ISSN: 2394-0026 (P)
ISSN: 2394-0034 (O)

Original Research Article

Inhibitory effect of various aqueous medicinal plant extracts on citrinin production and fungal biomass by *Penicillium notatum* and *Aspergillus niger*

Seema Zargar[1]*

[1]Department of Biochemistry, College of Science, King Saud University, Riyadh, KSA

*Corresponding author's email: **szargar@ksu.edu.sa**

How to cite this article: Seema Zargar. Inhibitory effect of various aqueous medicinal plant extracts on citrinin production and fungal biomass by *Penicillium notatum* and *Aspergillus niger*. IAIM, 2014; 1(3): 1-8.

Available online at www.iaimjournal.com

Received on: 05-11-2014 **Accepted on:** 08-11-2014

Abstract

Citrinin is a nephrotoxic mycotoxin produced by several species of the genus *Aspergillus*, *Penicillium* and *Monascus*. Citrinin is generally formed after harvest and occurs mainly in stored grains. The efficacy of different concentrations of aqueous leaf extracts of Aervalanata, Nerium oleander, Rhazya stricta Decne and Cleome amblyocarpa (5 to 10 mg/mL) on growth and citrinin production in two fungal strains Penicillium notatum and Aspergillus niger was investigated. Mycotoxin production and fungal biomass by the isolates was suppressed, depending on the concentration of the plant extract added to culture media at the time of spore inoculation. Citrinin production in fungal mycelia grown for 15 days in culture media containing 5 – 10 mg/mL of the aqueous extracts of A. lanata, N. oleander, R. stricta Decne and C. amblyocarpa showed inhibition of approximately 14.2 to 91.8 % in Penicillium notatum and 13.4 to 90.3% in Aspergillus niger. Among the all four extracts, Rhazya stricta Decne was more efficient than other tested plant extracts in inhibiting the citrinin production ranging from 22.4 to 91.8% in P. notatum and 32.6 to 93.2% in A. niger.

Key words

Penicillium notatum, Aspergillus niger, Citrinin, Fungal biomass.

Introduction

Mycotoxins, a group of structurally diverse secondary metabolites produced by various fungi, are toxic compounds that can contaminate foodstuffs, crops or human foods. The ingestion of these contaminated materials in several foods may be pathogenic in animals and humans as they may lead to health problems, such as liver, kidney or nervous system damage, immunosuppression and carcinogenesis [1]. Because fungi are

Copy right © 2014, IAIM, All Rights Reserved.

ISSN: 2394-0026 (P)
ISSN: 2394-0034 (O)

widespread in the environment, mycotoxins are considered unavoidable contaminants in foods and feeds. Therefore several studies should be done to combat the problem of contamination of these toxins in foods.

The mycotoxin citrinin is one of the toxic secondary metabolites produced. This mycotoxin was first isolated from filamentous fungus Penicillium citrinum [2]. It is also produced by other species of Penicillium [3], Aspergillus [4] and Monascus [5, 6, 7]. Due to its antibacterial potential, citrinin was used as an antibiotic [8], but relative toxicity studies showed that it has lot of side effects and acted in animals as a nephrotoxin [9], damaged the proximal tubules of the kidney [10], and was investigated as a potential causative agent in human endemica Balkan nephropathy [11]. It is difficult to establish widely acceptable limits for citrinin. Currently, there are no specific acceptable levels for this mycotoxin worldwide. The main reason may be its instability in foodstuffs. Efforts are being made to look for new antifungal materials from natural sources for preservation of food stuffs from production of mycotoxins [12, 13, 14]. Recently, Mossini and Kemmelmeier reported more than 90% reduction of citrinin production in Penicillium citrinum isolates by aqueous extracts of neem leaf [15]. After this no or very less attempts have been made to test the efficacy of other medicinal plant extracts against the growth of fungi and their subsequent citrinin production. Hence, the aim of this study was to test the various aqueous extracts obtained from medicinal plants from Saudi Arabia on growth of various fungi and subsequent citrinin production.

Material and methods

Microorganisms

Two citrinin-producing microorganisms namely Penicillium notatum and Aspergillus niger, were obtained from the culture collection of the Microbiology Laboratory of King Khalid University Hospital, King Saud University, Riyadh; KSA. All the fungal cultures were maintained on slants of potato dextrose agar medium at 4 °C.

Collection and preparation of aqueous plant extracts

Literature survey was done and taxonomic studies of the herbarium specimens of the medicinal plants available at the National Herbarium of Saudi Arabia (RIYADH), and the herbarium of King Saud University (Pharmacy), was done by using long arm stereo microscope. Leaves of all the four medicinal plants (**Table - 1**) were collected and washed under tap water. Then the leaves were dried at 60 °C in hot air oven for 5 days and ground to make a powder and passed through 20 mesh sieve. Ten grams of powdered leaves were made soluble with 100 ml distilled water at 200 rpm for 5 hour at room temperature [16]. The remaining insoluble material was filtered by Whatman No. 1 filter paper and centrifuged at 10,000 rpm for 10 min. The supernatant was collected and again passed through 0.22 µm filter (Millipore) and stored at -20 °C for further use.

Phytochemical analysis of medicinal plants

Phytochemical analysis of all the tested samples was done to determine the presence of active compounds as follows.

Molisch's test for the presence of Carbohydrates: 0.25 g of each powder was dissolved in 2.5 ml of distilled water separately and then filtered. Two or three drops of Molisch's reagent were added to each solution, followed by addition of 1 ml of concentrated H_2SO_4 by the side of test tube kept in slanting position. The mixture was allowed to stand for two minutes followed by dilution with 2.5 ml of distilled water. Formation of a red or dull violet

Copy right © 2014, IAIM, All Rights Reserved.

ISSN: 2394-0026 (P)
ISSN: 2394-0034 (O)

color at the inter phase of the two layers refers to presence of carbohydrates [17].

Test for presence of alkaloids: 0.1 g of powder was taken from each medicinal plant specimen and dissolved in 5 ml of methanol and then filtered. 2 ml of filtrate from each medicinal plant were stirred with 1:1 volume of 1% aqueous HCl in water bath and then again filtered. 1 ml from this filtrate from each sample was taken individually into 2 test tubes. To the first tube, few drops of Dragendorff's reagent were added. Occurrence of orange-red precipitate was taken as positive. To the second tube 1ml, Mayer's reagent was added and appearance of buff-colored precipitate signifies the presence of alkaloids [17].

Liebermann-Burchard test for steroids: Crude powder (0.1 g) from each sample was dissolved in 1 ml of acetic acid separately; the solutions were cooled on ice for 30 min followed by the addition of few drops of concentrated H_2SO_4 carefully. Color development from violet to blue or bluish-green signifies the presence of a steroidal ring [17].

Test for saponins: Crude powder (1 g) from each sample was dissolved in 5 ml of distilled water and boiled separately and then filtered. To each filtrate, 4 ml of distilled water was further added and shaked vigorously for 5 minutes. Persistence of frothing on warming was taken as an evidence for the presence of saponins [17].

Shinoda's test for flavonoids: 0.5 g of each powder was mixed with 5 ml of ethanol for each sample which was later warmed and then filtered. Three pieces of magnesium chips were then added to the filtrate followed by few drops of concentrated HCl. A pink, orange, or red to purple coloration indicates the presence of flavonoids [18].

Test for tannins: About 0.5 g each portion of crude powder was stirred with about 10 ml of distilled water separately and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of each filtrate. Presence of a blue-black, green or blue-green precipitate indicates the presence of tannins [18].

Fungal strains and culture conditions

The spores were suspended by growing the fungi on Petri dishes for 7 days at 25 °C with potato dextrose agar (PDA) containing 50 mg/L of streptomycin. Later spores were harvested by adding 10 ml of sterilized distilled water on each plate. The spore suspension hence obtained was filtered using cheese cloth, and spores were counted using a hemocytometer and brought to a final concentration of 105 conidia/ml.

Growth of fungi and subsequent citrinin production from both strains

Aliquots of 20 ml potato dextrose broth (PDB) were prepared in 250 ml conical flasks and sterilized by autoclaving. Four different concentrations (2.5, 5.0, 7.5 and 10.0 mg/ml) of each aqueous plant extracts were added to liquid broth cooled to 25 °C. 10 µl amount from suspension (containing 105 spore/ml of each strain of fungus) prepared was inoculated in each flask and kept in rotary shaker at 200 rpm for 10 days at 25 ± 2 °C. The control contained PDB broth and 10 µl of all the both strains of fungal suspension respectively. The fungal mycelium was harvested by filtration by muslin cheese cloth to separate from liquid culture. The filtered biomass was then dried at 60 °C for 24 hour. The dry weight of mycelium was determined. Filtrates were stored in 4 °C for carrying out citrinin extraction. All treatments consisted of three replicates, and experiments were repeated in triplicates and the averages were determined.

Copy right © 2014, IAIM, All Rights Reserved.

ISSN: 2394-0026 (P)
ISSN: 2394-0034 (O)

Determination of citrinin produced by P. notatum and A. niger in liquid media

The filtrates obtained from above experiment were used for extraction and estimation of citrinin. Briefly, the citrinin was extracted thrice with chloroform (1:1 v/v). All the three extractions were pooled and concentrated in vacuum at 40 °C using a rotary evaporator. The crude extract was then diluted in minimum amount of chloroform (2 ml) and citrinin was estimated by thin layer chromatography (TLC) according to Razak, et al. [16]. Subsets of citrinin obtained were estimated by High performance Liquid Chromatography (HPLC). The control contained PDB broth and 10 µl of fungal suspension. Briefly, different volumes (1 to 5 µl) of sample extracts were applied to precoated TLC plates (TLC Silica gel 60 F254, Merck, Germany) along with standard (that contained citrinin at 0.5 µg/ml) obtained from Sigma Chemical Co. (StLouis, MO, USA). The plates were allowed to develop in toluene/ethyl acetate/formic acid (6:4:0.5 v/v/v) in glass tanks and covered with aluminum foil [19]. The TLC plates were dried and observed under long wave length (365 nm). Citrinin appeared as a fluorescent yellow spot. The intensity of citrinin spots was compared with that of the spot from standard citrinin. The concentration of citrinin was calculated according to Younis and Malik [20].

High Performance Liquid Chromatography (HPLC) to validate citrinin concentration

HPLC analysis was performed to confirm spectrophotometric results. Residues of the extracts were dissolved in an appropriate volume of mobile phase (1 mL for all samples), filtered through a 0.45 µm disposable syringe filter (Micro Filtration Systems®) prior to injection into the chromatograph. Aliquots (10 µL) were injected on HPLC column and analysis were carried out using a Shimadzu® Liquid Chromatograph, equipped with an LC-20AD pump, a Rheodine® injector, an SPD-20A UV detector, a CBM-20 A-Communications Bus Module, and a LC Solutions Workstation system. A reverse-phase Atlantis® dC 18 column (150 × 3.9 mm, 5 µm) was used, at room temperature. The mobile phase used was acetonitrile-water and o-phosphoric acid (75:20:5 v/v/v) with a flow rate of 1 mL/min for an isocratic run of 10 min. Absorbance of samples and standard was detected at 360 nm. Retention times and peak areas were calculated by LC Solutions software. Evaluation of sample retention times with that of the standard identified the presence of citrinin in the samples. The relationships between peak height and area and the amount injected were linear over the ranges 2.5-50 µg.

Citrinin recovery

The extraction method was validated by the standard addition method of citrinin (1mg/ml of methanol: 10, 20 and 40 µL) to liquid PDB (3 mL), extracted, and were spotted on plates. The known amount of citrinin (5-50 µg) were also spotted, developed, detected, eluted, and estimated as samples to check on their recovery from TLC silica aluminum plates. The percentage of added citrinin recovery in PDB varied between 42% (for 10 µg) and 59% (for 40 µg), while recovery of known amounts of citrinin from plates averaged 99.34%.

Results and discussion

Two strains of fungus Penicillium notatum and Aspergillus niger were screened for the inhibition of growth of their biomass and citrinin production by various medicinal plant extracts from Saudi Arabia. The plant extracts used were as per **Table - 1**. Chemical composition of plant extracts was as per **Table - 2.** It was found that all extracts were positive for carbohydrates while as Cleome amblyocarpa was free of flavonoids and alkaloids. Aervalanata, Nerium oleander and Rhazya stricta Decne all showed negative test for saponins as per **Table - 2**. The

Copy right © 2014, IAIM, All Rights Reserved.

ISSN: 2394-0026 (P)
ISSN: 2394-0034 (O)

effect of medicinal plant extracts on mycellial dry weight on P. notatumis presented in **Table - 3**. All plant extracts showed effective reduction on growth of P. notatum biomass ranging from 2.0 to 77.2% as per **Table - 3**. Rhazya stricta Decne effectively reduced mycellial dry weight of P. notatum ranging from 8.5 to 77.2% and A. niger ranging from 16.5 to 73.2% as per **Table - 4**. All the other plant extracts inhibited growth of fungal biomass of both strains ranging from 2 to 68.8% as per **Table - 3** and **Table - 4**. These results were consistent with Mossini, et al. [19] who showed the significant reduction of Penicillium verrucosum and Penicillium brevicompactum using neem leaf extract and neem oil. The quantification of citrinin was also done by HPLC by comparing the retention times of the culture extracts with that of standard sample of citrinin. HPLC elution profiles from the broth of P. notatum and A. niger isolates from all the tested plant extracts showed the same retention time as of standard citrinin (retention time = 3.8 min).

This study for the first time showed the effect of selected medicinal plant extracts on growth and citrinin production of tested microorganisms. The effect of aqueous extracts of four medicinal plant extracts on citrinin production from both fungi was as per **Table - 5** and **Table - 6**. All the four plant extracts effectively decreased citrinin production ranging from 14.2 to 91.8% in P. notatum and 13.4 to 90.3% in A. niger. Amongst all the four extracts, Rhazya stricta Decne was more efficient than all in inhibiting the citrinin production ranging from 22.4 to 91.8% in P. notatum and 32.6 to 93.2% in A. niger as per **Table - 5** and **Table - 6**. It was also found that in case of P. notatum, the aqueous extract of Aervalanata was more effective ranging from 16.3 to 87% while as in A. niger it was comparatively less and the aqueous extract of Cleome amblyocarpa was effective ranging from 19.2 to 90.3% in A. niger while as it was 19.3 to 84.6% in P. notatum. This study for the first time reported inhibition of citrinin from medicinal plant extracts at these ranges. The present investigation showed that all the medicinal plant extracts were effective in decreasing the fungal biomass and citrinin production. Hence, these plant extracts can be used as fungicides in the stored food grains as these microorganisms most commonly grow on storage and produce citrinin under these conditions. The study emphasized on preventing the accumulation of toxic substances in food grains under storage.

Conclusion

The tested plants are being traditionally used for the treatment of various human ailments. In current research, we studied the potential of these plant extracts on inhibiting citrinin production by the fungus P. notatum and A. niger in culture medium. The fungi growth and citrinin was decreased, although there were differences between colony macroscopic characteristics in controls and treatments. Therefore, these plant extracts can be used in the storage of food grains to prevent them from the any fungal growth and mycotoxin (citrinin) production under such conditions. Additional research study is needed to determine the potential usefulness of these plant extracts in fungi control programs.

Acknowledgement

This research project was supported by a grant from the ‘Research Center of the Center for Female Scientific and Medical Colleges’, Deanship of Scientific Research, King Saud University.

References

1. Bennett JW, Klich M. Mycotoxins. Clinical Microbiology Reviews, 2003; 16, 3: 497–516.

Copy right © 2014, IAIM, All Rights Reserved.

ISSN: 2394-0026 (P)
ISSN: 2394-0034 (O)

2. Hetherington AC, Raistrick H. Studies in biochemistry of microorganism XI. On the production and chemical constitution of a new yellow colouring matter, citrinin, produced from glucose by Penicilliumcitrinum Thom. Philosophical Transactions of the Royal Society of London Series, 1931.
3. Ei-Banna AA, Pitt JI, Leistner L. Production of mycotoxins by Penicillium species. Systematic and Applied Microbiology, 1987; 10: 42–46.
4. Kurata H. Mycotoxins and mycotoxicoses. In A. E. Pohland, V. R. Dowell, J. L. Richards (Eds.), Microbial toxins in foods and feeds, New York, USA: Plenum Press, 1990, p. 249–259.
5. Blanc PJ, Loret MO, Goma G. Production of citrinin by various species of Monascus. Biotechnology Letters, 1995a; 17(3): 291.
6. Wani TA. Determination of reduction in citrinin production by response surface methodology using aqueous leaf extract of *Nepeta bracteata.* Int Res J Pharm. App Sci., 2013; 3: 1-9.
7. Li F, Xu G, Li Y, Chen Y. Study on the production of citrinin by Monascus strains used in food industry. Wei Sheng Yan Jiu, 2003; 32(6): 602–605.
8. Wong HC, Koehler PE. Production and isolation of an antibiotic from Monascuspurpureus and its relationship to pigment production. Journal of Food Science, 1981; 46: 589–592.
9. Betina V. Mycotoxins: Chemical, biological and environmental aspects. New York, USA: Elsevier, 1989a.
10. Phillips RD, Hayes AW, Berndt WO, Williams W. Effects of citrinin on renal function and structure. Toxicology, 1980b; 16: 123–127.
11. Frank HK. Citrinin. Zeitschrift fur Ernahrungswissenschaft, 1992; 31: 164–177.
12. Onyeagba RA, Ugbogu OC, Okeke CU, Iroakasi O. Studies on the antimicrobial effects of garlic (Allium sativumLinn) ginger (ZingiberofficinaleRoscoe) and lime (Citrus aurantifoliaLinn). Afr J Biotechnol., 2004; 3: 552-554.
13. Boyraz N, Ozcan M. Antifungal effect of some spice hydrosols. Fitoterapia, 2005; 76: 661-665.
14. Haciseferogullary H, Özcan M, Demir F, Calysyr S. Some nutritional and technological properties of garlic (Allium sativumL.). J Food Eng., 2005; 68: 463-469.
15. Mossini SAG, Kemmelmeier C. Inhibition of citrinin production in Penicillium citrinum cultures by Neem [Azadirachtaindica A. Juss (Meliaceae)]. Int J Mol Sci., 2008; 9: 1676-1684.
16. Razak MFA, Aidoo KE, Candilish AGG. Mixed herbs drugs: Inhibitory effect on growth of the endogenous mycoflora and aflatoxin production. Mycopathologia, 2009; 167: 273-286.
17. Sofowora A. Screening Plants for Bioactive Agents. In: Medicinal Plants and Traditional Medicinal in Africa. 2nd edition, Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria, 1993, p. 134-156.
18. Trease GE, Evans WC. Pharmacognosy. 15th edition, Saunders Publishers, London, 2002.
19. Mossini SAG, Arrotéia CC, Kemmelmeier C. Effect of neem leaf extract and neem oil on Penicilliumgrowth, sporulation, morphology and ochratoxin A production. Toxins, 2009; 1: 3-13.
20. Younis YH, Malik KM. TLC and HPLC assay of aflatoxin contamination in Sudanese peanuts and peanut products. Kuwait J Sci Eng., 2003; 30: 79-94.

Copy right © 2014, IAIM, All Rights Reserved.

ISSN: 2394-0026 (P)
ISSN: 2394-0034 (O)

Table – 1: Ethno botanical data of medicinal plants that were chosen for this study.

Family	Botanical name	Local name	Part used	Popular uses
Amaranthaceae	Aervalanata (L.)	Al-Athlab	Leaves	Used in treatment of cystitis
Apocynaceae	Nerium oleander L	Dafla	Leaves	Diuretic
Apocynaceae	Rhazya stricta Decne	Harmal	Leaves	Antibacterial, used in remedy for sore throat and fever
Capparidaceae	Cleome amblyocarpa Baratte and Murb	Khunayzah, ufaynach	Leaves	Antimicrobial, used against bacterial infections

Table - 2: Chemical composition of plant extracts.

Constituents	Aervalanata (L.)	Nerium oleander L	Rhazya stricta Decne	Cleome amblyocarpa
Carbohydrates	+	+	+	+
Steroids	-	+	+	-
Alkaloids	+	+	+	-
Saponins	-	-	-	+
Tanins	+	-	+	-
Flavonoids	+	+	+	-

Table - 3: Effect of medicinal plant extracts on growth of P. notatum in PDB broth.

Conc. (mg/ml of media)	Aervalanata		Nerium oleander		Rhazya stricta Decne		Cleome amblyocarpa	
	Mdw (mg)	Red. (%)	Mdw (mg)	Red. (%)	Mdw (mg)	Red. (%)	Mdw (mg)	Red. (%)
Control	246±2	0.0±0.0	246±2	0.0±0.0	246±2	0.0±0.0	246±2	0.0±0.0
2.5	239±3	2.0±1	241±1	2.0±3	225±1	8.5±3	229±1	6.9±1
5	202±4	17.8±2	212±3	13.8±2	198±3	19.5±1	212±3	13.8±2
7.5	183±1	25.6±3	193±1	21.5±4	153±2	37.8±0	199±2	19.1±0
10	93±2	62.1±1	75±3	69.5±1	56±1	77.2±3	101±1	58.9±4

(Conc. = Concentration, Red. = Reduction)

[The results are means of 2 experiments with 3 replicates each, determined 15 days after incubation. Values in parentheses are SD of 3 replicates.]

Copy right © 2014, IAIM, All Rights Reserved.

ISSN: 2394-0026 (P)
ISSN: 2394-0034 (O)

Table - 4: Efficacy of medicinal plant extracts on growth of A. Niger in PDB broth.

Conc. (mg/ml of media)	Aervalanata		Nerium oleander		Rhazya stricta Decne		Cleome amblyocarpa	
	Mdw (mg)	Red. (%)	Mdw (mg)	Red. (%)	Mdw (mg)	Red. (%)	Mdw (mg)	Red. (%)
Control	315±4	0.0±0.0	315±4	0.0±0.0	315±4	0.0±0.0	315±4	0.0±0.0
2.5	298±2	5.3±2	299±1	5.0±0	263±2	16.5±0	273±1	13.3±3
5	213±1	32.3±1	220±0	30.1±5	218±3	30.7±3	230±2	26.9±1
7.5	197±2	37.4±6	201±2	36.1±1	198±1	37.1±4	200±1	36.5±2
10	98±3	68.8±1	103±3	67.3±0	84±1	73.3±2	97±3	69.2±0

(Conc. = Concentration, Red. = Reduction)

Table -5: Efficacy of medicinal plant extracts on citrinin production by P. notatumin liquid media (HPLC analysis).

Conc. (mg/ml of media)	Aervalanata		Nerium oleander		Rhazya stricta Decne		Cleome amblyocarpa	
	CTN (μg/ml)	Red. (%)	CTN (μg/ml)	Red. (%)	CTN (μg/ml)	Red. (%)	CTN (μg/ml)	Red. (%)
Control	9.8±0.2	0.0±0.0	9.8±0.2	0.0±0.0	9.8±0.2	0.0±0.0	9.8±0.2	0.0±0.0
2.5	8.2±0.1	16.3±0.2	8.4±0.3	14.2±0.0	7.6±0.0	22.4±0.3	7.9±0.1	19.3±0.8
5	6.3±0.0	35.7±0.1	6.8±0.1	30.6±1.0	5.8±0.5	40.8±0.1	6.2±0.3	36.7±0.9
7.5	3.1±0.4	68.3±0.6	4.3±0.2	56.1±0.2	3.0±0.1	69.3±0.8	2.0±0.6	79.5±0.9
10	1.2±0.2	87.7±0.2	2.5±0.3	74.4±1.2	0.8±0.2	91.8±1.3	1.5±0.1	84.6±0.9

(Conc. = Concentration, Red. = Reduction)

Table – 6: Efficacy of medicinal plant extracts on citrinin production by A. niger in liquid media (TLC analysis).

Conc. (mg/ml of media)	Aervalanata		Nerium oleander		Rhazya stricta Decne		Cleome amblyocarpa	
	CTN (μg/ml)	Red. (%)	CTN (μg/ml)	Red. (%)	CTN (μg/ml)	Red. (%)	CTN (μg/ml)	Red. (%)
Control	10.4±0.6	0.0±0.0	10.4±0.6	0.0±0.0	10.4±0.6	0.0±0.0	10.4±0.6	0.0±0.0
2.5	8.7±0.2	16.3±0.4	9.0±0.2	13.4±0.6	7.0±0.3	32.6±0.9	8.4±0.7	19.2±0.3
5	7.6±0.5	26.9±0.1	7.9±0.6	24.0±0.3	4.9±0.5	52.8±0.8	5.3±0.3	49.0±0.6
7.5	4.3±0.3	58.6±0.5	5.6±0.1	46.1±0.3	2.6±0.3	75.0±0.9	4.1±0.3	60.5±0.7
10	1.4±0.1	86.5±0.3	1.9±0.0	81.7±0.2	0.7±0.1	93.2±0.6	1.0±0.2	90.3±0.8

(Conc. = Concentration, Red. = Reduction)

Source of support: Nil. **Conflict of interest:** None declared.

Copy right © 2014, IAIM, All Rights Reserved.