

Antibacterial activity of docynia fruit extract against antibiotic resistant bacterium causing upper respiratory tract infection (*Moraxella catarrhalis*)

Nguyễn Thị Minh Thư, Nguyễn Thị Phương Anh, Bùi Thị Việt Hà*

Faculty of Biology, VNU University of Science, 334 Nguyễn Trãi Str., Thanh Xuân Dist., Hanoi, Vietnam

Received 18 October 2012

Revised 31 October 2013; accepted 07 May 2013

Abstract. The bacterium *Moraxella catarrhalis* is the agent of upper respiratory tract infection. According to some Vietnamese folk remedies, the fermented broth made from *Docynia* fruit (*Docynia indica* (Wall.) Decne) can reduce the seriousness of the disease. This experience suggested to us a research on the antibacterial activity of *Docynia* fruit extract itself as well as of its fractional extracts toward *Moraxella catarrhalis*. It was found that the extract from *Docynia* fruit and its fractional extracts (PD1, PD2, PD3), all of them, showed antibacterial activity against its *Moraxella catarrhalis* and contained the substances belonging to families of flavonoid, tannin, alkaloid and glycoside. However, PD1 showed a stronger antibacterial activity than that of PD2, PD3. This fractional extract also contained more flavonoid and alkaloid than PD2 and PD3 did.

Keywords. *Docynia idica* (Wall.) Decne, *Moraxella catarrhalis*, antimicrobial compounds.

1. Introduction

Acute respiratory infection is a disease with mortality rate on top of 10 infectious diseases frequently seen in developing countries. Both upper and under respiratory tract are exposed to acute infections. Unlike form of lower respiratory infections, upper respiratory infections affect not only the respiratory function but also other agencies such as hearing, smell, and balancing.

Moraxella cattarrhalis is the bacteria responsible for most of the acute respiratory

infections, especially for serious complications such as otitis (*Streptococcus pneumoniae* and *Haemophilus influenza* are the two most popular agents) [1-3]. This bacterium *M. catarrhalis* is resistant to most of antibiotics which belong to beta-lactam family, and only remains sensitive to the second and third generation of cephalosporin, and quinolone.

Recent studies have shown that components of *Docynia idica* (Wall.) Decne fruit or vinegar made from extract of this fruit can bring benefit. Some compounds extracted from fruit of *Docinia* were found to be against the disorders of glucid and lipid metabolism [5]. A *Bacillus* strain isolated from *Docinia* vinegar

* Corresponding author. Tel: 84-4-38588856.
E-mail: buithivietha@gmail.com

presented antibacterial activity against *Moraxella catarrhalis* causing upper respiratory tract infection [6]. In the other hand, the extract from *Docynia* fruit can reduce seriousness of upper respiratory tract infection – according to some Vietnamese folk remedies. Based on these information, our present study was conducted to evaluate antibacterial activity of the *Docynia* fruit extract itself and its fractional extracts toward *Moraxella catarrhalis* causing upper respiratory tract infection – a dangerous antibiotic resistant bacterium.

2. Materials and Methods

2.1. Materials

- *Staphylococcus aureus*; *E. coli*; *Salmonella typhi*; *Shigella flexneri* were obtained from Vietnam Type Culture Collection, VNU Institute of Microbiology and Biotechnology. - *Moraxella catarrhalis* ATTC 25240 obtained from National Ear Nose Throat Hospital.

- *The fruit extract*: 1kg fry *Docynia* fruit was ground and extracted by lyophilization method.

2.2. Methods

Antimicrobial activity assays: Bacteria were cultivated on selected media in Petri plate. Antimicrobial activity was determined by Kirby-Bauer Zone of Inhibition test method.

Enzyme activity assays: Enzyme activities were determined by diffusion test on agar plates containing substrates such as CMC, chitin, starch, casein.

Freeze drying/ lyophilization: the samples are kept in vacuum condition.

Thin layer Chromatography (TLC): was carried out using a mixture of n-Hexane: Chloroform: Acetone: Methanol as mobile phase [1].

3. Results and Discussion

3.1. The antibacterial activity of *Docynia* fruit extract toward *Moraxella catarrhalis*

As shown in Table 1 and Fig 1, concentrations from 0.1 to 10 mg/ml, the extract presented no antibacterial effect. However, concentration of 50, 100 mg/ml, the activities were 12 and 22 (D-d, mm), respectively.

Table 1. Antibacterial activity *M. catarrhalis* of the *Docynia* fruit extract with different concentrations

Concentration (mg/ml)	Inhibition zone (D-d, mm) (<i>M. catarrhalis</i>)
0,1	-
1	-
10	-
50	12 ± 0.25
100	22 ± 0.55

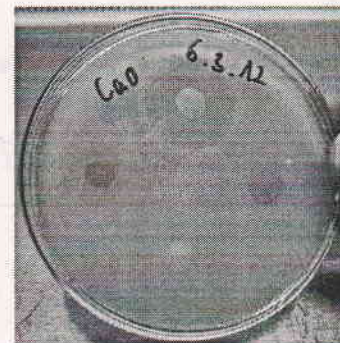


Figure 1. The disk diffusion test of antibacterial activity of *Docynia* fruit extract against *M. catarrhalis*.

This experiment was used to preliminary investigate whether or not the extracts from Docynia fruit contains the antibacterial activity. The result shown that the extract from Docynia fruit in the high concentration has the activity. In fact, there were antibacterial activity in the sample with high concentration of 50; 100 mg/ml (inhibition zone are 12 and 22, (D-d mm), respectively) but no at low concentration of 0,1; 1 and 10 mg/ml. Moreover, the antibacterial activity was much higher in the sample with concentration of 100mg/ml than in concentration of 50 mg/l (22 D-d, mm versus 12 D-d, mm). The Table 1 shows the highest antibacterial activity in the sample with concentration of 100 mg/ml. Thus, we can conclude that the concentration of the extract from Docynia fruit is related to antibacterial activity, the higher concentration, the stronger antibacterial activity.

3.2. Antibacterial activity of fractional extracts from Docynia fruit

By using TLC from Docynia fruit extract, we obtained 15 fractional extracts. 3 of them demonstrated antibacterial activity toward *M. catarrhalis* and named PD1, PD2, PD3 (table 2 and Figure 2).

Table 2. Antibacterial activity of fractional extracts against *M. catarrhalis*

Sample	Inhibition zone (D-d, mm) (<i>M. catarrhalis</i>)
PD1	27,5± 0.45
PD2	26,1 ± 0.25
PD3	18,0± 0.55

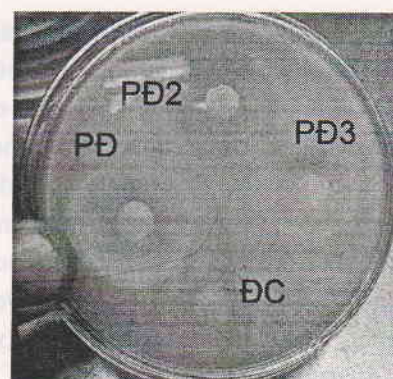


Figure 2. The disk diffusion test showing antibacterial activity of fractional extracts against *M. catarrhalis*.

The antibacterial activity of PD1 was the highest (27.5 mm of D0d), followed by PD2 and PD3 (26.1 and 18.0 mm, respectively).

3.3. Chemical composition of Docynia fruit extract

The result of the qualitative analysis of Docynia fruit extract were shown in Table 3 indicated that this extract contained compounds belonging to families of flavonoids, tannin, alkaloid and glycoside. Notice that the results of the quantitative analysis were not the same, depending on the indicator used. For example, with diazo as indicator, PD2 did not contain flavonoid (negative), while with NaOH – it contained (positive). Therefore, in order to detect one compound of these families (Table 3) some indicators were used simultaneously.

Table 3. Qualitative chemical composition of Docynia fruit extract

Family	Indicator	Result		
		PD1	PD2	PD3
Flavonoid	Shinoda	++	+	+
	Diazo	+	-	-
	NaOH 10%	+++	++	++
	H ₂ SO ₄	++	+	+
Tannin	Gelatin/ NaCl	+	-	-
	Lead Acetate	++	+	+
Alkaloid	Dragendorf	+++	+	+
Glycoside	Keller- Killian	+	+	++

As shown in Table 3, the fractional extract PD1 contained more flavonoid and alkaloid than PD2 and PD3 did. In addition, the data in Table 2 demonstrated that the antibacterial activity of PD1 was the highest.

These facts suggested that a relationship between the antibacterial activity and the presence of compounds belong to flavonoid and alkaloid families.

4. Conclusion

The extract of *Docynia* fruit had antibacterial activity toward *Moraxella cattarrhalis*. By using TCL, 3 fractional extract were isolated from fruit extract. All of them contained compounds belonging to families of flavonoid, tannin, alkaloid, glycoside.

The antibacterial activity of the extract seems to be related to the presence of flavonoid and glycoside compounds.

Acknowledgements

This study was supported by funds of the Vietnam National University, Hanoi for "Study on the effect of fermented *Docynia* fruit against antibiotic resistance bacteria causing human

upper respiratory tract infection (*Moraxella catarrhalis*)" Project (QG-11-17).

References

- [1] Karalus, R., and A.Campagnan (2000), "Moraxella catarrhalis, a review of an important human mucosal pathogen", *Microbes Infect*, 2, pp. 547 – 559.
- [2] Akinjogunla, O.J and Eghafona, N.O (2011), "Prevalence, Haemolytic activities and flouroquinolones susceptibility profiles of *Moraxella catarrhalis*, *Streptococcus pneumoniae* associated with acute otitis media", *Nature and Science*, 9(6), pp. 85 – 92.
- [3] Fang G., Fine M., Orloff J., (1990), "New and emerging etiologies for community – acquired pneumonia with implications for therapy", *Medicine*, 69, pp. 307 – 316.
- [4] Catlin BW. *Branhamella catarrhalis*: an organism gaining respect as a pathogen. *Clin Microbiol Rev.* 1990; 3:293–320.
- [5] Hoang Thi Minh Tan (2009), Some compounds extracted from *Docynia indica* (Wall.) Decne against disorders glucid and lipids metabolism, Biological Master's thesis, pp. 13 – 40.
- [6] Vu Thi Hue, Bui Thi Viet Ha (2010), "Antibacterial activity of *Bacillus* TM5.2 isolated from *Docynia* fruit vinegar against *Moraxella catarrhalis* causing upper respiratory tract infection", *J. Scien anh Technol*, 26(4), pp. 537-542.

Nghiên cứu khả năng ức chế vi khuẩn gây viêm đường hô hấp trên ở người (*moraxella catarrhalis*) đã kháng kháng sinh của dịch chiết quả táo mèo

Nguyễn Thị Minh Thu, Nguyễn Thị Phương Anh, Bùi Thị Việt Hà

Faculty of Biology, VNU University of Science, 334 Nguyễn Trãi Str., Thanh Xuân Dist., Hanoi, Vietnam

Tóm tắt. Theo như một vài phương pháp chữa bệnh ở Việt Nam thì dịch chiết từ quả Táo mèo (*Docynia indica* (Wall.) Decne) có thể làm giảm mức độ nghiêm trọng của bệnh viêm đường hô hấp trên. Cách chữa bệnh này đã đề ra một nghiên cứu về hoạt tính kháng khuẩn của các thành phần hóa học trong loại giấm này, có khả năng ức chế *Moraxella cattarrhalis* gây nhiễm trùng đường hô hấp trên.

Những kết quả đạt được đã chỉ ra rằng dịch chiết từ quả Táo mèo và các chiết xuất phân đoạn của nó có hoạt tính kháng khuẩn bao gồm các chất thuộc nhóm flavonoid, tannin, alkaloid và glucoside với những nồng độ khác nhau. Trong số các phân đoạn, những phân đoạn nào có tỷ lệ flavonoid và alkaloid cao hơn thì có hoạt tính kháng khuẩn mạnh hơn.

Từ khóa: *Docynia idica* (Wall.) Decne, *Moraxella catarrhalis*, antimicrobial compounds.