IDECEFYN

Molecular Medicinal Chemistry

vol 20 January-April 2010, xxx-xxx http//:www.idecefyn.com.ar ISSN 1666-888X

Anti-inflammatory and Antioxidant Activities of Argentine Northwest Fruits

Alberto MR[1,3,4]; Cardozo ML [1,4]; Ordóñez RM [1,2,3,4]; Zampini IC[1,2,3,4]; Isla MI.[1,2,3,4#]

[1]INQUINOA (CONICET). [2]Cátedra de Fitoquímica. Facultad de Bioquímica, Química y Farmacia. [3]Facultad de Ciencias Naturales e Instituto Miguel Lillo Universidad Nacional de Tucumán. [4]Fundación Miguel Lillo. Ayacucho 471. 4000- San Miguel de Tucumán. ARGENTINA

ABSTRACT

Many Northwest Argentinean fruits have been consumed since ancient times by native and rural communities because of their properties towards human health. In the present study, the antioxidant effect (free radicals scavenging capacity) and anti-inflammatory capacity (inhibitory effect on lipoxygenase enzyme) of regional fruits (*Psychotria carthagenensis*, *Prosopis alba*, *Prosopis nigra*, *Ziziphus mistol*, *Cereus forbesii*, *Myrcianthes pungens*, *Morus nigra* and *Eugenia uniflora*), were assayed. The results indicated that all fruits have DPPH radical scavenging activity in the following order: *E. uniflora* > *C. forbesii* > *M. pungens* > *P. carthagenensis* > *Z. mistol* >*M. nigra* > *P. nigra* > *P. alba*. The order of anti-inflammatory activity was: *E. uniflora* > *P. carthagenensis* > *Z. mistol* > *M. pungens* > *C. forbesii* > *M. nigra* > *P. nigra* > *P. alba*. A positive correlation was found between antioxidant activity and anti-inflammatory activity.

Keywords: anti-inflammatory activity, free radical scavenging capacity, fruits, phenolic compounds.

Corresponding author: **Dra. María Inés Isla,** INQUINOA- CONICET, Universidad Nacional de Tucumán, Ayacucho 471, 4000 - San Miguel de Tucumán. ARGENTINA, FAX: 54- 381-4248025 E-mail: misla@tucbbs.com.ar

Received: February 22, 2010. Accepted: March 10, 2010

Molecular Medicinal Chemistry

IDECEFYN

vol 20 January-April 2010, xxx-xxx http//:www.idecefyn.com.ar ISSN 1666-888X

Introduction

Free radicals are involved in different diseases and health conditions, including coronary heart disease, inflammation, stroke, diabetes mellitus, rheumatic disease, liver disorders, renal failure, cancer and aging (Cheng *et al.*, 2003). Among inflammatory cells, polymorphonuclear leukocytes are particularly adept at generating and releasing reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide anion, hydroxyl radical, hydrogen peroxide and nitric oxide (Ramos *et al.*, 1992). The excessively produced ROS can injure cellular biomolecules such as nucleic acids, proteins, carbohydrates and lipids, causing cellular and tissue damage, which in turn augments the state of inflammation (Trenam *et al.*, 1992).

The liberation of free arachidonic acid from cell membranes catalyzed by phospholipase A2 and the further metabolites of arachidonic acid catalyzed by cyclooxygenases (COXs) and lipoxygenases (LOXs) generate a range of important inflammatory mediators. LOXs are the key in the biosynthesis of leukotrienes (LT) and play a very important role in the physiopathology of the inflammatory and allergic diseases (Casey, 1999). The products of these enzymes, hydroperoxyeicosatetraenoic (HPETE), hydroxyeicosatetraenoic acids (HETE) and LT play a very important role in rheumatoid arthritis, psoriasis, asthma and immune disorders (Carter *et al*., 1991). There is also some evidence that LOX products could contribute to vascular changes in inflammation (Vane and Botting, 1987).

Consumption of fruits and vegetables has been associated with reduced risk of chronic diseases such as cardiovascular disease and cancer. Phytochemicals, particularly phenolic compounds, are suggested to be the major bioactive compounds in fruits and vegetables for the health benefits on oxidative stress and inflammatory process.

Our preliminary results demonstrated that some mature fruits obtained from plant species that grow in Northwestern Argentine have antioxidant activity, which could have beneficial effects in other pathologies, such as inflammation. There is a very close relationship between the compounds that have anti-inflammatory activity and antioxidant capacity. The anti-inflammatory activity of many plants has been related to the antioxidant properties of its polyphenols. The aim of the present paper was to compare the antioxidant effect (free radicals scavenging capacity), anti-inflammatory capacity (inhibitory capacity of LOX enzyme) and polyphenolic content (tannins and total phenolics) of Northwest Argentinean fruits: *Psychotria carthagenensis*, *Prosopis alba*, *Prosopis nigra*, *Ziziphus mistol*, *Cereus forbesii*, *Myrcianthes pungens*, *Morus nigra* and *Eugenia uniflora*

Experimental

Reagents

Linoleic acid was obtained from Fluka. Soybean lipoxygenase Type IB was purchased from Sigma chemical. All solvents were of analytical grade.

Plant Material

Fresh fruits were hand-harvested at mature stage. Afterwards, they were cleaned and stored at -20°C until use. The fruits analyzed were as follows: *Psychotria carthagenensis* (Moradillo), *Prosopis alba* (White algarrobo), *Prosopis nigra* (Black algarrobo), *Ziziphus mistol* (Mistol), *Cereus forbesii* (Ucle), *Myrcianthes pungens* (Mato), *Morus nigra* (Mulberry) and *Eugenia uniflora* (Arrayán).

Extracts preparation

Fruits (10 g) were homogenized with 100 ml of ethanol 96°, macerated during 7 days at room temperature and then, centrifuged during 20 min at 10000 xg. The supernatants were then filtered through Whatman Nº 1 paper and concentrated by vacuum in a rotary evaporator to dryness at 40°C. Dry extracts were re-dissolved with dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany) and stored at -20ºC.

Phenolic compounds and tannin content determination

Total phenolic compounds content were measured at 765 nm using Folin-Ciocalteu reagent (Singleton et al., 1999) and the results were expressed as µg gallic acid equivalent/mg dry extract (µg GAE/mg DE). Condensed tannins (proanthocyanidins) content was estimated as previously described by Porter et al. (1986) and the results were expressed as µg quebracho tannin equivalent/mg dry extract (µg QTE/mg DE).

Inhibition of lipoxygenase activity

LOX activity was determined using a continuous spectrophotometric method, based on the enzymatic oxidation of linoleic acid to corresponding hydroperoxide (Sircar *et al.*, 1983; Taraporewala and Kauffman, 1990). Soy LOX-1 is used as an *in vitro* biochemical model, since it resembles human LOXs in its substrate specificity

IDECEFYN
vol 20 January-April 2010, xxx-xxx http//:www.idecefyn.com.ar ISSN 1666-888X

and inhibition characteristics (Mahesha *et al*., 2007).

The assay mixture contained enzyme solution (0.9 nM), linoleic acid (50 µM), different dilutions of fruit extracts and 0.2 M borate buffer, pH 9.0 to reach a final volume of 1.8 ml. The corresponding controls were realized. The reaction was carried out at 25 °C and the absorption at 234 nm was recorded as a function of time during 3 min.

The anti-inflammatory effect was calculated as inhibition percentage of hydroperoxide production. Test compound concentration causing 50% inhibition hydroperoxide-release (IC_{50}) was calculated from the concentration-inhibition response curve by regression analysis. The extinction coefficient of 25 $mM^{-1}.cm^{-1}$ was used for quantification of lipid hydroperoxides.

DPPH free radical scavenging activity

The H-donor activity of plant extracts was measured by DPPH method according to Ordóñez *et al*., (2006). DPPH solution (1.5 ml of 300 µM in 96% ethanol) was incubated with the samples. The reaction mixture was shaken and incubated during 20 min at room temperature. Then, absorbance was measured at 515 nm. Butylated hydroxy-toluene (BHT) was used as reference compound.

The percentage (%) of radical scavenging activity (RSA) was calculated using the following equation:

$RSA\ \% = [(A_0 - A_s)/A_0] \times 100$.

Where A_0 is the absorbance of the control and A_s is the absorbance of the samples at 515 nm. SC_{50} values are expressed in mg of dry extract (mg DE) and denote the sample concentration required to scavenge 50% DPPH free radicals.

Statistical analysis

Results are mean values obtained from at least three independent experiments. The values were evaluated using GraphPad Prism 5.0 software.

Results and Discussion

LOXs are the family of the key enzyme in the biosynthesis of LT which is presumed to play an important role in the pathophysiology of several inflammatory diseases. Inhibition of the biosynthesis of inflammatory mediators by blocking of the LOXs activities would be an important treatment of many inflammatory diseases. This study elucidates the possible relationship between the free radical scavenger effect, LOX inhibitory activity, phenolics and tannins content of extracts from Northwest Argentinean fruits.

Fig 1 shows the scavenger effect of DPPH for all assayed fruit alcoholic extracts. According to SC_{50} values, the order of DPPH scavenging activity was the following: *E. uniflora* > *C. forbesii* > *M. pungens* > *P. carthagenensis* > *Z. mistol* > *M. nigra* > *P. nigra* > *P. alba*. SC_{50} values were between 0.04 and 2.10 mg DE/ml.

The concentrations of total phenolic compounds and tannins were also determined in order to analyze a possible relationship between them and the analyzed activities. The results are summarized in Table 1. The highest content of phenolic compounds and condensed tannins were found in *Eugenia uniflora* (51.37 ± 2.51 mg GAE/g DE) and *Prosopis alba* (397.93 ±35.45 mg QTE/g DE), respectively.

Table 1. Total phenolic compounds and condensed tannins content.

Fruits	Phenolics (mg GAE/g DE)	Tannins (mg QTE/g DE)
Eugenia uniflora	51.37 ± 2.51	112.37 ±10.43
Psychotria carthagenensis	21.18 ± 1.32	108.80 ±8.92
Ziziphus mistol	43.28 ± 2.46	127.31 ±7.65
Myrcianthes pungens	17.95 ± 0.98	341.87 ±12.56
Cereus forbesii	45.44 ± 3.20	24.87 ± 4.12
Morus nigra	14.04 ± 0.87	218.14 ±19.87
Prosopis nigra	10.32 ± 0.76	255.14 ±21.43
Prosopis alba	10.22 ± 0.68	397.93 ±35.45

A significant correlation ($P \leq 0.05$) was observed between the antioxidant potential determined by DPPH and total phenolic compounds concentration of all macerations (R^2= 0.6852). Our data are coincident with that reported by other authors (Holasova *et al*., 2002). In contrast, no significant correlation ($P \geq 0.1$) was found between radical scavenger activity and tannins content (R^2= 0.1674).

On the other hand, LOX activity was monitored as an increase in the absorbance at 234 nm, which reflects the formation of conjugated diene. The highest inhibitory effect on LOX activity was obtained for the extracts of *P. carthagenensis*, *E. uniflora*, *C. forbesii*, *M. pungens* and *Z. mistol* with IC_{50} values of 1.2; 1.4; 2.6; 3.1 and 4.4 mg DE/ml, respectively. The least inhibitory activity on LOX was observed for extracts from both *Prosopis* species and *Morus nigra*. All extracts

inhibit the enzyme activity in a concentration dependent manner.

At the level of 1.7 mg of DE/ml, *E. uniflora* showed the greatest inhibitory activity of LOX with a 92% inhibition followed by *P. carthagenensis*, *Z. mistol*, *M. pungens*, *C. forbesii* and *M. nigra* with 79, 28, 27, 10 and 2% respectively (Fig 2). In contrast, *P. nigra* and *P. alba*, did not show inhibitory effect on LOX activity. Any significant correlation ($P \geq 0.1$) was observed between anti-inflammatory activity with phenolic compounds (R^2= 0.3116) or tannins (R^2= 0.073). Our results indicate that other compounds could be responsible of LOX inhibition. However, there was a significant correlation ($P \leq 0.05$) between antioxidant and anti-inflammatory capacity (R^2= 0.5810).

Conclusion

In conclusion, this study demonstrated that macerations prepared with fresh fruits of *P. carthagenensis*, *E. uniflora*, *C. forbesii*, *M. pungens* and *Z. mistol* exhibit significant antioxidant and anti-inflammatory activities. These valuable properties could be due to phenolic compounds and other compounds present in this fruits. These findings suggest that the consumption of some regional fruits are beneficial for health and may be useful in developing new herbal medicine against oxidative stress diseases and might be relevant for clinical use for inflammatory diseases. Further studies are in progress to isolate the compounds responsible of functional properties of regional fruits.

Acknowledgements

The authors acknowledge the cooperation from the inhabitants of the areas of study and the financial support from Consejo de Investigación de la Universidad Nacional de Tucumán (CIUNT), Argentina, Agencia Nacional de Promoción Científica y Técnica (ANPCyT) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

References

-Carter GW, Young PR, Albert DH, Bouska J, Dyer R, Bell RL, Summers JB, Brooks DW, (1991). 5-Lipoxygenase inhibitory activity of Zileuton. *J Pharmacol Exp Ther* **256**: 929–937.

-Casey R, (1999). *Lipoxygenases.* In: Casey, R.; Shewry, P.R. ed. Seed proteins. London, Chapman and Hall.

-Cheng HY, Lin TC, Yu KH, Yang CM, Lin CC, (2003). Antioxidant and free radical scavenging activities of *Terminalia chebula*. *Biol Pharm Bull* **26**: 1331–1335.

-Holasova M, Fiedlerova V, Smrcinova H, Orsak M, Lachman J, Vavreinova S, (2002). Buckwheat-the source of antioxidant activity in functional foods. *Food Res Int* **35**: 207-211.

-Mahesha HG, Sridevi AS, Appu R, (2007). Inhibition of lipoxygenase by soy isoflavones: Evidence of isoflavones as redox inhibitors. *Arch Biochem Biophys* **461**: 176–185.

-Ordóñez AA, Gomez D, Vattuone MA, Isla MI, (2006). Antioxidant activity of *Sechium edule* (Jacq) Swartz. *Food Chem* **97:** 452-458.

-Porter LJ, Hrstich LN, Chan BC, (1986). The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochem* **25**: 223–230.

-Ramos CL, Pou S, Britigan BE, Cohen MS, Rosen GM, (1992). Spin trapping evidence for myeloperoxidase-dependent hydroxyl radical formation by human neutrophils and monocytes. *J Biol Chem* **267**: 8307–8312.

-Singleton VL, Orthofer R, Lamuela-Raventos RM, (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Method Enzymol* **299**: 152-178.

-Sircar UC, Shwender CJ, Johnson EA, (1983). Soybean lipoxygenase inhibition by nonsteroidal anti-inflammatory drugs. *Prostaglandins* **25**: 393-396.

-Taraporewala IB, Kauffman JM, (1990). Synthesis and structure-activity relationship of anti-inflammatory 9,10-dihydro-9-oxo-2-acridine-alkanoic acids and 4-(2-carboxyphenyl) aminobenzenealkanoic acids. *J Pharm Sci* **79**: 173-178.

-Trenam CW, Blake DR, Morris CJ, (1992). Skin inflammation reactive oxygen species and the role of iron. *J Invest Dermatol* **99**: 675–682.

-Vane J, Botting R, (1987). Inflammation and the mechanism of action of anti-inflammatory drugs. *FASEB Journal* **1**: 89–96.

Molecular Medicinal Chemistry

IDECEFYN

vol 20 January-April 2010, xxx-xxx http//:www.idecefyn.com.ar ISSN 1666-888X

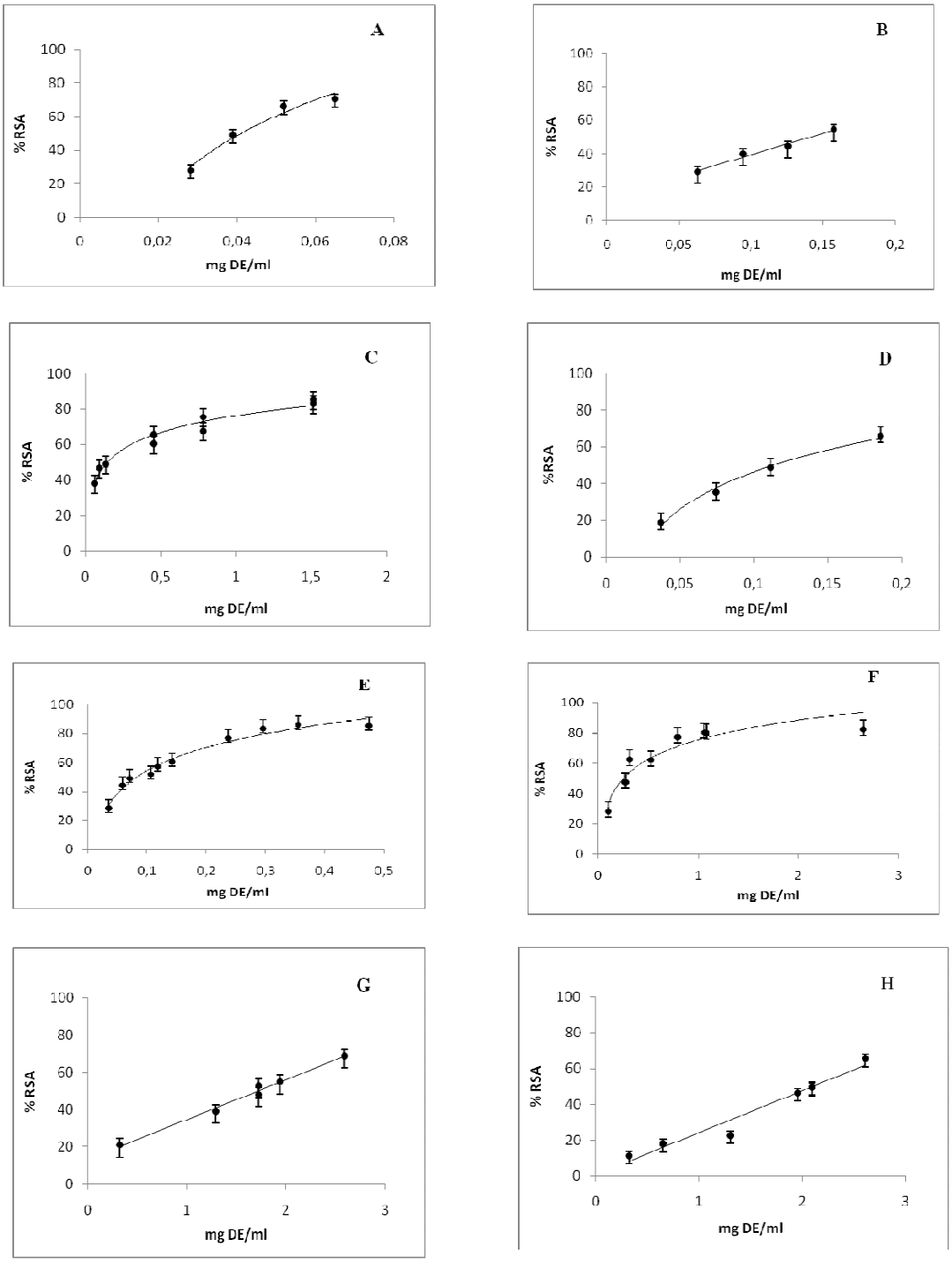

Figure 1 DPPH scavenging activity of fruit extracts: *E. uniflora* (A), *P. carthagenensis* (B), *Z. mistol* (C), *M. pungens* (D), *C. forbesii* (E), *M. nigra* (F), *P. nigra* (G), and *P. alba* (H) . The results are shown as mean ± SD, n=3.

Molecular Medicinal Chemistry

IDECEFYN

vol 20 January-April 2010, xxx-xxx http//:www.idecefyn.com.ar ISSN 1666-888X

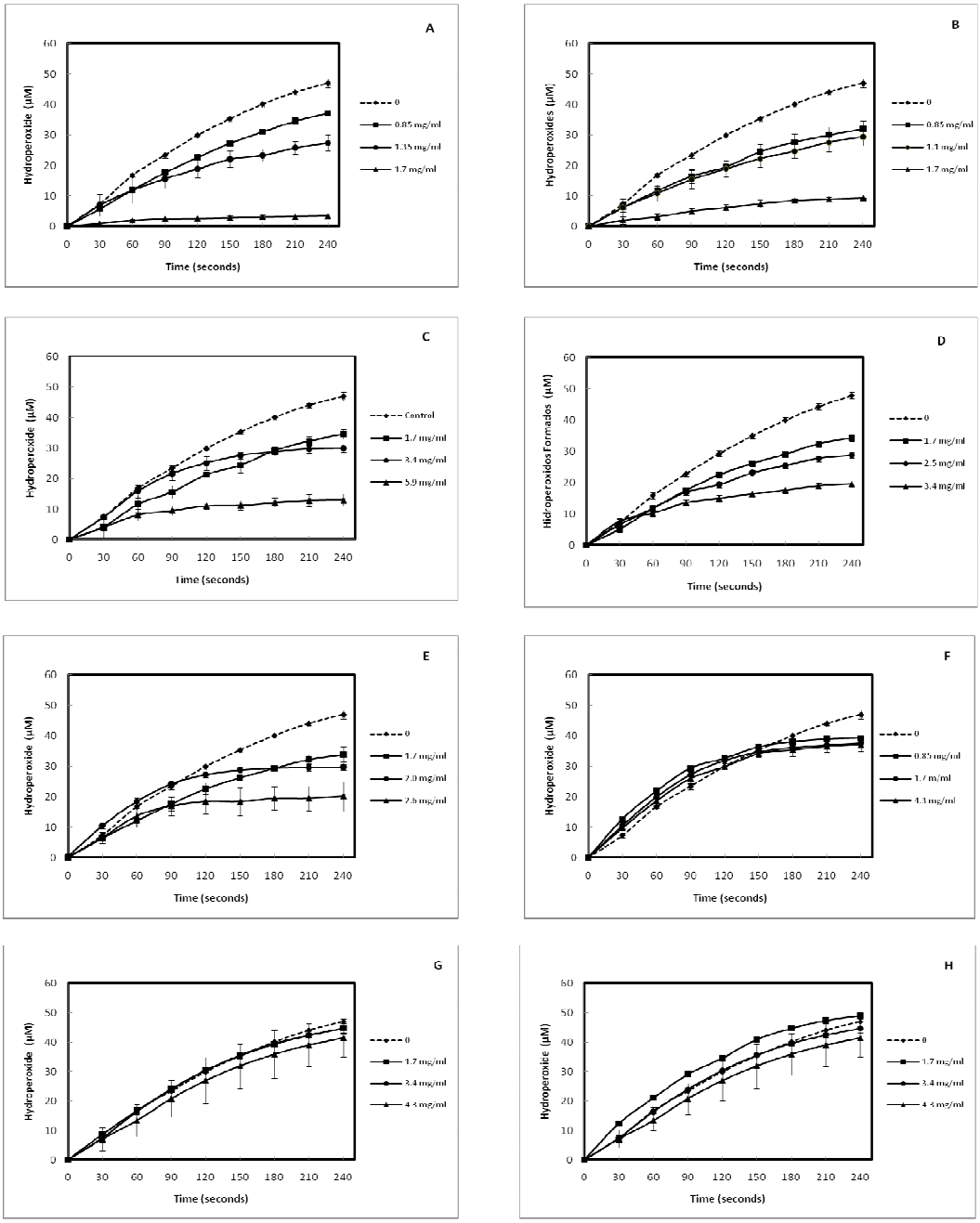

Figure 2: Lipoxygenase activity without fruit extracts (---●---) and in presence of different concentration of fruit extracts: *E. uniflora* (A), *P. carthagenensis* (B), *Z. mistol* (C), *M. pungens* (D), *C. forbesii* (E), *M. nigra* (F), *P. nigra* (G), *P. alba* (H).