NEW THERAPEUTIC FORMULATIONS WITH AN ANTIBACTERIAL EFFECT, BASED ON PLANT EXTRACTS

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Abstract
The antibacterial effects produced by anthocyanins and other bioactive plant compounds are weaker than those generated by antibiotics. In some cases, the combination of extract-antibiotic can cause synergistic effects, also the purpose of the research was to develop and test new antibiotic - plant extract formulations. New potential antimicrobial formulations was done by soaking discs impregnated with piperacillin or tetracycline with different extract. The tested microorganisms were: Staphylococcus aureus ATCC 25923, Streptococcus sp., Escherichia coli 820B, soil bacterium 23S, and Enterobacter cloacae. The combination of antibiotics with extracts determined, only for some of the microorganisms tested, better antibacterial effects than those caused by the antibiotic or the extract.

Keywords: peony extract, fern extract, antibiotic, new formulations.

1. INTRODUCTION

The products of secondary metabolism that are synthesized by plants are extremely important resources for the pharmaceutical, cosmetics, food, etc.. Researches on anthocyanins have demonstrated their beneficial effects in therapy due to their antioxidant, anti-inflammatory, anticarcinogenic, antiatherogenic, neuroprotective, antidiabetic, etc. properties. The antiviral and antibacterial properties of anthocyanins have been studied to a lesser extent.

Research conducted over the past 10 years have shown inhibitory effects on the growth of a number of microorganisms (Werlein et al., 2005; Okwui & Ukanwa, 2010), and also growth-enhancing effects on others (Puupponen-Pimia et al., 2001; Werlein et al., 2005). Extracts made from various organs of ferns have antibacterial activity, some even a strong one. Thus, antimicrobial properties were demonstrated against Gram-positive and Gram-negative bacteria, as well as on fungi (Vincent & Kanna 2007, Lee et al., 2009).

The genus *Dryopteris* has an especially strong antibacterial activity. *Dryopteris crassirhizoma* and *D. filix-mas* can be used against *Staphylococcus aureus* (Lee et al., 2009). Extracts obtained from the leaves of *Asplenium niponicum* and *Hypolepis punctata* are more efficient than those obtained from *Dryopteris crassirhizoma* against *Streptococcus mutans* and *S. sobrinus* (Shin, 2010). Most ferns contain antimicrobial substances such as polyphenols and flavonoids. In general, the antibacterial effects produced by anthocyanins or other bioactive plant compounds are weaker than those generated by antibiotics. In some cases, the combination of substances can cause synergistic effects, and the purpose of the present research was to develop and test new antibiotic - plant extract formulations.

2. MATERIAL AND METHOD

a. The plant material used: ethanol extract from petals of peony - *Paeonia officinalis*, and methanol extract from leaves of fern - *Dryopteris filix-mas*.

b. Obtaining new potential antimicrobial formulations was done by soaking discs impregnated with piperacillin (Pip) or tetracycline (Te) with 6 µl of extract. The following experimental antimicrobial formulations were tested:

- **in experiment 1:**
  - BB+Te = crude extract of peony + tetracycline,
  - BF+Te = peony extract after passage on the fluorisil column + tetracycline,
  - F1+Te = fraction one from the peony extract on passage on the C18 column + tetracycline,
  - BB+Pip = crude extract of peony + piperacillin,
  - BF+Pip = peony extract after passage on the fluorisil column + piperacillin,
  - F1+Pip = fraction one from the peony extract on passage on the C18 + piperacillin column,

- **in experiment 2:**
  - D+I = extract of *Dryopteris filix-mas* + tetracycline,
  - D+Pip = extract of *Dryopteris filix-mas* + piperacillin,

c. Testing the antibacterial properties of the new formulations obtained:

- microorganisms tested: *Staphylococcus aureus* ATCC 25923 (Sa), *Streptococcus sp*. (St), *Escherichia coli* 820B (Ec), soil bacterium 23S (Bs), in the first experiment and, in addition, *Enterobacter cloacae* (En) in the second experiment;
- culture medium: nutrient agar;
- the inoculum was prepared 24 hours before cultivation on the medium, in nutrient broth, incubated at 37°C;
- determining the antimicrobial activity of the extracts was done by the method of discs.

3. RESULTS AND DISCUSSIONS

Experiment 1

The extracts and the new formulations tested inhibit the growth of *Staphylococcus aureus* ATCC 25923. The crude extract of peony produces an inhibition zone (IZ) of 18 mm, while the extract resulting from the passing on fluorisil and fraction F1 have much weaker antibacterial effects (IZ = 10 mm). Compared with the effect of the antibiotic, the formulations tested on *Staphylococcus* do not have a better effect, except for F1+Te, which produces a greater inhibition zone. As far as the second antibiotic, piperacillin, is concerned, the effect of the new formulations is not more obvious than that of its components, as both Pip and BF+Pip and F1+Pip determine the same size of the inhibition zone (i.e. 25 mm).

Streptococcus sp. is sensitive to the extracts tested; crude peony extract produces the same inhibition area as in the case of the *Staphylococcus*. The new formulation BF+Te tested had a better antimicrobial effect (IZ = 33 mm) than that obtained for the antibiotic tested separately (ZI = 32 mm) (Table 1), whereas piperacillin formulations have a weaker effect.

The extracts and formulations tested inhibit the growth of *Escherichia coli* 820B; the crude extract of peony produces an inhibition zone of 18 mm, while the new formulations with tetracycline have a stronger effect compared with those using piperacillin. Yet, compared with the effect of the antibiotic, the BF+Pip formulation has a more marked effect (ZI = 30 mm, compared to ZI = 28).

Regarding the soil bacterium 23S, the extracts tested have weaker effects as against the negative control, represented by the alcohol where the extract was made. The new therapeutic formulations tested have effects similar to those seen in *Streptococcus* and *E. coli*. The piperacillin formulations have weaker effects than those with tetracycline, where F1+Te and F1+Te produce an inhibition zone larger than that of the antibiotic (Table 1).

Experiment 2

Staphylococcus aureus ATCC 25923 is sensitive to the antibiotics and formulations tested. Both formulation D+Te, and D+Pip can be noted, which produce inhibition zones larger than those of the antibiotics (Table 2), even though the extract had a weaker effect than the negative control.

Streptococcus sp. growth is inhibited by the antibiotics and the new formulations with a therapeutic potential tested, but the extract + antibiotic combinations fail to induce an inhibition zone diameter larger than that produced by the antibiotics (Table 2).

Table 1. Testing Form - experiment 1

Paeony extract/ formulations	Microorganism			
	Sa	St	Ec	Bs
	IZ	IZ	IZ	IZ
BB	18	18	18	10
BF	10	10	10.50	10
F1	10	9.50	10	10
M	9.50	7.50	9.50	11
Te	28	32	34	32
BB+Te	28	32	32	32
BF+Te	28	33*	32	33*
F1+Te	30*	32	32	33*
Pip	25	29	28	30
BB+Pip	NT	24	26	NT
BF+Pip	25	28	30*	30
F1+Pip	25	27	28	30
Pi+D	28	29*	28*	37*
Pi+BB	26	25	25	33

* - better antibacterial effect

Table 2. Testing Form - experiment 2

Paeony extract/ formulations	Microorganism				
	Sa	St	Ec	Bs	En
	IZ	IZ	IZ	IZ	IZ
D	8	7.50	8	9	6
Te	28	32	34	32	34
D+Te	30*	32	32	30	36*
Pip	25	29	28	30	31
D+Pip	26*	27	28	32*	36*
M	9.50	7.50	9.50	11	8

Escherichia coli 820B is sensitive to the antibiotics and the new formulations tested; the formulations used fail to produce an inhibition zone diameter larger than that produced by antibiotics (Table 2).

The growth of the bacterium isolated from the soil is inhibited by the antibiotics and formulations tested. The extract generates a smaller inhibition zone than that of the negative control, yet formulation D+ Pip produces an inhibition zone of 32 mm, while Pip has an IZ = 30 mm (Table 2).

Enterobacter cloacae is sensitive to the antibiotics and formulations tested. Formulation D+Te generates an inhibition zone of 36 mm, while the antibiotic generates an IZ = 34 mm. Formulation D+Pip produces an inhibition zone 5 mm larger than than that produced by Pip (Table 2).

In the experiments conducted by Parihar et al. (2006), the combination of antibiotics with various extracts obtained from leaves, rhizomes and roots of *Athyrium pectinatum* had better antibacterial effects for *Staphylococcus aureus* and *Agrobacterium tumefaciens* (Parihar et al., 2006). Unlike the results obtained by the authors mentioned, in the two experiments conducted, the new formulations with extract of *Dryopteris filix-mas* had an effect on *Escherichia coli* as well. Also, peony extract formulations: BB, BF and F1 had, in some cases, better therapeutic effects than those obtained only by means of the antibiotic.

4. CONCLUSIONS

The combination of antibiotics with extracts determined, only for some of the microorganisms tested, better antibacterial effects than those caused by the antibiotic or the extract. To obtain superior results it is necessary to tested other formulations as well, and those tests should include other microorganisms, too.

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