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ENZYMES SCREENING FROM CRUDE EXTRACT OF *PERGULARIA TOMENTOSA* L.

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Abstract: Screening of a large number of enzymes and determination of their quantities in aqueous extracts of a maceration of dried *Pergularia tomentosa* L. was performed in the present study. Results revealed β-amylase, protease, pepsin, lipoxygenase, L-asparaginase, polyphenol oxidase, tyrosinase and lipase activities in the extract of the defatted whole plant with different ranges and importance of activities.

INTRODUCTION

Pergularia tomentosa L., commonly known as BouHliba (Tunisia) and Oumou Ejloud (Hassaniya), belongs to the milky weed family Asclepiadaceae that includes 2000 to 3000 species is classified under 130 to 350 genera especially localized in tropical regions. It is generally founded in sandy, clayey to gravelly-stony soils and in hot deserts where rainfall does not exceed 100 mm (Benhouhou, 2005). In the Sahelien countries, it remains green all year round. It is a fetid smelling twinner herb, draw or shaken. Leaves are heart-shaped, fleshy and indented in kidney of an ashy gray. Stems are covered with hair and bear milky latex. Flowers are stalked and arranged in small axillaries umbels. Fruits looking like pickles are ovoid with a tomentose beak, short or long curved (Tahir, 1994). Seeds are flat and densely pubescent with a lighter and crenate margin (Goyder, 2006). Pharmacological studies have confirmed that the different parts of the plant have had a wide application in different folk medicine remedies, such as in case of diarrhea, bronchitis, constipation, skin diseases, tuberculosis and used as poultice, depilatory, laxative, anthelmintic, abortifacient… (Mossallam and BaZaid, 2000; Abou- Zeid et al., 2008; Clauzel, 2006; Hammiche and Maiza, 2006).

The fact that *P. tomentosa* represent a source of cardiotonic glycosides (Gohar et al., 2000; Hamed et al., 2006) and it is implied in different biological activities as molluscicidal (Hussein et al., 1994), antifungal (Hassan et al., 2007; Boulenouar et al.,2009), antibacterial (Dangoggo et al., 2002), insecticidal (Abdallah et al., 2004; Green Paul et al., 2011) and anti-tumor activities (Al-Said et al., 1989; Harms et al., 2008), which makes it an interesting object for different enzymes screening.

MATERIALS AND METHODS

Plant material

The collected plants were identified by Ferjani Ben Abdallah (Ben Abdallah et al., 2006) at the herbarium section, Department of Biology, Sfax Faculty of Sciences in Tunisia. To extract enzymes, defatted plants were macerated with 30% ethanol. In order to precipitate enzymes, ethanol was added to the supernatant successively with a percentage of 96 and 75 (Yotova et al., 2000).

Enzymes assays

The protein content of the enzyme preparations was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard. For β-amylase assay, the activity was read in a colorimeter at 540nm. A calibration curve was made with maltose (0.3 – 3.0 μmoles) to convert colorimeter readings to units of activity (Bernfeld, 1955). Concerning the caseinolytic activity (López et al., 2000), the reaction mixture contained 1 g/100 ml casein in Tris-HCl 0.1 mol/l buffer (pH 9.0) and the crude extract. Pepsin activity was based on the method of Anson (1938) with slight modification using denatured bovine hemoglobin. The absorbance was measured at 280 nm. Lipase activity was determined using p-nitrophenyl palmitate (pNPP) as substrate with slight modifications according to Ertuğrul et al. (2007) at λ=410 nm. Following the modified method of Axlerod, et al. (1981), the lipoxygenase activity was measured in borate buffer (0.2 M, pH 9.00), using linoleic acid as a substrate. The LOX activity was measured at 234 nm and expressed in absorbance per minute for 1 ml per quantity of protein in the plant extract. Cresolase activity was determined at 475 nm using L-tyrosine as a monophenolic substrate (Pérez-Gilabert et al., 2001). The activity of tyrosinase was determined at 30°C using a colorimetric assay adapted from literature (Behbahani et al., 1993; Duckworth and Coleman, 1970). The reaction was followed measuring the absorbance at intervals of 10 s for 5 min at 475 nm. To measure the asparaginase activity in the supernatant of the experiment extract, the concentration of ammonia, which is liberated from the enzyme action was determined by the method of direct Nesslerization (Ren et al., 2010).

RESULTS AND DISCUSSION

β-amylase hydrolyses the β-1,4-glucan bonds in amylosaccharide chains from the non-reducing ends and generates maltose. Maltose production is utilized in pharmaceutical industry for dispensing, production of maltose rich syrups and maltitol and utilized also in brewing industry. The check of the enzymatic activity of the crude extract of *Pergularia tomentosa* L. revealed that we had an activity of 0.652 U/mg of protein, which is less than *Saccharium offinacium* (Oyefuga et al., 2011) and seeds of *Trigonella Foenum-Graecum* (Srivastava and Kayastha, 2014).

Proteolytic enzymes are instrumental in regulating seed germination, apoptosis, root symbiosis (Takeda et al., 2007), plant defense and mobilization of storage proteins. Our results showed that the protease activity was high up to pH 7, followed by pH 5.0 and pH 9.0. Because its pH optimum is around 7.0, we think that we have a papain which belongs to the cysteine protease family. This enzyme is the most exploited plant protease in the brewing, baking industries and in cheese production.

The screening for the presence of pepsin in our plant showed that we have activity in the whole plant. Moreover, pepsin is the major digestive enzyme in stomach of animals. In fact, some plants have a similar activity to this enzyme: Bromelain, a protease found in juice and stem of *Ananas comosus*. Also, papain, derived from the latex of *Carica papya*, is known as "Vegetable Pepsin".

In many plant species, the activity of L-asparaginase is related to the level of potassium, and evidence suggests that asparagine is synthesized in leaves and is re-exported to the apex or fruits (Sieciechowicz et al., 1988). The low activity (0.073 U/mg) that we have detected can be explained by the fact that the higher protease activity is inversely proportional to L-asparaginase levels (Rajesh, 2011).

Lipoxygenase is involved in wounding and other stress responses. In plants, linolenic and linoleic acids are the most common substrates (Siedow, 1991). Defatted *Pergularia* showed an activity around 1.374 U/mg of protein with a different concentration of linolenic acid. Hydroperoxide substrates in the vegetative tissues of our plant can explain the mechanism of defense (Creelman and Mullet, 1997) and the antimicrobial activity (Croft et al., 1993).

Polyphenol oxidase is inducible by both biotic and abiotic stresses and plays an important role in various physiological reactions and in scavenging of free radicals in photo-synthesizing tissues (Heimdal et al., 1994). The activity 2.418 U/mg of protein can be influenced by the traces of ethanol in the extract and be related to the length of the storage period.

As a polyphenol oxidase, tyrosinase is responsible for the enzymatic browning of vegetables (Martinez and Whitaker, 1995) and it is involved in the regulation of the redox potential in plants (Walker and Ferrar, 1998). The screening of this activity in our extract showed a good activity with 1.319 U/mg proteins (Table 1).

Plant lipases are especially interesting because of their low cost, wide availability from natural sources (Freire and Castilho, 2000) and their high substrate selectivity and specificity. The ethanolic extract of *P. tomentosa* at pH 8.0 showed a lipase activity higher than at pH 4.8. Some research concerning lipolytic enzymes of Aslepiadaceae hypothesized that lipase was involved in the lipidic or terpenic metabolism of latex (Warnaar, 1987). By contrast, only few plant enzymes showing true lipase activity had been cloned so far.

Table 1. Enzyme activities obtained from our experiment with *Pergularia tomentosa* L.

Enzyme	Activity (U/mg of protein)
β-amylase	0.652
Protease	209.91 (pH 9.0) 299.872 (pH 7.0) 160.907 (pH 5.0)
Pepsin	185.409
L-asparaginase	0.073
Lipoxygenase	1.374
Polyphenol oxidase	2.418
Tyrosinase	1.319
Lipase	37.9 (pH 8.0) 8.91 (pH 4.8)

CONCLUSION

In this study, we report for the first time different enzymes screening in a plant belonging to the Asclepiadaceae family. This work opens a new window of research since *Pergularia tomentosa* L. is grown widely and in spontaneous way in Tunisia and offers a new biological material to be investigated as a source especially of proteases and polyphenol oxidase.

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