Screening of a hundred plant extracts as tyrosinase and elastase inhibitors, two enzymatic targets of cosmetic interest

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Abstract

In search for natural products of cosmetic interest, a hundred plant extracts were *in vitro* tested against elastase and tyrosinase. The inhibitors of these enzymes find application as skin whitening, anti-ageing, anti-wrinkle agents as well as in the treatment of dermatological disorders.

Among the tested samples, seventeen extracts resulted strongly active. In particular, eleven out of them were capable to inhibit both enzymes, five showed a strong activity only against tyrosinase and one only against elastase. The IC_{50} values of the selected samples ranged from 7 to 100 µg/mL and from 20 to 100 µg/mL against elastase and tyrosinase, respectively. Leaves extract of *Pistacia lentiscus* emerged as the most potent elastase inhibitor and, together with *Cytinus hypocistis* (aerial parts) and *Limonium morisianum* (aerial parts), it showed also the lowest IC_{50} of tyrosinase inhibition.

The tested plants were collected in India, Africa and Mediterranean area. Interestingly, among the most active ones, two are endemic and exclusive of Sardinia Island (Italy), namely: *Limonium morisianum* and *Hypericum scruglii*, moreover, the latter resulted the only plant which hydroalcoholic extract was capable to inhibit elastase selectively.

Moreover, a positive correlation was established among the potency of enzymatic inhibitions and the total phenolic and flavonoid content of the samples. The presence of these aromatic compounds in the most active plants confers them a potential additional value as skin protectors from oxidative damage.

Keywords

Skin ageing, tyrosinase, elastase, phytocosmetics, polyphenols, *Hypericum scruglii*

1. Introduction

Skin ageing processes are generally divided into intrinsic and extrinsic, both responsible for drastic changes in skin structure and elasticity. The intrinsic or chronological skin ageing is irremediably related to the passage of time, although it is also influenced by the inherited genes. Conversely, the extrinsic skin ageing is caused by environmental factors, such as chronic exposure to sunlight (photoageing) or pollutants, and it is influenced by miscellaneous lifestyle components (i.e. smoking and diet) (Farage et al., 2008). In particular, photoageing is caused by overexposure to UV radiations, which increases the production of reactive oxygen species (ROS) (Rittié and Fisher, 2002), causing lipid peroxidation, DNA damage, and proteins alterations. Moreover, ROS can also contribute to skin ageing by direct activation of enzymes responsible for the cleavage of extracellular matrix (ECM) components (Mukherjee et al., 2011; Rittié and Fisher, 2002).

Natural products from plants are widely used as cosmetic or cosmeceutical ingredients because of their capability to slow down the intrinsic skin ageing processes and to contrast the extrinsic ones. Plants anti-ageing properties are generally attributed to their antioxidant metabolites, which minimize free radical activity and protect skin against solar radiations (Sahu et al., 2013). Additionally, several plant metabolites are also reported to modulate the activity of enzymes involved in the ageing processes (Cefali et al., 2016; Mukherjee et al., 2011). Among these enzymatic targets of cosmetic interest, elastase and tyrosinase are of remarkable importance.

Elastase belongs to chymotrypsin family of proteases and it is responsible for the breakdown of elastin and other proteins, such as collagen and fibronectin, which are fundamental for the ECM elastic properties (Imokawa and Ishida, 2015). Misregulations of this enzyme are involved in skin ageing processes (Korkmaz et al., 2010). In fact, the excessive hydrolysis of the dermal elastin fiber network leads to the loss of skin elasticity and consequent skin sagging (Thring et al., 2009). On this basis, elastase inhibitors are endowed with anti-wrinkles activity promoting the preservation of skin elasticity.

Tyrosinase is a copper-containing enzyme, also known as polyphenol oxidase (PPO). It catalyzes two distinct reactions, namely: the hydroxylation of a monophenol and the conversion of an *o*-diphenol to the corresponding *o*-quinone. This enzyme is responsible for the rate-limiting first two steps of melanin biosynthetic pathway, and thus, for skin, hair, and eyes color in humans (Pillaiyar et al., 2017). Tyrosinase misregulated expression and/or activity causes skin pigmentation disorders such as: lentigo senilis, urticaria pigmentosa, and age-related skin hyperpigmentation (Slominski et al., 2004). Therefore, tyrosinase inhibitors are candidate skin-whitening agents.

In this work, aimed at identifying natural products endowed with anti-ageing potential, the *in vitro* tyrosinase and elastase inhibitory activity of a hundred hydroalcoholic plant extracts was evaluated. Moreover, the total phenolic and flavonoid content of the tested extracts was also determined, considering the importance of these compounds as antioxidants. In order to investigate on the involvement of these classes of phytochemicals in the tested bioactivities, total phenolic and flavonoid content was also statistically correlated to the percentages of enzymatic inhibitions.

2. Methods and materials

2.1. Plant material

The Indian plants (used in Ayurveda tradition), dried and powdered, were kindly supplied by Maharishi Ayurveda Product Italy (Verona, Italy). They were collected in Ram Bagh (Rajasthan, India) and authenticated by Dr. MR Uniyal, Maharishi Ayurveda Product Ltd., Noida, India.

The samples of African plants were collected in six villages of Baskoure and Songretenga communes (Burkina Faso) and identified by Prof. Joseph Issaka Boussim. Among the Mediterranean plants, the ones collected in Sardinia Island (Italy) were identified by Dr. Cinzia Sanna and Prof. Andrea Maxia, while the two *Sedum* species were collected in Emilia Romagna (Italy) and identified by Prof. Ferruccio Poli. The other Mediterranean plants samples were kindly supplied by Biokyma S.r.l, Anghiari (AR) Italy, and identified by Dr. Franco Maria Bini. Vouchers of crude drugs of the Indian

plants and Mediterranean plants were deposited in Department of Pharmacy and Biotechnology, University of Bologna (Via Irnerio 42, Bologna, Italy). Vouchers of the African plants were deposited in Herbarium of the Botanical Laboratory of the University of Ouagadougou (Burkina Faso). Vouchers of the Sardinian plants were deposited at the General Herbarium of the Department of Life and Environmental Sciences, University of Cagliari and vouchers of the two *Sedum* species were deposited in the Herbarium of the Department of Pharmacy and Biotechnology, University of Bologna. All the information (including vouchers) of the considered plants are reported in Table 1.

2.2. Preparation of the extracts

Thirty mg of dried and powdered plant material were extracted by sonication for 30 minutes using 1.5 mL of MeOH/H_2O (1:1). Subsequently, the samples were centrifuged for 20 min, the supernatant was separated from the pellet and dried to yield the crude extracts.

2.3. Tyrosinase inhibitory assay

The enzymatic inhibitory assay was performed according to Venditti et al. (2013) with slight modifications. Mushroom tyrosinase (2 mU) and sample (50 μg/mL) were incubated for 5 min in 0.1 M sodium phosphate buffer pH 6.8, in 0.1 mL of final volume. L-DOPA (final concentration 2 mM) was added up to a final reaction volume of 0.2 mL. The formation of dopachrome was immediately monitored for 5 min at 490 nm in a microplate reader (Victor^{TM} *X*3 PerkinElmer, Waltham, Massachusetts, United States) under constant temperature of 30°C. The IC_{50} (concentration necessary for 50% inhibition of enzyme activity) was calculated by constructing a linear regression curve showing extracts concentrations (from 1 to 250 μg/mL) on the *x*-axis and percentage inhibition on the *y*-axis. A negative control was obtained by adding water instead of extracts, while kojic acid (solubilized in water) was used as positive control, finding an IC_{50} of 3±0.37 μg/mL (21 μM).

The percentage of enzyme inhibition was calculated using the following formula:

$$\%\text{Inhibition} = [1 - (\Delta\text{Abs/min}_{\text{sample}} / \Delta\text{Abs/min}_{\text{negative control}}) \times 100]$$

In order to determine the kinetic parameters for the enzymatic reaction the Lineweaver-Burk plot was built, using substrate concentration in the range from 0.5 to 4 mM. In the assay conditions, the obtained K_M value was of 0.2 mM and V_{max} of 10 µmol/min (ΔAbs/min=0.03), considering dopachrome ε at 490 nm = 3.6201 mM^{-1} cm^{-1} and a light path length of 0.8 cm.

2.4. Elastase inhibitory assay

The assay was performed according to the method of Liyanaarachchi et al. (2018) whit some modifications. Porcine pancreatic elastase (1.5 mU) and extract sample (50 µg/mL) were incubated for 5 min in 0.1 M TRIS buffer pH 8.1, in 0.1 mL final volume. Substrate N-succinyl-Ala-Ala-Pro-Phe p-nitroanilide (2 mM) was added to start the reaction in a final volume of 0.2 mL. The variation of absorbance was monitored for 5 min at 420 nm in the microplate reader under constant temperature of 30°C. For the IC_{50} calculations, samples and quercetin (positive control) were tested at different concentrations ranging from 1 to 250 µg/mL. In the case of quercetin the assay was performed in 2% DMSO, thus a proper negative control in the same conditions was used for the IC_{50} calculation.

Lineweaver-Burk plot was built, using substrate concentration in the range of 0.25 - 2 mM. In the assay conditions, the obtained K_M value was of 0.2 mM and V_{max} of 6 µmol/min (ΔAbs/min=0.04), considering ε of p-nitroanilide at 420 nm = 8.8 mM^{-1} cm^{-1} and a light path length of 0.8 cm.

2.5. Total phenolic and flavonoid content

The assays were performed in Spectrophotometer Jasco V-530 as described by Di Pompo et al. (2014) with slight modifications. Briefly, for total phenolic content analysis a calibration curve was constructed using 50 µL of different gallic acid stock solutions prepared in MeOH 80% (from 10 to 200 µg/mL) mixed with 250 µL of Folin-Ciocalteu reagent (diluted 1:10) and 500 µL of H_2O. Different stock solutions of extracts were prepared in water (from 0.05 to 0.2 mg/mL) and 50 µL of each stock were mixed with the same reagents as described above. Both calibration curve and samples were incubated at room temperature for 5 min before adding 800 µL of sodium carbonate solution

(Na_2CO_3 20%). After 30 min of incubation at 40°C, absorption was recorded at 760 nm. Total phenolic content was calculated by interpolation in the calibration curve and expressed as: mg GAE (gallic acid equivalent)/g of extract (dried weight).

Total flavonoid content was determined using rutin to perform the calibration curve. Different stock solutions of extracts were prepared in water (from 0.05 to 0.2 mg/mL) and 50 µL of each one were mixed with 450 µL of methanol and 500 µL of $AlCl_3$ (2% w/volume of methanol). The absorption at 430 nm was recorded after incubation (15 min) at room temperature. The calibration curve was obtained using 50 µL of different rutin stock solutions prepared in DMSO (from 1 to 100 µg/mL). Total flavonoid content of the extracts was calculated by interpolation in the calibration curve and expressed in terms of mg RE (rutin equivalent)/g of extract (dried weight).

2.6. Statistical analysis

Values were expressed as the mean ± SD of three independent experiments (each one performed in duplicate). Statistical analyses were performed using Graph Pad Prism 4 software (La Jolla, CA, USA). Samples were compared by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post-hoc test, considering significant differences at P values <0.05. Pearson correlation coefficient (r) was evaluated in order to determine the correlation between total phenolic and flavonoid content and enzymatic activities.

3. Results

A first screening of tyrosinase and elastase inhibitory activity was carried out on the extracts at the fixed concentration of 50 µg/mL. The obtained results (reported in Table 1) allowed the selection of seventeen extracts, which, at the tested concentration, highlighted a marked inhibitory activity (percentage of inhibition higher than 30%). In particular, the following samples were selected: *Arbutus unedo* L. (leaves), *Azadirachta indica* A. Juss. (leaves), *Cistus monspeliensis* L. (aerial parts), *Cistus salvifolius* L. (aerial parts), *Cochlospermum tinctorium* Perrier ex A. Rich. (leaves), *Cytinus*

169 *hypocistis* (L.) L. (aerial parts), *Hypericum hircinum* L. (aerial parts), *Hypericum scruglii* Bacch.,
170 Brullo & Salmeri (areal parts), *Khaya senegalensis* (Desv.) A. Juss (fruits), *Limonium morisianum*
171 Arrigoni (aerial parts), *Myrtus communis* L. (fruits and leaves), *Pistacia lentiscus* L. (fruits and
172 leaves), *Pistacia terebinthus* L. (leaves), *Vitellaria paradoxa* C.F. Gaertn. (leaves and roots).

173 Those samples were more deeply investigated by calculating the IC_{50} of enzymatic inhibition and
174 comparing them by statistical analysis.

175 Regarding elastase inhibition, the IC_{50} values of the twelve selected samples ranged from 7.17±1.36
176 to 101.07±20.74 μg/mL (Fig. 1A). These results are particularly interesting considering that the
177 positive control (quercetin) showed an IC_{50} value of 61 μg/mL (202 μM). Among the twelve samples,
178 the extract obtained from the leaves of *Pistacia lentiscus* resulted the most potent elastase inhibitor.

179 Regarding the activity against tyrosinase, the IC_{50} values calculated for the sixteen most active
180 extracts ranged from 20.35±0.24 to 101.41±7.46 μg/mL (Fig. 1B). The extracts of *Cytinus hypocistis*
181 (aerial parts), *Limonium morisianum* (aerial parts) and *Pistacia lentiscus* (leaves) resulted the most
182 potent and no significant differences among their IC_{50} values were highlighted by the statistical
183 analysis.

184 As highlighted by the results of the first screening (Table 1), three samples showed a percentage of
185 tyrosinase inhibition little lower than 30%, thus, although they were not selected among the most
186 promising plants, their IC_{50} was also calculated. In particular, *Cassia siberiana* D.C. showed an IC_{50}
187 of 165 μg/mL, while *Lavandula stoechas* L. and *Hypericum scruglii* were proved only poorly active.
188 In fact, for these two plants, even at the highest tested concentration (250 μg/mL) the percentage of
189 inhibition was much lower than 50%.

190 Polyphenols and flavonoids are considered important natural active principles and, in particular, they
191 are well known for their antioxidant properties. In the present study, the total content of these classes
192 of metabolites was evaluated in all the samples. The seventeen extracts, selected as more promising

as enzymatic inhibitors, proved also enriched in flavonoids (ranging from 7.8±0.1 to 86.6±0.9 mg RE/g of extract) and phenolics (ranging from 41.8±0.7 to 147±1.4 mg GAE/g of extract).

Moreover, considering that several polyphenols and flavonoids (i.e. chalcones, flavanones, resveratrol derivatives, ellagic acid) are reported to inhibit tyrosinase and elastase (Pillaiyar et al., 2017; Xing et al., 2016; Wittenauer et al., 2015), the relations between enzyme inhibitory activities and total phenolic and flavonoid content were statistically investigated.

In particular, Pearson correlation test was performed to correlate the percentage of enzymatic inhibition (showed by the extracts at 50 μg/mL) to the phenolic and flavonoid content, respectively. Although the found correlations were not strong, in all cases *r* was comprised between 0 and 1, indicating a positive correlation between increasing total phenolic and flavonoid content and both enzymatic inhibitory activities (Fig. 2A and B). The highest positive correlation (r=0.3535 and P=0.0003) was found between tyrosinase inhibition and total phenolic content.

4. Discussion

In search for natural products endowed with elastase and tyrosinase inhibitory activity, a hundred plant extracts were *in vitro* tested against these two enzymes.

The samples were harvested in different geographical areas (Table 1), and the majority of them are plants of ethnobotanical relevance (Khare, 2014; Guarrera, 2006; Nadembega et al., 2011).

A documented ethnobotanical use is not available only for five out of the tested plants, namely: *Centaurea horrida* Badarò, *Hypericum scruglii*, *Ferula arrigonii* Bocchieri, *Limonium morisianum* and *Plagius flosculosus* (L.) S. Alavi & V. H. Heywood, which are endemic plants of Sardinia Island (Italy).

Seventeen, out of a hundred samples, were selected as the most promising and their IC_{50} of enzymatic inhibition were investigated. Among them, eleven resulted strongly active on both enzymes; five were able to inhibit only tyrosinase and one was strongly active only against elastase. Leaves extract

of *Pistacia lentiscus* emerged as the most potent elastase inhibitor and, together with *Cytinus hypocistis* (aerial parts) and *Limonium morisianum* (aerial parts), it showed also the lowest IC_{50} of tyrosinase inhibition.

P. lentiscus is used in Mediterranean traditional medicine in form of infusion or decoction to treat a wide number of diseases, such as stomachache, eyes infections, burn skin, bronchitis (Bouasla and Bouasla, 2017). Flavonoids, phenolic acids, and their derivatives such as myricetin glycoside, catechin, β-glucogallin, quercitrin gallate were identified as the most abundant phytoconstituents of this plant (Rodríguez-Pérez et al., 2013). Those compounds might play a role in the elastase inhibitory activity showed by this plant (Melzig et al., 2001). Interestingly, *L. morisianum* is an endemic and exclusive plant of Sardinia and recently some information about its phytochemical profile and anti HIV-1 activity were reported (Sanna et al., 2018a). Myricetin, myricetin 3-*O*-rutinoside, myricetin-3-*O*-(6″-*O*-galloyl)-β-d-galactopyranoside, (-)-epigallocatechin 3-*O*-gallate, tryptamine, ferulic and phloretic acids were isolated from its aerial parts.

Some of the tested samples were obtained from plant species belonging to the same genus, this allowed further considerations concerning their bioactivities. In particular, according to the statistical analysis, *Pistacia lentiscus* leaves resulted more potent elastase inhibitor than leaves of *Pistacia terebinthus* ($P<0.05$) (Fig. 1A), while no differences were found between their activity against tyrosinase (Fig. 2A). *Cistus salvifolius* was significantly more potent against elastase than *Cistus monspeliensis*, while, also in this case, no differences were found between their tyrosinase inhibitory activities. *Hypericum hircinum* was significantly more active against elastase than *Hypericum scruglii* ($P<0.05$). *Hypericum scruglii* was found not active against tyrosinase, thus it is more promising to develop a cosmetic product endowed with selective anti-wrinkle activity.

H. scruglii resulted enriched in phloroglucinols, which were proved able to inhibit the HIV-1 replication in cell based assays (Sanna et al., 2018b).

241 Moreover, *Hypericum perforatum* L. was also included in the initial screening, showing only a weak
242 percentage of inhibition on both enzymes. In fact, it was not selected among the most active plants.
243 The phytochemical profiles of these three *Hypericum* species were already reported to be significantly
244 different; in the same study their inhibitory activity against α-glucosidase was investigated and also
245 in that case, *H. perforatum* proved to be less potent than the other two *Hypericum* species (Mandrone
246 et al., 2017). The lack of cytotoxicity already reported for the hydroalcoholic extracts of these
247 *Hypericum* species (Mandrone et al., 2017) make them even more promising for cosmetic purposes.

248 A further discussion deserved to be done also on the differences in bioactivity showed by extracts
249 obtained from different organs of the same plant source (Table 1). In particular, both extracts of fruits
250 and leaves of *Myrtus communis* were tested. Both fruits and leaves were active against tyrosinase,
251 even though fruits resulted more active ($P<0.05$) (Fig. 1B), and only fruits were found active against
252 elastase. Conversely, whereas *Arbutus unedo* leaves exhibited remarkable elastase and tyrosinase
253 inhibitory activities, no enzymatic inhibition was shown by the extract obtained from its fruits.

254 *A. unedo* is a source of arbutin, a glycosylate hydroquinone, which is already known as skin-
255 whitening agent (Degen et al., 2016). However, it inhibits the monophenolase function of this enzyme
256 (Hori et al., 2004), while, in this work, the inhibition of its diphenolase function was evaluated. This
257 data suggests that the presence of active metabolites other than arbutin (i.e. flavonoids) (Castaldi et
258 al., 2009) might contribute to *A. unedo* (leaves) anti-ageing and skin-whitening properties.

259 In the case of *Pistacia lentiscus*, fruits and leaves extracts were both strongly active against tyrosinase,
260 with no significant differences in their IC_{50} values, while only leaves were found active against
261 elastase.

262 Roots and leaves of *Vitellaria paradoxa* were both selected among the most active samples, showing
263 no significant differences between their IC_{50} values of tyrosinase and elastase inhibition (Fig. 1A and
264 1B). *Vitellaria paradoxa* is known as shea tree and it is very important for food and cosmetic
265 industries. The most investigated and important product obtained from this plant is the butter

extracted of the kernel, which is endowed with anti-inflammatory and antioxidant properties (Honfo et al., 2014). Saponins, tannins, and alkaloids were found in its roots, stem bark, and leaves even though these organs remain still poorly investigated (Ndukwe et al., 2007).

Phenolic and flavonoid content of all the samples was evaluated, and the plants selected as promising enzymatic inhibitors showed also to be enriched in these classes of natural compounds. These results suggest that the selected plants might have an additional value as skin protectors and anti-ageing agents, due to flavonoids and polyphenols antioxidant potential.

A linear correlation was found between enzymatic activities and increasing phenolic and flavonoid content. Specific class of polyphenols might act against tyrosinase through a competitive mechanism of inhibition, consistently with the biological role of this enzyme, which, in fact, is a polyphenoloxidase.

However, compounds, other than flavonoids and polyphenols, might be responsible for the activity against the considered enzymes, and further experiments are ongoing in order to acquire more information.

5. Conclusions

A hundred extracts obtained from plants collected in India, Africa and Mediterranean area were screened as elastase and tyrosinase inhibitors. Seventeen extracts were selected as the most promising, and among them eleven resulted strongly active on both enzymes; five were able to inhibit only tyrosinase and one was strongly active only against elastase. Noteworthy, among the most active plants selected, two are endemic of Sardinia Island, namely: *Hypericum scruglii* and *Limonium morisianum*.

The plants active against both enzymes are potentially suitable to develop skin-whitening agents, endowed with additional anti-wrinkles effect. In particular, the following 10 plants potently inhibited both enzymes: *Arbutus unedo* (leaves), *Cistus salvifolius* (aerial parts), *Cistus monspeliensis* (aerial

parts), *Cytinus hypocistis* (aerial parts), *Hypericum hircinum* (aerial parts), *Limonium morisianum* (aerial parts), *Pistacia terebinthus* (leaves), *Pistacia lentiscus* (leaves), *Myrtus communis* (fruits), and *Vitellaria paradoxa* (leaves and roots).

Hypericum scruglii (aerial parts) resulted a strong and selective elastase inhibitor, suggesting its potential use as ingredient for selective anti-wrinkles cosmetics.

Azadirachta indica (leaves), *Cochlospermum tinctorium* (leaves), *Khaya senegalensis* (leaves), *Myrtus communis* (leaves) and *Pistacia lentiscus* (fruits) showed activity only against tyrosinase, resulting of particular interest to develop skin-whitening agents with no anti-wrinkle effect, eventually ideal for youngest skins.

Moreover, the most bioactive plants resulted also enriched in polyphenols and flavonoids, conferring them additional antioxidant properties. The total phenolic and flavonoid content showed a linear correlation with the enzymatic inhibitory activities. In order to identify the metabolites responsible for the activities, further biological and phytochemical studies are ongoing on the selected plants.

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Figures captions

Fig. 1. IC_{50} values of elastase inhibition (A) and IC_{50} values of tyrosinase inhibition (B) obtained for the most active extracts. Different letters within the same assay indicate significant differences in ANOVA test ($P<0.05$). Results are expressed ad means ± SD of three independent experiments.

Ai=*Azadirachta indica*; AuL=*Arbutus unedo* (leaves); Csa=*Cistus salvifolius*; Ct=*Cochlospermum tinctorium*; Cym=*Cistus monspeliensis*; Cyh=*Cytinus hypocistis*; Hh=*Hypericum hiricinum*; Hs=*Hypericum scruglii;* Ks=*Khaya senegalensis*; Lm=*Limonium morisianum*; McF=*Myrtus communis* (frutits); McL=*Myrtus communis* (leaves); Pit=*Pistacia terebinhtus*; PlF=*Pistacia lentiscus* (fruits); PlL=*Pistacia lentiscus* (leaves); VpL=*Vitellaria paradoxa* (leaves); VpR=*Vitellaria paradoxa* (roots).

Fig. 2. A: Correlation between the total phenolic content and the percentages of enzymatic inhibitions. Total phenolic content is expressed in mg GAE/g. For elastase *r2* was: 0.06207 and *P* value: 0.0124, while Pearson coefficient (*r*): 0.2491. For tyrosinase *r2* was: 0.1249; *P* value: 0.0003 and *r*: 0.3535. **B:** Correlation between the total flavonoid content and the percentages of enzymatic inhibitions. Total flavonoid content is expressed in mg RE/g. For elastase *r2* was: 0.07369, *P* value:

0.0063 and *r*: 0.2715. For tyrosinase *r2* was: 0.07438, *P* value: 0.0060 and Pearson coefficient: 0.2727.

Fig. 1

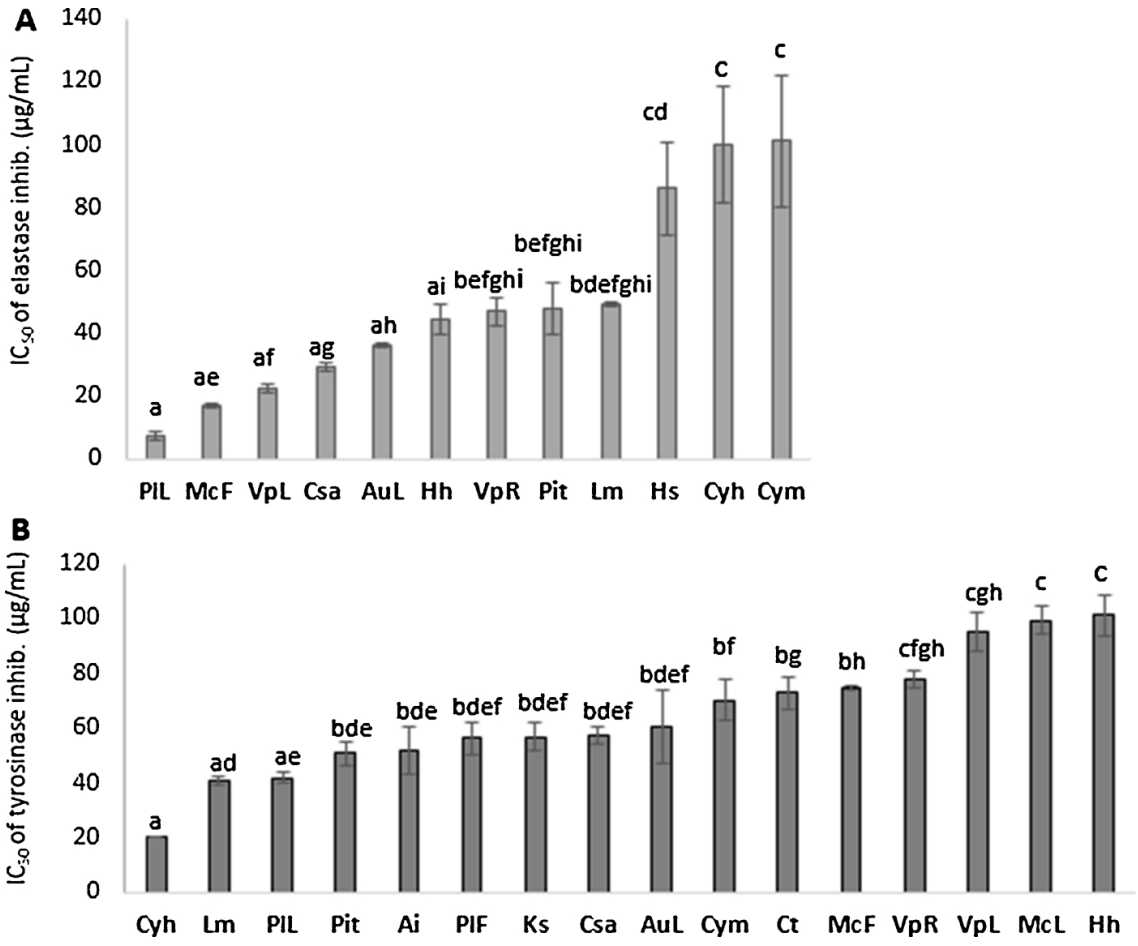

Fig. 2

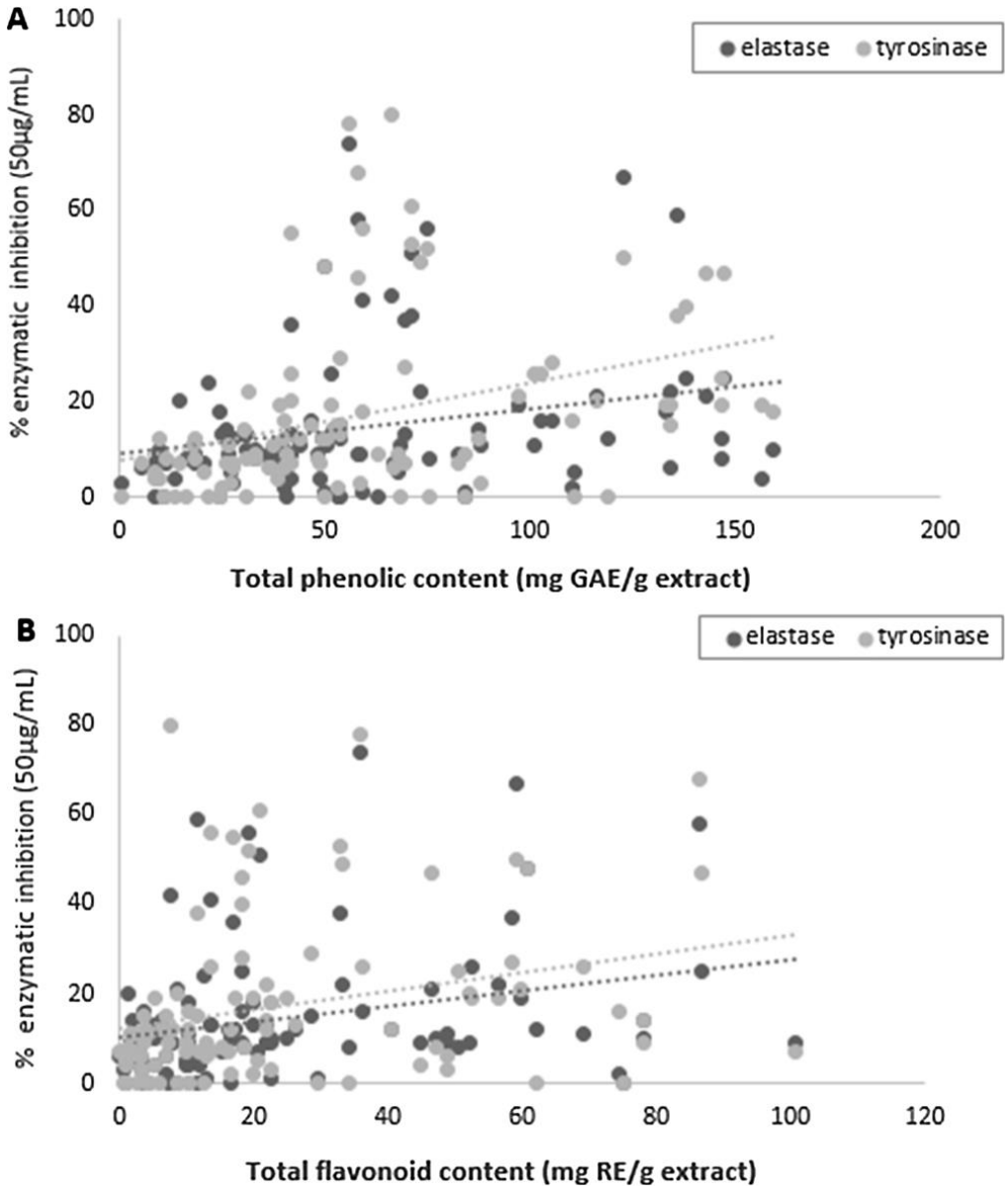
A
elastase
tyrosinase
% enzymatic inhibition (50µg/mL)
Total phenolic content (mg GAE/g extract)
B
elastase
tyrosinase
% enzymatic inhibition (50µg/mL)
Total flavonoid content (mg RE/g extract)

Table 1. The table reports all the plants used in this study, their botanical name, voucher number, family, the plant part used, their origin, and the percentage of elastase and tyrosinase inhibitory activity tested at 50 μg/mL.

Traditional Medicine	Plant Name	Family	Plant Part	Elasatse Inhibition	Tyrosinase Inhibition
Ayurveda	*Aconitum heterophyllum* Wall. ex Royle (#MAPL 0402)	Ranunculaceae	roots	8%	11%
	Aegle marmelos (L.) Corrêa (#MAPL 0089)	Rutaceae	leaves	1%	9%
	Alstonia scholaris (L.) R. Br. (#MAPL 0430)	Apocynaceae	bark	0%	9%
	Asparagus racemosus Willd. (#MAPL 0451)	Asparagaceae	tuberous root	0%	4%
	Azadirachta indica A. Juss. (#MAPL 0158)	Meliaceae	leaves	21%	47%
	Bacopa monnieri (L.) Wettst. (#MAPL 4278)	Plantaginaceae	whole plant	4%	16%
	Boerhavia diffusa L. (#MAPL 5188)	Nictagynaceae	whole plant	5%	3%
	Boswellia serrata Roxb. ex Colebr. (#MAPL 0827)	Burseraceae	resin	6%	7%
	Centella asiatica (L.) Urb. (#MAPL 1814)	Apiaceae	whole plant	9%	8%
	Chlorophytum borivilianum Santapau & R.R.Fern. (#MAPL 0001/06)	Asparagaceae	tuberous roots	7%	6%

Commiphora mukul (Hook. ex Stocks) Engl. (#MAPL 3847)	Burseraceae	resin	5%	0%
Convolvulus prostratus Forssk. (#MAPL 7028)	Convolvulaceae	whole plant	0%	2%
Crateva nurvala Buch. Ham. (#MAPL 3563)	Capparaceae	bark	3%	6%
Curculigo orchioides Gaertn. (#MAPL 2045)	Hypoxidaceae	tuberous root	5%	9%
Embelia ribes Burm. f. (#MAPL 3194)	Primulaceae	fruits	2%	6%
Phyllanthus emblica L. (#MAPL 0338)	Phyllanthaceae	pericarp	6%	15%
Ficus religiosa L. (#MAPL 6442)	Moraceae	resin	4%	7%
Hemidesmus indicus (L.) R. Br. ex Schult. (#MAPL 3904)	Apocynaceae	roots	14%	12%
Mimosa pudica L. (#MAPL 5108)	Leguminosae	leaves	0%	15%
Mucuna pruriens (L.) DC. (#MAPL 0044)	Leguminosae	seeds	4%	19%
Moringa oleifera Lam. (#MAPL 0415)	Moringaceae	seeds	0%	9%
Pueraria tuberosa (Willd.) DC. (#MAPL 1421)	Leguminosae	roots	20%	7%
Rosa centifolia L. (#MAPL 4527)	Rosaceae	petals	8%	25%
Rubia cordifolia L. (#MAPL 4548)	Rubiaceae	roots	9%	6%
Swertia chirata Buch.-Ham. ex Wall. (#MAPL 4536)	Gentianaceae	whole plants	9%	7%

	Terminalia arjuna (Roxb. ex DC.) Wight & Arn. (#MAPL 0722)	Combretaceae	stem bark	18%	19%
	Terminalia bellirica (Gaertn.) Roxb. (#MAPL 2103)	Combretaceae	fruits	12%	19%
	Terminalia chebula Retz. (#MAPL 2104)	Combretaceae	pericarp	10%	18%
	Tinospora cordifolia (Lour.) Merr. (#MAPL 2050)	Menispermaceae	stem	4%	7%
	Withania somnifera (L.) Dunal (#MAPL 3203)	Solanaceae	roots	4%	0%
African Traditional Medicine	*Vitellaria paradoxa* C.F.Gaertn. (Herbarium OUDG 6736)	Sapotaceace	leaves	67%	50%
	Vitellaria paradoxa C.F.Gaertn. (Herbarium OUDG 6736)	Sapotaceace	roots bark	59%	38%
	Cassia sieberiana D.C. (Herbarium OUDG 4890)	Leguminosae	roots bark	16%	28%
	Chrysanthellum indicum subsp. *afroamericanum* B.L. Turner. (Herbarium OUDG 2381)	Compositae	whole plant	2%	16%
	Cochlospermum planchonii Hook.f. ex Planch (Herbarium OUDG 4865)	Bixaceae	tuber	21%	20%
	Cochlospermum tinctorium Perrier ex A. Rich (Herbarium OUDG 3410)	Bixaceae	leaves	25%	47%

	Euphorbia paganorum A. Chev. (Herbarium OUDG 4792)	Euphorbiaceae	leaves and branches	0%	0%
	Gardenia sokotensis Hutch (Herbarium OUDG 4389)	Rubiaceae	leaves	8%	0%
	Gardenia sokotensis Hutch (Herbarium OUDG 4389)	Rubiaceae	stem bark	11%	0%
	Khaya senegalensis (Desv.) A. Juss. (Herbarium OUDG 7831)	Meliaceae	fruit	25%	40%
	Panicum subalbidum Kunth (Herbarium OUDG 5989)	Poaceae	roots	13%	0%
Mediterranean Tradition	*Agrimonia eupatoria* L. (BKY-H001)	Rosaceae	aerial parts	22%	19%
	Alchemilla vulgaris L. (BKY-H601)	Rosaceae	aerial parts	12%	0%
	Althaea officinalis L. (BKY-I900)	Malvaceae	roots	0%	0%
	Asparagus officinalis L. (BKY-L601)	Asparagaceae	roots	7%	6%
	Betula pendula Roth. (BKY-U606)	Betulaceae	leaves	11%	3%
	Calendula officinalis L. (BKY-M100)	Compositae	petals	15%	0%
	Centaurium erythraea Rafn. (BKY-G600)	Gentianaceae	aerial parts	1%	0%
	Coriandrum sativum L. (BKY-I400)	Apiaceae	fruits	8%	0%
	Equisetum arvense L. (BKY-H900)	Equisetaceae	stem	3%	0%

	Galium verum L. (BKY-B800)	Rubiaceae	aerial parts	0%	0%
	Gentiana lutea L. (BKY-Q001)	Gentianaceae	roots	18%	0%
	Hypericum perforatum L. (BKY-G200)	Hypericaceae	aerial parts	19%	21%
	Marrubium vulgare L. (BKY-S400)	Lamiaceae	aerial parts	16%	15%
	Medicago sativa L. (BKY-C106)	Leguminosae	aerial parts	10%	4%
	Parietaria officinalis L. (BKY-V900)	Urticaceae	aerial parts	13%	7%
	Pinus sylvestris L. (BKY-C101)	Pinaceae	gems	14%	7%
	Primula veris L. (BKY-B001)	Primulaceae	roots	11%	12%
	Sedum hispanicum L. (Herbarium BOLOHSFI104208)	Crassulaceae	aerial parts	9%	4%
	Sedum sexangulare L. (Herbarium BOLOHSFI104210)	Crassulaceae	aerial parts	12%	12%
	Thymus serpyllum L. (BKY-R100)	Lamiaceae	aerial parts	11%	26%
	Thymus vulgaris L. (BKY-C900)	Lamiaceae	leaves	16%	26%
	Zingiber officinalis Roscoe (BKY-H600)	Zingiberaceace	roots	10%	11%
	Verbena officinalis L. (BKY-S010)	Verbenaceae	aerial parts	9%	3%
Mediterranean Tradition Collected in Sardinia	*Arbutus unedo* L. (Herbarium CAG 878)	Ericaceae	fruits	9%	11%
	Arbutus unedo L. (Herbarium CAG 878)	Ericaceae	leaves	56%	52%
	Asphodelus ramosus L. (Herbarium CAG 1405)	Xanthorrhoeaceae	bulbs	7%	5%

Asphodelus ramosus L. (Herbarium CAG 1405)	Xanthorrhoeaceae	leaves	9%	12%
Carlina gummifera (L.) Less. (Herbarium CAG 770)	Compositae	leaves	9%	12%
Centaurea calcitrapa L. (Herbarium CAG 781)	Compositae	aerial parts	12%	7%
Centaurea horrida Badarò (Herbarium CAG 777) [a, d]	Compositae	aerial parts	13%	2%
Centaurea napifolia L. (Herbarium CAG 784)	Compositae	aerial parts	7%	8%
Cistus monspeliensis L. (Herbarium CAG 135)	Cistaceae	aerial parts	38%	53%
Cistus salviifolius L. (Herbarium CAG 135/C)	Cistaceae	aerial parts	51%	61%
Cynara cardunculus L. (Herbarium CAG 790)	Compositae	aerial parts	9%	22%
Cytinus hypocistis (L.) L. (Herbarium CAG 1200)	Cytinaceae	aerial parts	42%	80%
Ferula arrigonii Bocchieri (Herbarium CAG 612/A) [a, d]	Apiaceae	leaves	7%	8%
Ferula arrigonii Bocchieri (Herbarium CAG 612/A) [a, d]	Apiaceae	roots	7%	5%
Galactites tomentosa Moench (Herbarium CAG 789)	Compositae	aerial parts	11%	12%

Genista corsica (Loisel.) DC. (Herbarium CAG 286) [b]	Leguminosae	aerial parts	10%	8%
Glechoma sardoa (Bég.) Bég. (Herbarium CAG 1104) [a]	Lamiaceae	aerial parts	13%	26%
Hypericum hircinum L. (Herbarium CAG 232)	Hypericaceae	aerial parts	48%	48%
Hypericum scruglii Bacch., Brullo & Salmeri (Herbarium CAG 239/C)[a, d]	Hypericaceae	aerial parts	37%	27%
Lavandula stoechas L. (Herbarium CAG 1067)	Lamiaceae	aerial parts	15%	29%
Limonium morisianum Arrigoni (Herbarium CAG 909/G) [a, d]	Plumbaginaceae	aerial parts	41%	56%
Myrtus communis L. (Herbarium CAG 514)	Myrtaceae	fruits	36%	55%
Myrtus communis L. (Herbarium CAG 514)	Myrtaceae	leaves	22%	49%
Pistacia lentiscus L. (Herbarium CAG 280)	Anacardiaceae	fruits	9%	46%
Pistacia lentiscus L. (Herbarium CAG 280)	Anacardiaceae	leaves	74%	78%
Pistacia terebinthus L. (Herbarium CAG 796)	Anacardiaceae	leaves	58%	68%
Ptilostemon casabonae (L.) Greuter (Herbarium CAG 743)[c]	Compositae	aerial parts	10%	19%

Plagius flosculosus (L.) S.Alavi & V.H.Heywood (Herbarium CAG 743)[b, d]	Compositae	aerial parts	1%	18%
Rosmarinus officinalis L. (Herbarium CAG 1091)	Lamiaceae	aerial parts	26%	19%
Santolina corsica Jord. & Fourr. (Herbarium CAG 732/A) [b]	Compositae	aerial parts	13%	14%
Scolymus hispanicus L. (Herbarium CAG 812)	Compositae	aerial parts	10%	12%
Silybum marianum (L.) Gaertn. (Herbarium CAG 801)	Compositae	aerial parts	8%	8%
Smilax aspera L. (Herbarium CAG 1414)	Smilacaceae	aerial parts	12%	13%
Stachys glutinosa L. (Herbarium CAG 1099)	Lamiaceae	aerial parts	10%	9%
Tanacetum audibertii (Req.) DC. (Herbarium CAG 737/A)[b]	Compositae	aerial parts	9%	20%
Thymus herba barona Loisel. (Herbarium CAG 1065)[b]	Lamiaceae	aerial parts	14%	14%

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