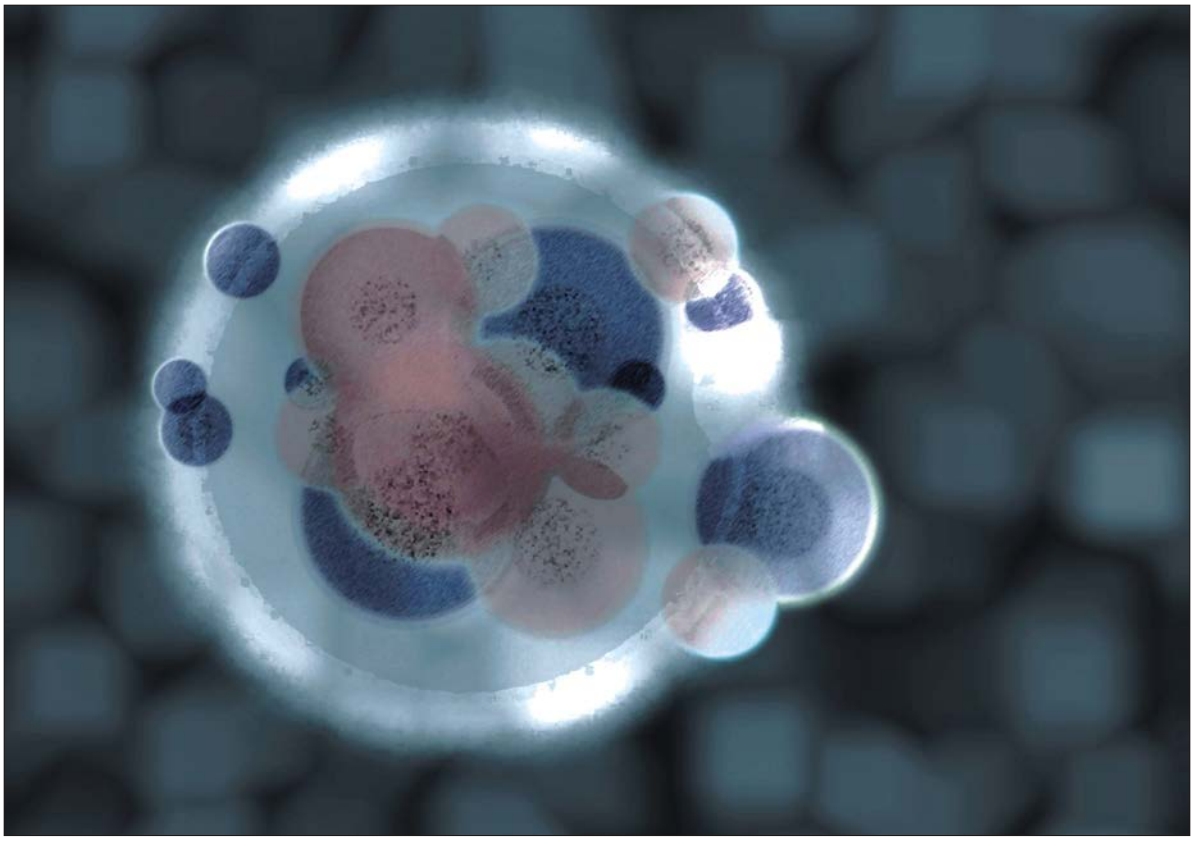

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Enzymatic Mechanisms Related to Antimicrobial Activity of *Rutaceae* Extracts

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Abstract: Rutaceae extracts enzymatic activities were studied in order to find out their possible relation with the antimicrobial activity that they possess as well as the improvement of other productive aspects. Several enzymatic activities were detected (Acid phosphatase, alkaline phosphatase, esterase, lipase, leucine arylamidase, trypsine, valine arylamidase, cystine arylamidase, α-fhymotrypsine, naphtol-AS-BI-phosphohydrolase, α-fucosidase, α and β galactosidase, β-glucuronidase, α and β glucosidase, α-mannosidase and N-acetyl-β-glucosaminidase) and special attention is paid to those enzymes that have direct effect on either the bacterial cell structure or the bacterial metabolism.

Key words: Enzymatic activity, Rutaceae, antimicrobial activity, natural extracts

INTRODUCTION

Plant extracts have been used all through the centuries as treatment for several diseases due to the antibacterial and antifungal activities that many of them possess.

These antimicrobial properties are mainly due to some of their components and amongst them we may cite: terpens, essential oils, cumarines and flavonoids (Cutter, 2000; Kim *et al*., 1995; Lis-Balchin *et al*., 1998; Mau *et al*., 2001; Vargas *et al*., 1999).

About 1340 plants are known to be potential sources of antimicrobial components (Gould, 1996), but more than 250000 species contain a wide diversity of bioactive components.

Very often the biologic activity of natural extracts is due to the synergism among the different components because when separated, their activity is lower than when being together. It is considered that extracts toxicity decreases when they are used as a crude extract than when the components are purified and isolated. This fact is known as buffering (Poppenga, 2001; Smith-Schalkwijk, 1999).

The mechanisms of action of several natural extracts is thought to be due to the overcharge that they produce on the microorganisms cell wall providing their bacteriostatic or bactericide activity although it is not well known. This fact determines the control and integrity loss. The exact action mechanisms of several natural extracts are not well known, however it is acknowledged that their bacteriostatic or bactericide activity is due to the overcharge that they produce on the microorganisms cell wall. This factor determines the control and integrity loss. Apart from the antimicrobial activity of some natural extracts, they also possess other biological activities, for instance related to the enzymatic, improving appetite and optimizing the nutrient absorption in mammals fed with them (Kamel, 2002).

The plant extracts are part of a group of additive substances that are tolerated as additives from the legal point of view. The natural extracts would belong to the additives group classified as aromatic and flavoring substances, in which are included all the natural and synthetic products and can be used in all the animal species, with no restriction neither in the age nor the product dosage. These products are well accepted by the customers and represent one of the most promising alternatives to growth promoters antibiotics and therefore the research for new substances stands for an important investigation area in the feed additives field.

The active principles from the plants are usually: essential oils, resins and glycoside alkaloids. The 8th edition of the French Pharmacopea defines essential oils as products with a general composition quite complex that contain the volatile principles that are found in vegetables more or less modified during their preparation.

The essential oils only are specially found in plants. It is thought that more or less exist 17500 aromatic species. These oils are chemical products that form the odoriferous essences of a wide range of vegetables. They are widely distributed in about 60 plant families that include: *Compositae*, *Labiatae*, *Lauraceae*, *Mirtaceae*,

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Panaceae, *Rosaceae*, *Rutaceae*, *Umbeliferae*, etc. (Peris and Asensio, 2001; San Martín, 1977) and can be isolated from different parts of the plant.

The main objective of this study is to establish the enzymatic activities from *Rutaceae* extracts and their possible relation with the antimicrobial activity that possess, as well as the capability of increasing the digestibility of the products used for animal feed, improving, therefore, the productivity efficiency.

MATERIALS AND METHODS

The following assays have been performed using *Rutaceae* extracts. With the aim of evaluating the enzymatic activity of *Rutaceae* extracts, API ZYM® methodology (Biomérieux, Marcy-l'Etoile, France) was used according the modifications performed by Calvo, (1985). This system is a semi-quantitative micromethod designed for the research of nineteen enzymatic activities: acid phosphatase, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, lipase, valine arylamidase, cystine arylamidase, trypsine, α-Chymotrypsine, Naphtol-AS-BI-phosphohydrolase, α-fucosidase, α and β galactosidase, β-glucuronidase, α-mannosidase, α and β glucosidase and N-acetyl-β-glucosaminidase.

Briefly, the modified API ZYM® methodology consists of inoculating each product to test against a gallery containing twenty cupules, each with the base consisting of the enzymatic substrate and its buffer.

The gallery is kept in environmental temperature for 4 h, in order to reach a complete enzyme-substratum reaction. After that, a drop of ZYM A and ZYM B reagents are added, letting to a color development for at least five minutes. Results are checked according to the colorimetric patterns provided with the kit.

RESULTS AND DISCUSSION

The enzymatic activities detected in the tested products, determine that when they come into contact with the bacterial cells, these remain exposed to them and therefore may induce adverse reactions that contribute to the bacterial inhibition. Taking into account the different enzymatic activities detected, we can state that N-acetyl-β-glucosaminidase contributes to the bacterial wall destruction, due to the fact that a basic compound of the bacterial wall is N-acetyl-glucosamine. In addition, phosphatases may inhibit teicoic acids formation, which are part of the Gram positive wall and may also interrupt glucose degradation. Other enzymes that were quite active were esterase and lipase. They both have effect on lipids, which are important compounds of the Gram negative bacteria as well as Gram positive spores. The protein synthesis is also affected because leucine arylamidase and cystine arylamidase produce interference in this anabolic process (Table 1).

Galactosidases, glucosidases, glucuronidases, mannosidases and fucosidases were also detected

Table 1: Semi-quantification of the *Rutaceae* extract enzymatic activities

Enzyme assayed	Substrate	pH	Results according to colour pattern	Nanomoles of hydrolysed substrate
Control	-	-	-	-
Alkaline phosphatase	2-naphthyl phosphate	8.5	2	10
Esterase (C4)	2-naphthyl butyrate	6.5	2	10
Esterase lipase (C8)	2-naphthyl caprylate	7.5	0	0
Lipase (C14)	2-naphthyl myristate	7.5	2	10
Leucine arylamidase	L-leucyl-2-naphthylamide	7.5	1	5
Valine arylamidase	L-valyl-2-naphthylamide	7.5	0	0
Cystine arylamidase	L-cystyl-2-naphthylamide	7.5	2	10
Trypsin	N-benzoyl-DL-arginine-2-naphthylamide	8.5	0	0
α-chymotrypsin	N-glutaryl-phenylalanine-2-naphthylamide	7.5	0	0
Acid phosphatase	2-naphthyl phosphate	5.4	1	5
Naphtol-AS-BI-phosphohydrolase	Naphtol-AS-BI-phosphate	5.4	1	5
α-galactosidase	6-Br-2-naphthyl-αD-galactopyranoside	5.4	2	10
β-galactosidase	2-naphthyl-βD-galactopyranoside	5.4	2	10
β-glucuronidase	Naphthol-AS-BI-βD-glucuronide	5.4	0.5	2.5
α-glucosidase	2-naphthyl-αD-glucopyranoside	5.4	1	5
β-glucosidase	6-Br-2-naphthyl-βD-glucopyranoside	5.4	2	10
N-acetyl-β-glucosaminidase	1-naphthyl-N-acetyl-βD-glucosaminide	5.4	3	20
α-mannosidase	6-Br-2-naphthyl-αD-mannopyranoside	5.4	0.5	2.5
α-fucosidase	2-naphthyl-αL-fucopyranoside	5.4	0.5	2.5

although in lower levels. However, they inhibit carbohydrate formation and intervene in a negative way in energy uptake by bacteria.

It is also remarkable to point that the presence of α and β galactosidase, β glucosidase, N-acetyl-β-glucosaminidase and proteases may help the digestibility of those feed products that contain these natural extracts.

All these factors seem to lead to a higher microbiological quality as well as an improvement of some important productive aspects as digestibility and nutrient absorption optimization (Table 1).

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