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# Biological Evaluation of Certain Plants of Family Salicaceae and Arecaceae

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## Abstract

**Objective:** There is an increasing interest in the use of natural products to oppose human diseases. The leaves of *Flacourtia rukam* Zoll and A. Mortizi, *Archontophoenix alexandrae* (Wendl. and Drude), and *Dictyosperma album* (Bory) H. Wendl. and Drude ex. Scheff were selected for phytochemical and biological screening to search for new natural drugs. Total ethanolic extracts of the mentioned plants were subjected to preliminary phytochemical screening followed by screening their cytotoxic, antimalarial, antimicrobial, and anti-inflammatory activities. **Materials and Methods:** The extracts were tested for the presence of various phytochemicals. The cytotoxic activity was determined against five human cancer cell lines: melanoma, breast, oral, ovarian, and cervical cancers and two noncancerous cell lines. The antimalarial activity was determined against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum* depending on the plasmodial lactate dehydrogenase activity. Antimicrobial screening was continued using the modified version of the CLSI method whereas anti-inflammatory activity was determined by measuring the activity of inducible nitric oxide synthase (iNOS). **Results:** Preliminary phytochemical screening indicated the absence of alkaloids, anthraquinones, and saponins in all the extracts. The latter showed no toxicity against the tested cancer cell lines and no activity against the tested microbes. The extract of *D. album* lacks the activity against D6 and showed a moderate activity against W2 *P. falciparum* ($IC_{50}$ = 41.7 µg/mL). *D. album* extract showed no inhibition for iNOS as contrary to *F. rukam* and *A. alexandrae* extracts which showed a good inhibition ($IC_{50}$ = 20 and 100 µg/mL, respectively). **Conclusion:** All tested extracts lack cytotoxic and antimicrobial activities.

**Keywords:** Antimicrobial, Archontophoenix, cytotoxic, Dictyosperma, Flacourtia, inducible nitric oxide synthase

## Introduction

Cancer involves abnormal cell growth with the potential of metastasis. It is one of the diseases that cause morbidity and mortality all over the world. The number of cases is expected to increase by about 70% over the next two decades.[1] The economic impact of cancer is significant and is increasing.[1] Chemotherapy, radiotherapy, and surgery are the main typical cancer treatment approaches.[2] Chemotherapy efficacy and safety remain a primary objective as their toxicity and other side effects are severe. Moreover, multidrug resistant cancer is even a bigger challenge.

Furthermore, infectious diseases such as HIV, tuberculosis, and malaria are among the main causes of morbidity and mortality worldwide due to drug-resistant microorganisms and the emergence of unknown diseases which caused by microbes.[3] In 2010, malaria was estimated to cause about 216 million incidents of illness and 655,000 deaths a year.[4] Countries with high incidence of malaria showed lower economic growth rates.[5] Malaria in humans is caused by four species of parasites belonging to the genus *Plasmodium*: *Plasmodium falciparum, Plasmodium ovale, Plasmodium malaria,* and *Plasmodium vivax*, where *P. falciparum* is the parasite causing most deaths.[6]

Induced nitric oxide (NO) is the end product of inducible NO synthase (iNOS) enzyme. It is an important mediator in some of human diseases. Inhibitors of iNOS have been suggested to be useful in inflammatory diseases.[7]

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Many studies have been carried out on different plant species to discover compounds of medicinal application against fungal and bacterial infections, malaria, and cancer. Among these, several studies have focused on the biological and phytochemical properties of different species of the family Salicaceae[8] and Arecacea.[9]

*Flacourtia rukam* Zoll and A. Mortizi belongs to family Salicaceae and is known as governor's plum, Indian plum, Indian prune, and rukam.[10] It is native to Madagascar and Malesia and was introduced into some tropical regions. The fruits of *F. rukam* have been used for the treatment of diarrhea, dysentery, and dysmenorrhea. The leaves have been conventionally used in inflamed eyelids, smallpox, and headache and dusted over the wounds. The root decoction is used in skin allergies, abdominal colic, pneumonia, and liver ailments.[11]

*Archontophoenix alexandrae* (Wendl. and Drude) has different common names: Alexander palm, Alexandra palm, King Alexander palm, King palm, and northern bangalow palm. It is endemic to North Queensland, Australia. It occurs in the rainforests of tropical and warm temperate regions.[12]

*Dictyosperma album* (Bory) H. Wendl. and Drude ex. Scheff has two common names: princess palm due to its graceful appearance and hurricane palm because of its ability to withstand strong hurricane force winds. It is native to Reunion and Mauritius. It is widely cultivated in tropics and subtropics as an ornamental. Root decoction is used as diuretic.[13]

The objective of our work is to test the alcoholic extracts of the leaves of three plants, cultivated in Egypt, for their potential antimalarial, antimicrobial, cytotoxic, and potential anti-inflammatory activities. The plants were selected for investigation based on the few reports about their phytochemistry and biological activities.[14,15]

## Materials and Methods

### Plant material

The leaves of *F. rukam*, *A. alexandrae,* and *D. album* were collected in 2015 from different gardens in Egypt (Botanical Garden, Aswan; El-Zohorya Garden, Cairo; and El-Orman Public Garden, Giza, respectively). The plants were kindly identified by Dr. Abd El-Halim A. Mohammed, Horticultural Research Institute, Department of Flora and Phytotaxonomy Researches, Dokki, Cairo, Egypt, and Dr. Mohamed Gibali (Senior Botanist), Agriculture Research Center, Ministry of Agriculture, Dokki, Giza. Voucher specimens (BUPD-63, BUPD-64, and BUPD-65) were deposited in Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt.

### Preparation of extracts

The air-dried powdered leaves of *F. rukam*, *A. alexandrae*, and *D. album* were exhaustively percolated with 70% ethanol. The solvent was evaporated under reduced pressure to afford crude extracts (23.6%, 11.2%, and 15.3%, respectively).

### Preliminary phytochemical screening

The three extracts were subjected to preliminary phytochemical screening tests for carbohydrates, glycosides, alkaloids, saponins, steroids, triterpenoids, phenolics, flavonoids, and anthraquinones. The qualitative tests were performed according to Dash[16] and Raaman.[17]

### Biological activities

#### *Cytotoxic activity*

*In vitro* cytotoxic activity for the total ethanolic extracts was determined against five human cancer cell lines: melanoma (SK-MEL), breast cancer (BT-549), oral cancer (KB), ovary cancer (SKOV-3), and cervical cancer (HeLa) and two noncancerous kidney cell lines (LLC-PK1 and VERO). All cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The assay was performed in 96-well tissue culture-treated microplates. The cells were seeded at a density of 25,000 cells/well and incubated for 24 h. Samples at different concentrations were added and the cells were again incubated for 48 h. At the end of incubation, the cell viability was determined using neutral red dye.[18] Doxorubicin was used as a positive control, while DMSO was used as the negative (vehicle) control.

#### *Antimalarial activity*

*In vitro* antimalarial activity was determined against D6 (chloroquine-sensitive) and W2 (chloroquine-resistant) strains of *P. falciparum,* which were obtained from the Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC, USA. The assay is based on the determination of plasmodial lactate dehydrogenase activity.[19] $IC_{50}$s were obtained from the dose–response curves. Artemisinin and chloroquine were used as drug controls and dimethyl sulfoxide as a vehicle control.

#### *Antimicrobial activity*

All organisms used in this study were obtained from the American Type Culture Collection (Manassas, VA, USA) and consisted of the fungi *Candida albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 204305 and the bacteria *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staph. aureus* (MRSA) ATCC 33591, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068. Susceptibility testing was performed using a modified version of the CLSI (formerly NCCLS) method.[20] *M. intracellulare* was tested using a modified Franzblau method.[21] Microbial inocula were prepared by correcting the OD630 of microbe suspensions in incubation broth to give final target inocula. Drug controls (ciprofloxacin® [ICN Biomedicals, Solon, OH, USA] for bacteria and amphotericin B [ICN Biomedicals, Ohio] for fungi) were included in each assay. All organisms were read at 530 nm or 544ex/590em (*M. intracellulare* and *A. fumigatus*) before and after incubation. $IC_{50}$ values were determined from dose–response curves of percentage decrease in cell viability against test concentrations. Ciprofloxacin was used as

antibacterial positive control against *S. aureus*, MRSA, *E. coli*, *P. aeruginosa*, and *M. nteracellulare* and exhibited $IC_{50}$ value of 0.10, 0.06, 0.01, 0.06, and 0.214 µM, respectively. Amphotericin B was used as antifungal positive control against *C. albicans, C. glabrata, C. krusei, A. fumigatus,* and *C. neoformans* and exhibited $IC_{50}$ value of 0.17, 0.22, 0.57, 1.30, and 0.17 µM, respectively. DMSO was used as the negative control.

### *Anti-inflammatory activity*

Excessive generation of NO contributes significantly to the progress of inflammation.[22] Intracellular NO production can be reduced by inhibition of iNOS. The ability of the extracts under investigation to inhibit the activity of iNOS was assessed in mouse macrophages (RAW 264.7 cells), as described earlier.[22] The cells were seeded at a density of 50,000 cells/well in 96-well plates and grown for 24 h. Total 70% ethanolic extracts were added to the cells after incubating with samples for 30 min, lipopolysaccharides (LPS) (5 µg/mL) was added, and the cells were further incubated for 24 h. The activity of iNOS was determined by measuring the level of nitrite in the cell culture supernatant with Griess reagent. The degree of inhibition of nitrite production was calculated in comparison to the vehicle control. $IC_{50}$ values were obtained from dose–response curves. Parthenolide was used as a positive control. Cytotoxicity of test samples to macrophages was also determined in parallel to check if the inhibition of iNOS was due to cytotoxic effects.[23]

## Results

Preliminary phytochemical screening indicated the absence of alkaloids, anthraquinones, and saponins and the presence of carbohydrates, glycosides, sterols, triterpenes, flavonoids, and phenolic compounds in all tested extracts.

The total ethanolic extracts did not show any cytotoxicity against the used human cancer cell lines (SK-MEL, KB, BT-549, SKOV-3, and HeLa) and the two noncancerous kidney cell lines (LLC-PK1 and VERO) up to a concentration of 200 ug/mL. Furthermore, none of them showed any activity against the used microbes. In antimalarial screening, *F. rukam* total extract showed higher activity against both *P. falciparum* D6 and *P. falciparum* W2 ($IC_{50}$ = 21.4 and 14.4 µg/mL, respectively) with selectivity index of 2.2 and 3.3 than that of *A. alexandrae* (33.2 and 20.5 µg/mL, respectively) with selectivity index ranging from 1.4 to 2.3, whereas *D. album* showed decreased activity against *P. falciparum* D6 and moderate activity against *P. falciparum* W2 ($IC_{50}$ = 41.7 µg/mL, with selectivity index of 1.1). The total ethanolic extracts of *F. rukam* and *A. alexandrae* showed good inhibition to the iNOS ($IC_{50}$ = 20 and 100 µg/mL, respectively) while that of *D. album* showed no activity, using parthenolide as a positive control with $IC_{50}$ value of 0.3 µg/mL.

## Discussion

There are few reports in the literature about the chemistry and biological activities of the three mentioned plants: *F. rukam*, *A. alexandrae*, and *D. albuM*; therefore, a study aiming to explore the chemical and biological potential of these plants should be encouraged. Taking this into account, preliminary phytochemical screening has been carried out and showed the presence of sterols, triterpenes, flavonoids, and phenolic compounds. As these phytochemical classes give promising biological activities,[24,25] this encouraged us to evaluate cytotoxic, antimalarial, antimicrobial, and anti-inflammatory activities for the mentioned leaves' ethanolic extracts.

The findings of the current study showed that none of the extracts exhibited cytotoxicity against the used human cancer cell lines (skin melanoma SK-MEL, oral cancer KB, breast cancer BT-549, ovarian cancer SKOV-3, and cervix carcinoma HeLa) and the two noncancerous kidney cell lines (LLC-PK1 and VERO). This finding corresponds to previous study where *F. rukam* showed no activity against breast cancer cell lines.[26] Further cytotoxic studies on other cell lines are required for the tested extracts. Moreover, it was revealed that the extracts were unable to inhibit the growth of the used microbes: *Candida albicans*, *C. glabrata*, *C. krusei*, *C. neoformans*, *A. fumigatus*, *S. aureus*, MRSA, *E. coli*, *P. aeruginosa*, and *M. intracellulare*, so antimicrobial activity using other microbes is encouraged for future study on the tested extracts.

The preliminary phytochemical screening revealed the presence of phenolics and flavonoids, which are compounds reported to act as antimalarial[27] and anti-inflammatory.[28] Hence, it is not surprising that the extracts of *F. rukam* and *A. alexandrae* leaves showed activity against both *P. falciparum* D6 and *P. falciparum* W2. Furthermore, *F. rukam* and *A. alexandrae* extracts showed a good inhibition for iNOS which may account for the expected anti-inflammatory activity, because iNOS-derived NO plays an important role in numerous pathophysiological conditions such as inflammation.

## Conclusion

Ethanolic extracts of *F. rukam* and *A. alexandrae* leaves showed moderate antimalarial activity. The ethanolic extract of *F. rukam* demonstrated inhibition to the iNOS activity in LPS-induced macrophages. Hence, further chemical investigation is needed for all mentioned extracts.

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### Conflicts of interest

There are no conflicts of interest.

## References

1. Stewart BW, Wild CP. World Cancer Report. Lyon, France: International

Agency for Research on Cancer; 2014.
2. Bell RM. A review of complementary and alternative medicine practices among cancer survivors. Clin J Oncol Nurs 2010;14:365-70.
3. Iwu MW, Duncan AR, Okunji CO. New Antimicrobials of Plant Origin. Alexandria: ASHS Press; 1999.
4. Weil DN. Economic Growth: International Edition: Pearson Education. New York, NY: USA; 2008.
5. Cooper AF, Besada H. Moving Health Sovereignty in Africa: Disease, Governance, Climate Change. USA: Routledge; 2016.
6. Dronamraju KR, Arese P. Malaria: Genetic and Evolutionary Aspects. New York, NY: USA: Springer Science & Business Media; 2006.
7. Cirino G, Distrutti E, Wallace JL. Nitric oxide and inflammation. Inflamm Allergy Drug Targets 2006;5:115-9.
8. Vieira G, Goncalves T, Regasini L, Ferreira P, Lotufo L, do O Pessoa C, *et al*. New clerodane diterpene from the twigs of *Casearia obliqua* (*Salicaceae*). Planta Med 2008;74:PB19.
9. Hughes AF, Lima FG, Lucchese AM, Góes Neto A, Uetanabaro AP. Antimicrobial activity of *Syagrus coronata* (Martius) Beccari. Braz Arch Biol Technol 2013;56:269-74.
10. Kiple KF. The Cambridge World History of Food. 2. USA: Cambridge University Press; 2001.
11. Lim T. *Flacourtia rukam*. Edible Medicinal and Non-Medicinal Plants. New York and London: Springer; 2013. p. 776-9.
12. Uhl NW. Genera Palmarum: A classification of palms based on the Work of Moore HE Jr. Lawrence: Allen Press; 1987.
13. Grubben GJ. Plant Resources of Tropical Africa (PROTA). Wageningen, Netherlands: Prota; 2008.
14. Williams CA, Harborne JB, Clifford HT. Negatively charged flavones and tricin as chemosystematic markers in the Palmae. Phytochemistry 1973;12:2417-30.
15. Moustafa SM, Menshawi BM, Wassel GM, Mahmoud K, Mounier MM. Screening of some wild and cultivated Egyptian plants for their free radical scavenging activity. Int J Pharmtech Res 2014;6:1271-8.
16. Dash PR. Phytochemical Screening and Pharmacological Investigations on *Hedychium coronarium*, Hamburg, Anchor Academic Publishing; 2016.
17. Raaman N. Phytochemical Techniques. India: New India Publishing; 2006.
18. Borenfreund E, Babich H, Martin-Alguacil N. Rapid chemosensitivity assay with human normal and tumor cells *in vitro*. *In Vitro* Cell Dev Biol 1990;26:1030-4.
19. Mohamed NM, Makboul MA, Farag SF, Jain S, Jacob MR, Tekwani BL, *et al*. Triterpenes from the roots of *Lantana montevidensis* with antiprotozoal activity. Phytochem Lett 2016;15:30-6.
20. Ferraro MJ. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. National Committee for Clinical Laboratory Standards; 2000.
21. National Committee for Clinical Laboratory Standards, Woods GL. Susceptibility Testing Mycobacteria, Nocardia, and Other Aerobic Actinomycetes: Tentative standard; 2000.
22. Quang DN, Harinantenaina L, Nishizawa T, Hashimoto T, Kohchi C, Soma G, *et al*. Inhibition of nitric oxide production in RAW 264.7 cells by azaphilones from xylariaceous fungi. Biol Pharm Bull 2006;29:34-7.
23. Zaki MA, Balachandran P, Khan S, Wang M, Mohammed R, Hetta MH, *et al*. Cytotoxicity and modulation of cancer-related signaling by (Z)- and (E)-3,4,3',5'-tetramethoxystilbene isolated from *Eugenia rigida*. J Nat Prod 2013;76:679-84.
24. Sayed HM, Mohamed MH, Darwish FM, Mohamed AM. Phytochemical and biological investigations of *Flacourtia cataphracta* roxb. Cultivated in Egypt. Bull Pharm Sci 2006;29:371-87.
25. Sengab AE, El Dina M, Elgindi MR, Elsaid MB. Biological studies of isolated triterpenoids and phenolic compounds identified from *Wodyetia bifurcata* family Arecaceae. J Pharmacogn Phytochem 2015;3:67-73.
26. Moustafa SM, Menshawi BM, Wassel GM, Mahmoud K, Mounier M. Screening of some plants in Egypt for their cytotoxicity against four human cancer cell lines. Int J Pharm Tech Res 2014;6:1074-84.
27. Kaou AM, Mahiou-Leddet V, Canlet C, Debrauwer L, Hutter S, Laget M, *et al*. Antimalarial compounds from the aerial parts of *Flacourtia indica* (*Flacourtiaceae*). J Ethnopharmacol 2010;130:272-4.
28. Calixto JB, Campos MM, Otuki MF, Santos AR. Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. Planta Med 2004;70:93-103.