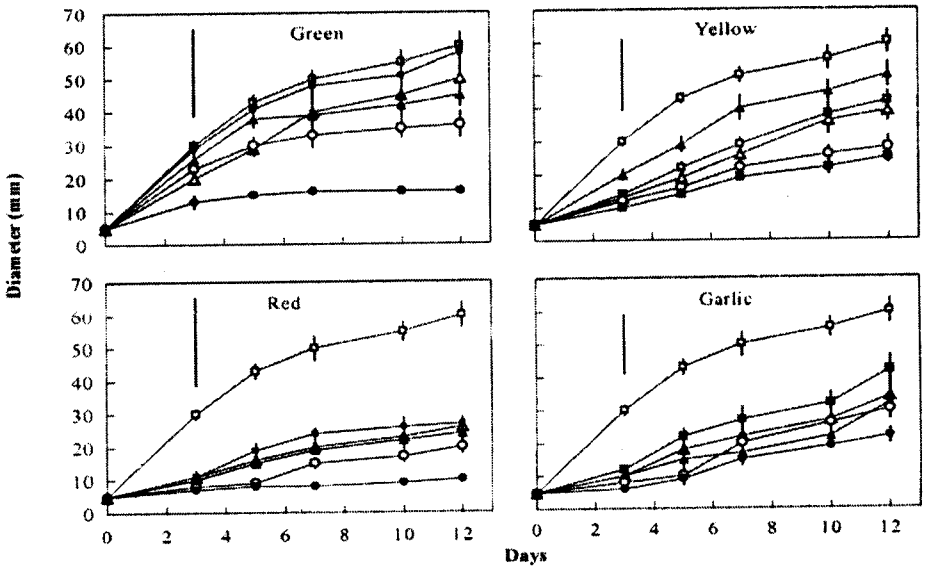

Fig. 1: Effect of phoenic compound extracts of onion and garlic on growth of Aspergillus nigey (□ control, ■ 50, △ 100, ▲ 200, ○ 300 and • 500 mL L^{-1} concentrations) (LSD at $p<0.05$).

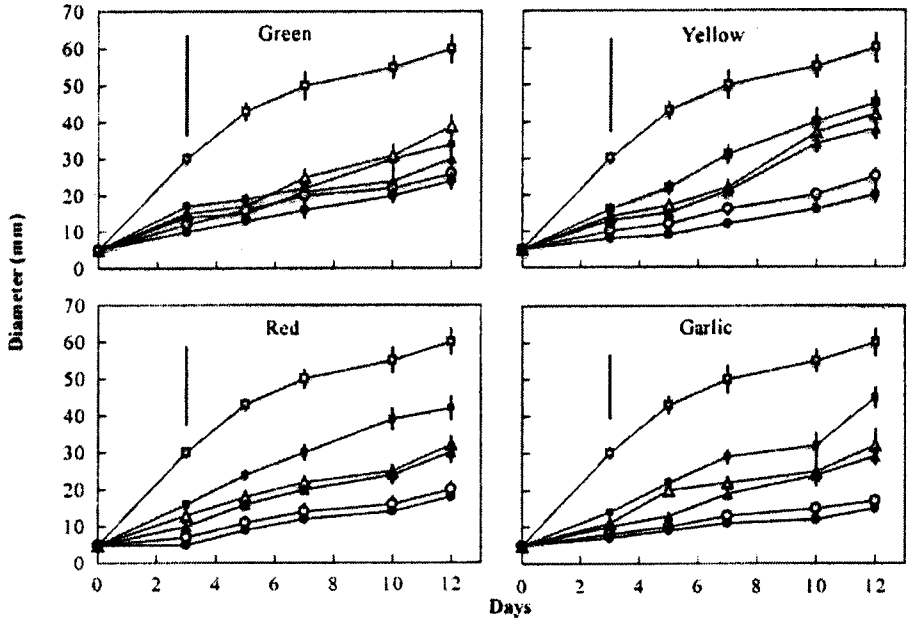

Fig. 2: Effect of phoenic compound extracts of onion and garlic on growth of Fusarium Oxysporum (□ control, ■ 50,△ 100,▲ 200,○ 300 and • 500 mL L^{-1} concentrations) (LSD at $p<0.05$).

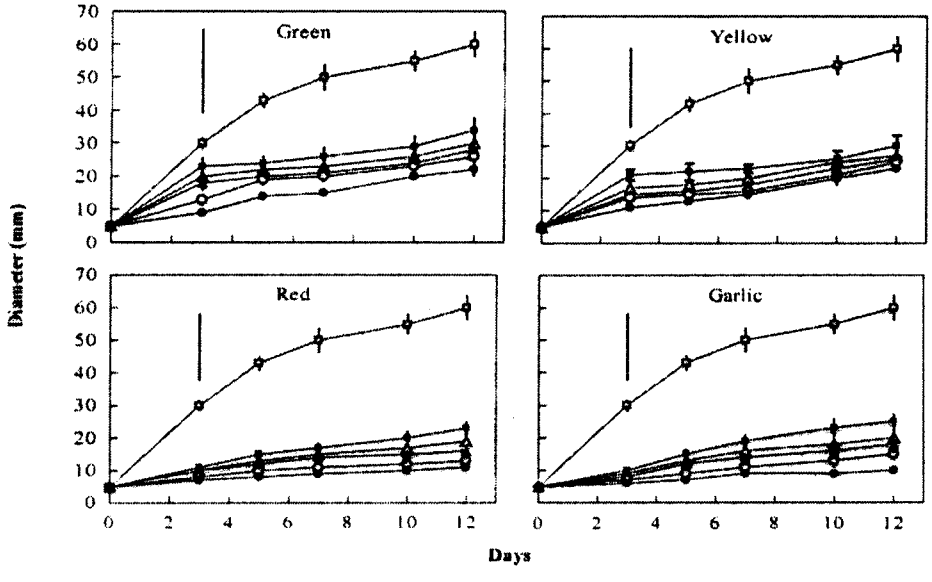

Fig. 3: Effect of phoenic compound extracts of onion and garlic on growth of Penicillium Cyclopium (□ control, ■ 50,△ 100,▲ 200,○ 300 and • 500 mL L^{-1} concentrations) (LSD at $p<0.05$).

Discussion

The inhibitory activity of phenolic compound extracts of onions or other *Allium* plants have not been widely investigated. Wymand and Van Etten (1977) tested six isoflavonoids which showed different inhibitory activities against 20 phytopathogenic and saprophytic species. Some phenolic compounds of *Helichrysum* species, identified as flavones derivatives, showed high antimicrobial activity (Thomàs-Barberàn *et al.*, 1990; Wang *et al*, 1989) and similar antibacterial activity of Chinese chive extract was observed (Hsieh *et al.*, 2001). Locher *et al.* (1995) also reported that some Polynesian plant extracts showed marked antibacterial activity against many pathogenic species including *S. aureus*, *E. coli* and *P. aeruginosa*.

Although the paper disk assay is a practical approach to study potential antibacterial compounds, using the size

of inhibition zone to indicate relative antibacterial activity of the phenolic compound is not adequate, because the zone must be affected by the solubility and rate of diffusion in agar medium; and thus the results could be affected. The inhibitory activity of phenolic compounds extracts of *Allium* plants against mould was also scarcely investigated; however phenolic compounds are in general more effective inhibitors of fungi than of bacteria (Naidu *et al.*, 2000; Zaika, 1988). Flavonoids possess marked inhibitory activity against some *Aspergillus* species (Weidenbörner *et al.*, 1990). In view of phytoalexin properties, isoflavonoids were the first phenolic compounds investigated for their antimicrobial activity (Van Etten, 1967), and different isoflavones were tested against *Aspergillus ochraceus*, *Penicillium digitatum* and *Fusarium culmorum* and these species showed differential susceptibilities to isoflavones (Kramer *et al.*, 1984).

Finally, its is concluded from the results of this investigation that phenolic compound extracts of common onions and garlic were found to inhibit bacterial and mould growth. However, the effectiveness of this inhibition was strongly related to the type of onions or garlic extracts used. The results also showed that a significant ($\tilde{n} < 0.05$) effect was obtained when concentrations of PCEs were above 20%. On the other hand, it will also be of interest to examine the different compounds of the extracts and their antimicrobial activities against a wide range of a commonly food contaminating bacteria and fungi to maintain their qualities. However, despite that *Allium* plants could be a potential source for inhibitory substances, to date unfortunately, the relative biochemical instability of the phenolics and related compounds, and the brown color resulting from their reactions seem to limit their use as a practical food additives or preservatives.

References

Bilyk, A., P. L. Cooper and G. M. Sapers, 1984. Varietal difference in distribution of quercetin and kaempferol in onion (*Allium cepa*) tissue. J. Agric. and Food Chem., 32: 274-276.

Kaur, C. and H. Kapoor, 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. Int. J. Food Sci. and Technol., 37: 153-161.

Locher, C. P., M. T. Burch, H. F. Mower, J. Berestecky, H. Davis, B. Van Poel, A. Lasure, D. A. Vanden Berghe and A. J. Vlietinck, 1995. Antimicrobial activity and anti-complement activity of extracts obtained from selected Hawaiian medicinal plant. J. Ethnopharmacol., 49: 23-32.

Tomà s-Barberàn, F., E. Iniesta-Sanmartn, F. Tomàs-Lorente and A. Rumbero, 1990. Antimicrobial phenolic compounds from three Spanish of Helichrysum species. Phytochem., 29: 1093-1095.

Sivam, G. P., J. W. Lampe, B. Ulness, S. R. Swanzy and J. D. Potter, 1997. Helicobacter *pylori* - in vitro susceptibility to garlic (Allium sativum) extract. Nut. Cancerol., 27: 118-121.

G. Griffiths, L. Trueman, T. Crowther, B. Thomas and B. Smith, 2002. Onions-A global benefits to health. Phytotherapy. Res., 16: 603-615.

H. D. Van Etten, 1967. Antifungal activity of pterocarpans and other selec. isoflavon. Phytochem., 15: 655-659.

J. G. Wyman and H. D. Van Etten, 1977. Antibacterial activity of selec. isoflavonoids. Phytopathol., 68: 583-589.

K. R. Price and M. J. C. Rhodes, 1997. Analysis of the major flavonol glycosides present in four varieties of onion (*Allium cepa*) and changes in composition resulting from autolysis. J. Sci. Food. and Agric., 74: 331-339.

L. L. Zaika, 1988. Spices and herbs: their antimicrobial activity and its determ. J. Food Safety, 9: 97-118.

L. Bravo, 1999. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nut. Review, 56: 317-333, 1999.

M. Weidenbörner, H. Hindorf, C. H Jha and P. Tsotsonos, 1990. Antifungal activity of flavonoids against storage fungi of the genus Aspergillus. Phytochem., 29: 1103-1105.

Naidu, A. S., Natural food antimicrobial systems. CRC Press, Boca Raton, Florida, 2000.

Naidu, A. S., W. R. Bidlack and A. T. Crecelius, 2000. Flavonoids, in: Naidu A. S. (Ed.), Natural food antimicrobial systems, CRC Press, Boca Raton, Florida, pp: 325-348:

N. Benkeblia, 2000. Phenylalanine ammonia-lyase, peroxidase, pyruvic acid and total phenolics variation in onion tissues during storage. Lebensm. Wiss. und Technol., 33: 112-116.

P. C. Hsieh, J. L. Mau and S. H. Huang, 2001. Antimicrobial effect of various combination of plant extracts. Food Microbiol., 18: 35-43.

P. M. Ward, S. Fasitsas and S. E. Katz, 2002. Inhibition, resistance development, and increased antibiotic and antimicrobial resistance caused by neutraceuticals. J. Food Prot., 65: 528-533.

P. R. Kramer, H. Hindorf, C. H. Jha, J. Kallage and F. Zilliken, 1984. Antifungal activity of soybean and chickpea isoflavones and their reduced derivatives. Phytochem., 23: 2203-2205.

R. S. Rivlin, 2001. Historical perspective on the use of garlic. J. Nut., 131: 951S-954S.

V. Lattenzio, V. De Cicco, D. Venere, G. Lima and M. Salerno, 1994. Antifungal activity of phenolics against fungi commonly encountered during storage. It. J. Food Sci., 1: 23-30.

Y. Wang, M. Hamburger, J. Gueho and K. Hostettmann, 1989. Antimicrobial flavonoids from Psiadia *trinervia* and their methylated and acetylated derivatives. Phytochem. 28: 2323-2327.