

Phytochemical screening and antimicrobial activity of Indian almond leaf extracts

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Abstract: The antimicrobial activity of Indian almond leaf extracts, prepared by extraction using water, 70% ethyl alcohol and 95% ethyl alcohol, were tested for their efficacy by paper disc diffusion method on Muller Hinton Agar (MHA). The results showed that all three extracts could inhibit mainly the growth of *Staphylococcus aureus* with the inhibition zones of 4.33 mm, 3.33 mm and 6.67 mm, respectively. No growth inhibition was observed with other microbial cultures of *Escherichia coli*, *Klebsiella* sp., *Citrobacter* sp. and *Enterobacter* sp. Chemical analysis by Gas Chromatography–Mass Spectrometry (GC-MS) revealed that there were 31 compounds that were matched with the data in the library, of which, benzenethiol -1,2,3benzenetriol or “Pyrogallol” was found to be the most abundance.

Keywords: Antimicrobial activity, Indian almond leaf extract, Phytochemical analysis, GC-MS

Introduction

Indian almond tree (*Terminalia catappa*) is a Combretaceous plant belonging to the tropical almond family. It is widely distributed throughout all regions of Thailand. In aquaculture, the Indian almond leaves have been claimed to have active compound(s) that promote wound healing. It has been used in Thailand for curing injured siamese fighting fish, presumably to increase thickness of keratin layer of the fish. In the literature, the leaf part of the plant has been shown to have a potential to use as an alternative treatment for chemical substances and antibiotics [1]. The objectives of this study were to evaluate the antibacterial activity of the Indian almond leaf extracts in order to get the most effective extracts that could inhibit the growth of microbial pathogens, and to evaluate the potential antimicrobial compounds in the Indian almond leaf extracts by Gas Chromatography–Mass Spectrometry (GC-MS) analysis.

Materials and Methods

Extraction of Indian almond leaves

The Indian almond leaves, collected from School of Agriculture and Natural Resources, University of Phayao, were washed with deionized water and immediately dried in an oven at 50 °C for 24 hours. The dried leaves were then ground into powder by using mortar and pestle. The leaf powder was then extracted separately with three types of solvent, namely water, 70% ethanol and 95% ethanol for 3 days (100 g per 1000 ml). After filtration, the extract was evaporated by using a rotary evaporator to dryness. The extracts were kept at 37°C until being used.

Antibacterial activity of Indian almond leaf extracts

Antibacterial tests were performed using disc agar diffusion method. The bacterial suspension of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* sp., *Citrobacter* sp. and *Enterobacter* sp. was each diluted to 1.5×10^8 CFU/ml and swabbed on the surface of MHA agar. Plates were incubated for 24 hours at room temperature. Antimicrobial activity was detected by measuring the inhibition zone around the disc (in mm.). Tetracycline (TE 30 µg/disc) was used as positive control.

Phytochemical analysis of Indian almond leaf extracts by gas chromatography – mass spectrometry (GC-MS)

The Indian almond leaf extracts were subjected to Gas Chromatography–Mass Spectrometry (GC-MS) analysis and compounds were matched with data in the library.

Result & Discussion

The efficacy test for antimicrobial activity of the leaf extracts was conducted by disc diffusion method on Muller Hinton agar (MHA). The lowest concentrations of the three Indian almond leaf extracts prepared by using water, 70% ethanol and 95% ethanol were 31.25, 31.25 and 15.63 mg/ml, respectively. The results obtained from the disc diffusion method showed that *Staphylococcus aureus* the inhibition zone could only be observed with *Staphylococcus aureus* with the diameters of 4.33 mm for the water extract, 3.33 mm for the 70% ethanol and 6.67 mm for the 95% ethanol (Table 1). No significant inhibition zones were observed with other microbial cultures, including *Escherichia coli*, *Klebsiella* sp., *Citrobacter* sp. and *Enterobacter* sp. Therefore, it was suggested that the antimicrobial activities of the extracts are relatively specific.

From the Gas chromatography – mass spectrometry (GC-MS) analysis, there were 31 compounds detected in the 95% Indian almond leaf extract (Figure.1). Among these, only the top 10 constituents with the highest abundance were listed (Table 2). The lowest concentrations of Indian almond leaves extracted by water, 70% ethanol and 95% ethanol that could inhibit the growth of *Staphylococcus aureus* at were found to be 31.25, 31.25 and 15.63 mg/ml, respectively. GC-MS studies suggested that pyrogallol tannin might be the active compound which is similar to the results reported previously Sundaram Poongulali (2016) and Muthuraman Sundararaman (2016).

Table 1. Inhibition zone of Indian Almond leaf extracts against pathogenic bacteria.

Solution	Concentration of the extract (mg/ml)	inhibition zone (mm)				
		<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella</i> sp.	<i>Citrobactor</i> sp.	<i>Enterobacter</i> sp.
Tetracycline (control)		≥19	29.67	-	20	20
95% eth	125	19.67	-	-	-	-
	62.5	15	-	-	-	-
	31.25	12.67	-	-	-	-
	15.63	6.67	-	-	-	-
70% eth	125	20	-	-	-	-
	62.5	14.33	-	-	-	-
	31.25	3.33	-	-	-	-
	15.63	-	-	-	-	-
H ₂ O	125	23	-	-	-	-
	62.5	17.33	-	-	-	-
	31.25	4.33	-	-	-	-
	15.63	-	-	-	-	-

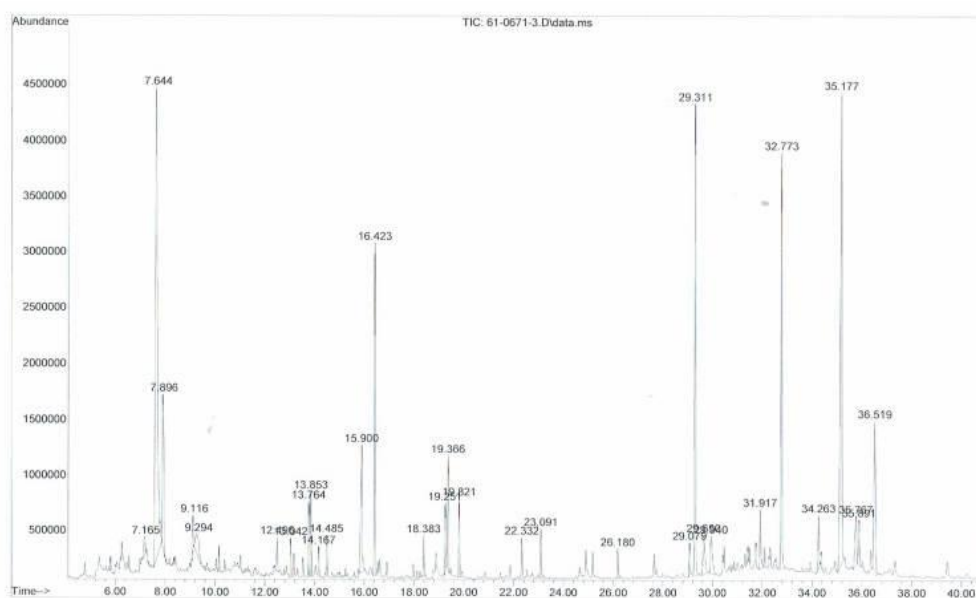


Figure 1. A typical chromatogram of the chemical constituents present in 95% ethanolic crude extract of *Terminalia catappa*.

Table 2. Major constituents of Indian almond leaves extracted by 95% ethanol based on by Gas chromatography – mass spectrometry (GC-MS).

No.	R.T. min	Compounds	Molecular formula	Molecular weight	% peak area
1	7.644	Benzenetriol Benzene-1,2,3-triol Pyrogallol	C ₆ H ₆ O ₃	126.111 g/mol	100.00%
2	35.177	Stigmast-5-en-3-ol	C ₂₉ H ₅₀ O	414.718 g/mol	74.31%
3	29.311	Tetracosahexaene	C ₂₄ H ₃₈	326.568 g/mol.	48.60%
4	32.773	Vitamin E	C ₂₉ H ₅₀ O ₂	430.706 g/mol	42.40%
5	16.423	Hexadecanoic acid ethyl ester	C ₁₈ H ₃₆ O ₂	284.48 g/mol	27.45%
6	7.896	1,3a-epoxy-3ah-indene	-	-	22.24%
7	36.519	Aristol-a-en-8-one	C ₁₅ H ₂₂ O	218.34 g/mol	22.47%
8	15.9	Hexadecanoic acid Hexadecanoate	C ₁₆ H ₃₂ O ₂	256.4 g/mol	19.71%
9	19.366	Ethyl-9-octadecenoate	C ₂₀ H ₃₈ O ₂	310.522 g/mol	10.23%
10	35.767	Olean-12-en-3-ol	C ₃₂ H ₅₂ O ₂	468.766 g/mol	8.72%

Conclusion

The antimicrobial efficacy of Indian Almond leaf extracts prepared by using three kinds of solvent water, 70% ethanol and 95% ethanol were conducted by testing the ability of the extracts to inhibit the growth of bacteria. The results showed that the 95% ethanol extract are the best in inhibiting the growth of *Staphylococcus aureus*. Water is lower ability to inhibit *Staphylococcus aureus* and 70% ethanol is the lowest. But it has not affect the inhibition of *Escherichia coli*.

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References

- Francisco José Mininel, Carlos Sérgio Leonardo Junior, Livia Greggi Espanha, Flávia Aparecida Resende, Eliana Aparecida Varanda, Clarice Queico Fujimura Leite. et al. 2014. Characterization and Quantification of Compounds in the Hydroalcoholic Extract of the Leaves from *Terminalia catappa* Linn. (Combretaceae) and Their Mutagenic Activity. Evidence-Based Complementary and Alternative Medicine : Hindawi Publishing Corporation.
- K.-T. CHUNG, Z. LU, M. W. CHOU. 2006. Mechanism of Inhibition of Tannic Acid and Related Compounds on the Growth of Intestinal Bacteria. Food and Chemical Toxicology. 36(12), 1053-60.
- Nantarika Chansue and Nongnut Assawawongkasem. 2008. The *in vitro* Antibacterial Activity and Ornamental Fish Toxicity of the Water Extract of Indian almond leaves (*Terminalia catappa* Linn.). KKU veterinary journal. 18(1), 36-45.
- Sundaram Poongulali and Muthuraman Sundararaman. 2016. Antimycobacterial, Anticandidal and Antioxidant Properties of *Terminalia catappa* and Analysis of Their Bioactive Chemicals. International Journal of Pharmacy and Biological Sciences. 6(2), 69-83.
- W. Purivirojkul and N. Areechon. 2006. Antibacterial Activity and Toxicity of Indian Almond (*Terminalia catappa*) extract in Siamese Fighting Fish (*Betta splendens* Regan). Proceedings of 44th Kasetsart University Annual Conference: Fisheries. 109-116.