
بسم الله الرحمن الرحيم

In-vitro Anti-Microbial and Brine-Shrimp Lethality Potential of the Leaves Extract of Nahar (*Mesua ferrea*) Plant

Presented by

AHMED IDRIS ADEWALE (G0910689)

Bio-environmental Research Unit (BERU), Biotechnology Engineering Department, Kulliyyah of Engineering, International Islamic University Malaysia, Gombak, 53100, Kuala Lumpur, Malaysia.

RESEARCH COMMITTEE

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MUYIBI SULEYMAN AREMU (PROF. DR.)

JAMAL IBRAHIM DAOUD (ASSIST. PROF. DR.)

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MAIN OUTLINE

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Introduction

Mesua ferrea L. (Guttiferae)

COMMON NAMES: Ceylon ironwood, Indian rose chestnut, Cobra's saffron, Penaga Lilin, Na, Nahar/Nahor, Nāga

FAMILY: Clusiaceae (Guttiferae).

NATIVITY: Tropical Sri Lanka (also Assam, Southern Nepal, Indochina, the Malay Peninsula)

VEGETATION: Bears flowers between April and July

Fruits between October and November (Dennis and Kumar, 1998).

USES: Well-known medicinal plant (used in indigenous system of medicine for the treatment of fever, dyspepsia, and renal diseases) (Nadkarni,1954)

RESOUCEFULNESS: Antimicrobial activity of plant oils

other extracts applications (raw and processed food preservation, pharmaceuticals, alternative

medicine and natural therapies (Lis-Balchin and Deans, 1997)

Problem statement and its significance

Increasing interest in human health, concern over pathogenic and spoilage microorganisms in foods and increase in outbreaks of food borne disease

→ Increasing need to search for natural antimicrobials from non-conventional sources to augment the available ones and also to meet specific applications.

Infectious diseases represent an important cause of morbidity and mortality among humans

→ The use of medicinal plants as screening pool for novel antibiotics has several advantages related to safety, availability, and minimizing the risk of side effects and addiction (Lee *et al*., 2003).

Bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents (Nascimento *et al*. 2000)

→ The importance of identifying new effective antimicrobial agents cannot be overemphasized (Ghaleb *et.al,* 2009)

Efforts are being encouraged towards the substitution of synthetic non-biodegradable polymers by fully or partially biodegradable polymers of semi-synthetic or bio-origin due to several environmental concerns (Suvangshu et al., 2010)

Research Objectives

1. Evaluation of the antimicrobial activity of the leaves extract

2. Cytotoxicity analysis of the extract

Methodology

Leaves pretreatment and sample preparation (oven drying @ 45°C for 2 days)

Nahar trees

Extraction of Nahar leaves: oven shaker set at 37°C and 200rpm for 24 hours

Oven shaker

Methodology...cont'd

Anti-microbial assay of Nahar leaves extracts on *E. coli, P. aeruginosa, B. subtilis and S. aureus* using disc diffusion methods

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Determination of Minimum Inhibitory Concentration (MIC) and Minimum bactericidal Concentration (MBC) using broth dilution

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Cytotoxicity test using Brine Shrimps Lethality Bioassay

RESULTS AND DISCUSSIONS

NL crude extract yields

Ethanol gave higher crude extract's yield (about 10.2%) than methanol.

Solvents	Yields of crude extract (g)
Ethanol	6.71±0.25
Methanol	6.09±0.22

This confirmed the earlier report of Wang and Helliwell [23], that ethanol is superior to methanol and acetone for extracting biologically-active components (e.g., flavonoids) from tea. Besides, ethanol is considered as safe (GRAS solvent).

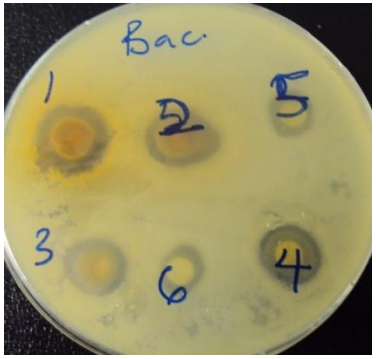
Zone of inhibition (mm) of Nahar leaves extract

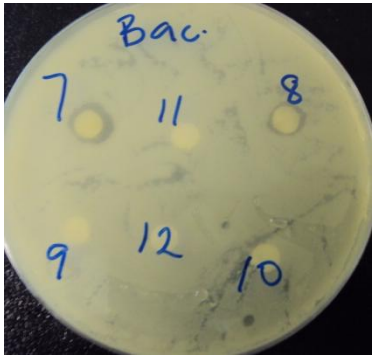
Bacteria	Ethanol extract	Methanol extract	Chl.	Tet.	Str.	Gen.	Van.	DMSO	Methanol	Ethanol
E. coli	17.5±0.5	18.0±0.5	23.0	20.0	24.0	19	0	0	0	0
P. aeruginosa	17.0±0.5	17.5±0.5	23.0	23.0	20.0	21.0	19.0	0	0	0
S. aureus	17.0±0.5	16.0±0.5	25.0	26.0	20.0	23.0	20.0	0	0	0
B. subtilis	18.0±0.5	18.0±0.5	24.0	26.0	21.0	24.0	20.0	0	0	0

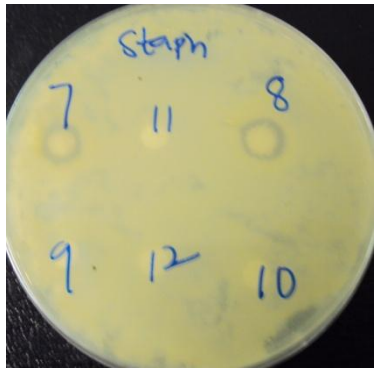
Antibacterial activity

The extract showed a remarkable antibacterial property against all the selected microbes (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis and Staphylococcus aureus*) with the inhibition zones ranging from 16.0±0.5mm to 18.0±0.5mm for all the tested bacteria.

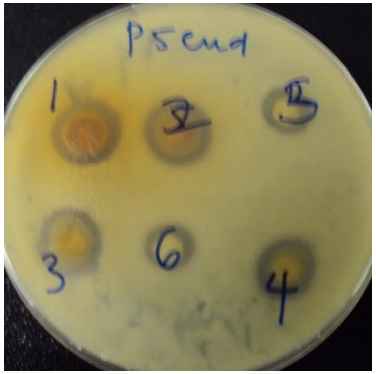
Gram-positive bacteria

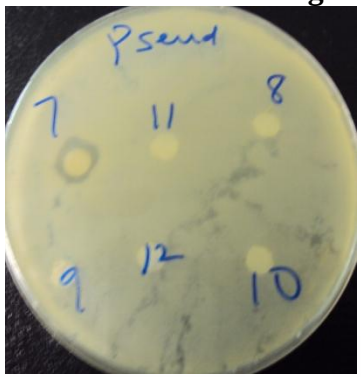






Gram-negative bacteria





MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF NAHAR SEED KERNELS' OIL

- The result obtained from the MIC and MBC determinations showed that the active extracts were found to be both bacteriostatic and bactericidal with the gram-positive bacteria showing less resistance.

- The MIC range of 0.625 - 2.5 mg/ml with MBC value of 5 mg/ml was obtained for the gram-negative bacteria while MIC range of 0.313 - 1.3 mg/ml with MBC value of 2.5 mg/ml was obtained for the gram-positive bacteria.

- It could be deduced from this that the Gram-positive bacteria appeared to be more sensitive, more susceptible and less resistant, while the Gram negative bacteria are less sensitive, less susceptible and more resistant.

- This also justifies the fact that is generally known, which is that Gram negative bacteria have an outer membrane consisting of lipoprotein and lypopolysaccharide, which is selectively permeable and thus regulates access to the underlying structures

Brine shrimp lethality bioassay of methanol extract of NL

Concentration (µg/ml)	1000	500	250	125	62.50	31.25	15.63	7.81	3.91	1.95
No. of shrimps per test sample	20	20	20	20	20	20	20	20	20	20
No. of survivors	8	10	12	12	14	16	16	18	20	20
No. of death	12	10	8	8	6	4	4	2	0	0
Percentage mortality (%)	60	50	40	40	30	20	20	10	0	0

LC_{50} = 500ppm (µg/ml)

- The extract was found to be moderately cytotoxic to the Brine shrimps at high concentration with LC_{50} of 500ppm (µg/ml).

- Ayo *et al.* (2007) reported that LC_{50} values lower than 1000 µg/ml are considered bioactive. Therefore, the methanol extracts of *M. ferrea* leaves may have some significant biological activity.

- The results of this present work may be of importance in the elucidation of the potential and medicinal uses of the extracts.

Conclusion

The antimicrobial and cytotoxic activity of Nahar leaves extracts, found in this study, may explain some of the traditional medicinal uses of the plants. These could also be of particular interest in relation to find out its untapped efficacy and can also be a potential of chemically interesting and biologically important drug candidates.

Future challenges!!!

1. Characterization of the bioactive compounds of the leaves extracts
2. Detailed physico-chemical properties of the extract
3. Incorporation of the extract in water disinfection, food packaging materials and storage stability
4. And so on…

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الجامعة الإسلامية العالمية ماليزيا
INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA
يونيورسيتي إسلام انتارابغسا ملايسيا

Bioenvironmental Engineering Research Unit (BERU),
Department of Biotechnology Engineering,
Faculty of Engineering,
International Islamic University Malaysia,
50728 PO. Box 10, Gombak,
Kuala Lumpur, Malaysia.
Tel: +603-61964440 Fax: +603-61964442

Website: http://eng.iiu.edu.my/~beru

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Publications

Ahmed, I. A., Elwathig Mohamed, S. M., Aremu, M. S., Jamal, I, D. and Mikail, M. A., (2010). Potential of Nahar (*Mesua Ferrea*) Seeds' Oil for Industrial Applications. Proceeding of The 2nd International Biotechnology & Biodiversity Conference (BIOJOHOR 2010), pp. 75-79.

Ahmed, I. A., Elwathig Mohamed, S. M., Aremu, M. S., Jamal, I, D. and Mikail, M. A., (2010). Proximate Composition of Nahar (*Mesua Ferrea*) Leaves and Antibacterial Assay of its Extracts. Proceeding of the International Postgraduate Conference on Engineering (IPCE 2010) 16-17 October 2010. Universiti Malaysia Perlis (UniMAP).

Ahmed, I. A., Elwathig Mohamed, S. M., Aremu, M. S., Jamal, I, D. and Mikail, M. A., (2010). Extraction, Composition and Antimicrobial Activity of Nahar (*Mesua Ferrea*) Seeds' Oil. ACT-Biotechnology Research Communication 1:1 (2011) 28-32.

Ahmed, I. A., Elwathig Mohamed, S. M., Aremu, M. S., Jamal, I, D. and Mikail, M. A., (2011). Nahar (*Mesua ferrea*) Seed Kernel: Oil Extraction, Applications and Commercialization Potential. IRIIE, 2011 (Silver Medal).

Ahmed, I. A., Elwathig Mohamed, S. M., Aremu, M. S., Jamal, I, D. and Mikail, M. A., (2011). In-vitro Anti-Microbial and Brine-Shrimp Lethality Potential of the Leaves Extract of Nahar (*Mesua ferrea*) Plant. ICBioE, 2011 (Paper accepted).

Ahmed, I. A., Elwathig Mohamed, S. M., Aremu, M. S., Jamal, I, D. and Mikail, M. A., (2011). Disinfection kinetics of Nahar (*Mesua ferrea*) seed kernel's oil using Pour Plate Method and Heterotrophic Plate Count. ICBioE, 2011 (Paper accepted).

Ahmed, I. A., Elwathig Mohamed, S. M., Aremu, M. S., Jamal, I, D. and Mikail, M. A., (2011). In-vitro Anti-Microbial and Brine-Shrimp Lethality Potential of Seed Kernels' Oil of Nahar (*Mesua ferrea*) Plant. 102nd AOCS Annual Meeting & Expo in Cincinnati, OH, USA (Paper accepted).

References

- **Ainie, K., Siew, W. L., Tan, Y. A., Noraini, I., Mohtar, Y., Tang, T. S. and Nuzul, A. I. 2005. MPOB test methods, a compendium of test on palm oil products, palm kernel products, fatty acids, food related products and others, pp. 308-317. Malaysia: Malaysia Palm Oil Board (MPOB).**
- **Akpan u. G., Jimoh A., and Mohammed A. D. 2006. Extraction, Characterization and Modification of Castor Seed Oil. Leonardo Journal of Sciences ISSN 1583-0233 Issue 8, January-June 2006 p. 43-52, Bombay, India, 1954, p. 792.**
- **AOAC. 1995. Official methods of analysis. 16th Edn. Gaithersburg, M.: AOAC International.**
- **Bailey's Industrial Oil and fat products, 6th Edition, edited by Fereidoon Shahidi. "A Wiley-Interscience Publication". TP670.S46, 2004.**
- **Bashar Mudhaffar Abdullah. 2009. Physicochemical Characteristics of Malaysian Rubber (*Hevea Brasiliensis*) Seed Oil. European Journal of Scientific Research ISSN 1450-216X Vol.31 No.3 (2009), pp.437-445 © Euro Journals Publishing, Inc.**
- **Denis T.J. and Kumar K. A., 1998. Constituents of *Mesua ferrea.* FITOTERAPIA. LXIX: 291-303**
- **Ghaleb Mohamma Adwan, Bassam Ali Abu-shanab and Kamel Mohammad Adwan, 2009. *In vitro* activity of certain drugs in combination with plant extracts against *Staphylococcus aureus* infections. African Journal of Biotechnology Vol. 8 (17), pp. 4239-4241, 1 September 2009. ISSN 1684–5315 © 2009 Academic Journals.**
- **Nada Č. Nikolić, Suzana M. Cakić, Sanja M. Novaković, Mirjana D. Cvetković, Mihajlo Z. Stanković. 2009. Effect of Extraction Techniques on Yield and Composition of Soybean Oil. Macedonian Journal of Chemistry and Chemical Engineering, Vol. 28, No. 2, Pp. 173–179.**

➢ **Nascimento GGF, Locatelli J, Freitas PC, Silva GL., 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz. J. Microbiol. 31: 247-256.**

➢**Nazish Badar, Muhammad Arshad and Umer Farooq. 2008. Characteristics of *Anethum Graveolens* (Umbelliferae) Seed Oil: Seed Oil: Extraction, Composition And Antimicrobial Activity. International Journal of Agriculture & Biology ISSN Print: 1560–8530; ISSN Online: 1814–9596 07–384/SAE/2008/10–3–329–332.**

➢**Nzikou J.M., Kimbonguila A., Matos L., Loumouamou B., Pambou-Tobi N.P.G., Ndangui C.B., Abena A. A., Silou Th., Scher J. and Desobry S., 2010. Extraction and Characteristics of Seed Kernel Oil from Mango (*Mangifera indica*). Research Journal of Environmental and Earth Sciences 2(1): 31-35, 2010. ISSN: 2041-0492.**

➢**Rudy Nurdin, Kaida Khalid, Zainal Abidin Talib and Aspollah Md. Sukari (2006). Development of essential oil extraction by microwave assisted process from a local plant. *Solid State Science and Technology, Vol. 14, No 1 (2006) 107-114* ISSN 0128-7389.**

➢**Singh R.K. and Saroj K. Padhi, 2009. Characterization of Jatropha oil for the preparation of biodiesel. Natural Product Radiance, Vol. 8 (2), 2009, pp 127-132.**

➢**Suvangshu Dutta, Niranjan Karak, Jyoti Prasad Saikia & Bolin Kumar Konwar. 2010. Biodegradation of Epoxy and MF Modified Polyurethane Films Derived from a Sustainable Resource. J. Polym. Environ. DOI 10.1007/s10924-010-0161-8**