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Potential Anti-Inflammation Activity of Ethanol Leaf Extract of Calophyllum Inophyllum from Papua

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ABSTRACT

Inflammation is the body's defense mechanism as a tissue response to injury and other external factors. Flavonoids in Bintangur leaves (Calophyllum inophyllum) are thought to have anti-inflammatory effects. This study aims to determine the anti-inflammatory activity of Bintangur leaves. Also, the effective dose of Bintangur leaves in reducing edema in the legs of mice injected with carrageenan. Anti-inflammatory testing was carried out on male white mice. The results of the anti-inflammatory test, in % DAI of the treatment group, obtained the most significant percent value of anti-inflammatory power, namely at a dose of 400 mg/kg BW of 8.83%. Followed by a dose of 200 mg/KgBW of 6.18% and 100 mg/KgBW of 5.36%. The ethanol extract of Bintangur leaves at a dose of 200 mg/kg BW and 400 mg/kg BW has the potential as an anti-inflammatory against carrageenan-induced mice. A 400 mg/kg/bw dose has the highest ability to reduce edema with an average value of % inflammation inhibition of 60.01% at the 300th minute and AIP of 8.83%. The data obtained were analyzed using ANOVA with a significant value of 0.02 ($p<0.05$), which showed a difference in edema with a 95% confidence level in at least one pair of groups. This study shows anti-inflammatory activity in the ethanol extract of Bintangur leaves; the higher the dose, the more anti-inflammatory activity will increase.

KEYWORDS: Anti-inflammatory, *Calophyllum inophyllum*, Carrageenan, Edema

ARTICLE DETAILS

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I. INTRODUCTION

Calophyllum inophyllum is a medicinal plant belonging to the Calophyllaceae family. This plant generally grows in tropical rainforest ecosystems, with a medium-sized tree diameter, reaching 20 m in height, rough bark, and white flowers averaging 25 mm in width that bloom year-round. This tree produces round, green drupes with a diameter of 24 cm (Vittaya et al., 2023).

C.C. inophyllum generally grows along the coast and islands in the Pacific Ocean. C. inophyllum is a medicinal plant with the potential to cure various diseases. The parts of the plant used in traditional medicine are the fruit, leaves, flowers, bark, and seeds (Farida et al., 2024). In Indonesia, C. inophyllum leaves are empirically used as a herbal remedy for cancer (Budiarti & Sholihah, 2018). Ethnobotanically, C. inophyllum leaves have been utilized by the Papuan people; boiled water can treat skin infections, and the white sap from the leaves is used to treat eye irritation (Chrystomo et al., 2016).

The emergence of a disease is closely related to the inflammatory process. Inflammation is a body defense response; acute and chronic inflammation can be fatal and lead to cancer (Yi, 2023). Inflammation is the body's protective reaction against foreign substances entering the body due to infection or tissue damage. Inflammation can be treated with synthetic steroidal or non-steroidal anti-inflammatory drugs. However, long-term use of these drugs can cause side effects.

Treatment using herbal plants is the best solution to date. This is because herbal medicine has fewer side effects. The results of preliminary tests through in silico studies suggest that the bioactive compounds of ethanol extract of C. inophyllum leaves against the EGFR (Epidermal Growth Factor Receptor) receptor which is a receptor involved in the

inflammatory process where one of them is the EGFR-ERK pathway shows that the phenolic compound 2H-Benzo(cd)pyrene-2,6(1,H)-dione, 3,5,7,10-tetrahydroxy-1 with the chemical formula C22H16O6 shows potential in inhibiting the EGFR receptor with a docking value of 55,427 greater than the standard drug (Jaikumar, 2017).

The content of polyphenol compounds in the polar extract of C. inophyllum leaves is the largest. The total phenolic content in the water extract of C. inophyllum leaves is 97 ± 9.2, and the methanol extract is 140.28 ± 17.1 mg/g. In addition, the total flavonoid content in the water extract of C. inophyllum leaves is 88.94 ± 2.94 and the methanol extract is 177.06 ± 5.29 mg/g (Dutta & Ray, 2014). The potential of C. inophyllum as a herbal medicine in the treatment of inflammation is enormous, as this is correlated with the levels of polyphenol compounds found in the polar extract of this plant. This study explains the potential of the ethanol extract of C. inophyllum leaves as an alternative traditional medicine when the body experiences inflammation, testing anti-inflammatory activity using the carrageenan-induced paw edema model method on white male mice.

II. MATERIALS AND METHODS

A. Sample

Calophyllum inophyllum leaves were obtained from Base-G Beach, North Jayapura District, Jayapura City, Papua Province.

B. Simple ingredients

5 kg of fresh leaves are sorted into wet and dry forms, and the medicinal plants are dried at room temperature and protected from direct sunlight. The dried medicinal plants are ground and sieved using a 60-mesh sieve. They are stored in a clean, tightly closed container.

C. Extraction

500 g of the crude drug was macerated using 70% ethanol (1:5) for 24 hours. Re-maceration was carried out for 3 days to maximize extraction. Continuous stirring and filtration were carried out during the maceration process to obtain the macerate. The macerate was concentrated into a crude extract, and its yield was calculated.

D. Solvent-Free Test

The ethanol solvent must be ensured to have evaporated completely, leaving only the secondary metabolite compounds. The test is performed by adding two drops of H2SO4 and 1 mL of 2% K2Cr2O7 to the extract. The extract, free from the ethanol solvent, does not change color.

E. Phytochemical Analysis

A color test was used to identify bioactive compounds and determine the flavonoid, alkaloid, tannin, and steroid/triterpenoid compounds present in the ethanol extract of Calophyllum inophyllum leaves.

F. Test Solution

The test solution included 1% carrageenan, 1% Na-CMC, Na-Diclofenac, C. inophyllum extract with P1 (100 mg/kgBW), P2 (200 mg/kgBW), P3 (400 mg/kgBW).

G. Anti-Inflammation Test

Male mice were acclimatized for 7 days by fasting for 18 hours while still being given drinking water. The paw volume of mice was measured before being induced by carrageenan with a plestismometer (Vo). Mice were grouped into five groups. Activity testing was done by injecting 0.1 mL of 1% carrageenan subplantarly. After 60 minutes, they were given 1% Na-CMC as a negative control group, Na-diclofenac as a positive control group, and P1 extract (100mg/kgBW), P2 (200mg/kgBW), and P3 (400mg/kgBW) orally, while the normal group was not given any treatment. The paw volume of mice was measured 60 minutes after administration of the test preparation. Edema volume was measured every 60 minutes or 1 hour; measurements were carried out for up to 300 minutes. The percentage of edema volume, inflammation inhibition, AUC, and %API were calculated.

H. Data Analysis

The data obtained were then tested by the Shapiro-Wilk test for normality and the Levene test for homogeneity. If the data were normally distributed and homogeneous, a One-Way ANOVA with a 95% confidence level was used, followed by the LSD (Least Significant Difference) test to determine whether there were any significant differences.

III.DISCUSSION

A. Calophyllum inophyllum Leaf Extract

Extraction is an essential first step in the analysis of bioactive compounds. The conventional method that can be used to extract bioactive compounds is maceration. The extraction yield from Calophyllum inophyllum leaves obtained was 57.35 g, yielding 11.44%. The extraction result in percent yield indicates a measure of the efficiency of the solvent in extracting specific components from the original material. The higher the yield value, the more bioactive compounds are contained in the sample (Senduk et al., 2020). Research by Hapsari et al., (2022) obtained a percent yield of bintangur leaves of 2.18%, 2.51% and 2.58% using methanol solvents with concentrations of 50%, 80% and 100%, and also 3.38% for 100% ethanol solvent. The percent yield of methanol extract of C. inophyllum leaves originating from India was 74.44% based on multistage extraction. This indicates that the dominant compounds in C. inophyllum leaves are polar (Indrakumar, 2012). Therefore, the yield value of a plant can be influenced by the solvent and solvent concentration used and the extraction method chosen.

Table 1. Results of Calophyllum inophyllum Leaf Extraction

Simplex Weight (g)	Solvent Volume (mL)	Extract Weight (g)	Yield (%)
500	7500	57.35	11.44

B. Ethanol Solvent Free Test

The ethanol-free test was conducted by reacting a thick extract of bintangur leaves with K2Cr2O7 and H2SO4. The principle of the reaction that occurs is an oxidation reaction between ethanol and K2Cr2O7 in an acidic environment, with the following reaction:

$3C2H5OH + 2K2Cr2O7 + 8H2SO4 \longrightarrow 3CH3COOH + 2Cr2(SO4)3 + 11H2O + 2K2SO4$

Samples that do not change color are declared free from ethanol solvent. In this study, the initial color of the extract was brownish orange. The color of the sample did not change when reacted with H2SO4 and K2Cr2O7, which indicates that the sample is free from ethanol. La et al. (2023) stated that extracts that still contain ethanol solvent will change color from orange to bluish green and vice versa; no color change occurs if the extract is free from ethanol.

C. Phytochemical Test

Phytochemical screening is a process of examining the secondary metabolite compounds present in a natural product. The goal is to determine whether a particular plant species has the potential for use. Secondary metabolites in natural products include alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids, quinones, and others (Sa'adah and Nurhasnawati, 2017).*The results of the study by Fajriaty et al. (2018) showed that the secondary metabolite compounds flavonoids, saponins, terpenoids/steroids, phenols, and tannins were detected in bintangur leaves, while alkaloids and quinones were not detected. The difference in results between the previous study and this study could be due to the type of solvent and the concentration of the solvent used.*

The extract *C. inophyllum's phytochemical results contain alkaloids, glycosides, saponins, phenols, tannins, flavonoids, proteins & amino acids, steroids, and coumarin (Indrakumar, 2012). The roots, stems, leaves, flowers, fruits, and seeds of C. inophyllum contain secondary metabolite compounds including flavonoids, coumarins, fatty acids and xanthones, which are believed to have various bioactivities such as analgesic, antiaging, anti-arthritic, anticancer, anti-polyverative, anti-diabetic, antimicrobial, anti-HIV, and specifically anti-inflammatory (Ferdosh, 2024). Research by Cassein et al. (2021) suggests that extracts and secondary metabolite compounds from isolated results of C.inophullum are reported to have antioxidant, anti-inflammatory, antimicrobial, and antimycobacterial activities.*

D. Anti-Inflammation Test

The anti-inflammatory testing method in this study used a plethysmometer to measure the volume of edema in the paws of mice. Edema is formed due to carrageenan induction, a substance that plays a role in stimulating the release of inflammatory mediators such as histamine, which can lead to inflammation. The mechanism of carrageenan in causing edema is by stimulating mast cell lysis, resulting in the release of inflammatory mediators, resulting in vasodilation, which leads to capillary wall exudation and phagocyte migration to the inflamed area, resulting in edema (Yusuf et al., 2021).

The highest percentage of edema was in the negative control group given 1% Na-CMC, with an average percentage of edema of 12.74% and the lowest percentage of edema was in the positive control group given 50 mg of diclofenac sodium, with an average percentage of edema of 3.39%. As the dose of ethanol extract increases, the percentage of edema decreases. This is shown in the extract group in P1 (100 mg/KgBW) the highest percentage of edema occurred at the 180th minute at 10.71%; P2 (200 mg/KgBW) the highest percentage value at the 180th minute at 8.82% and P3 (400 mg/KgBW) reached a maximum value at the 180th minute at 6.48%. The percentage of edema in the positive control and extract groups was smaller than in the negative control group. The percentage of edema shows the swelling in the soles of the mice's feet after being injected with carrageenan (Figure 1).

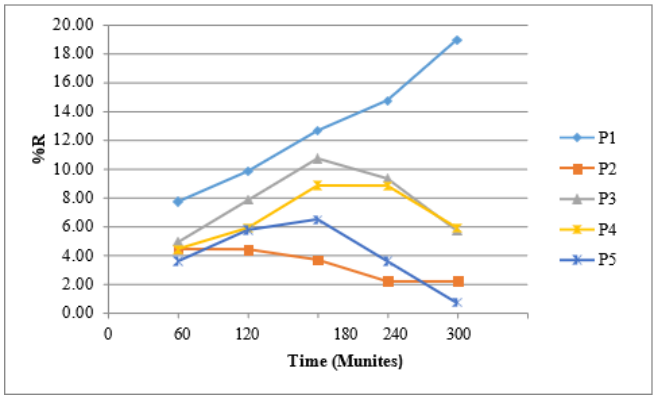

Figure 1. Graph of Percentage of Edema in Mice's Feet

The percentage of inflammation inhibition shows the ability to reduce the volume of edema caused by inflammation between the comparator (sodium diclofenac) and the ethanol extract of bintangur leaves (Suwandi, et al., 2022). Based on the LSD test, it was found that the treatment groups of EEDB 200 mg/KgBB and EEDB 400 mg/KgBB showed significant differences with K(-) and EEDB 100 mg/KgBB ($p<0.05$) from the 60th, 120th, 180th, 240th and 300th minutes but did not differ significantly with K(+) ($p>0.05$), this indicates that the ethanol extract of bintangur leaves at doses of 200 mg/KgBB and 400 mg/KgBB has the potential to reduce the volume of edema (Figure 2).

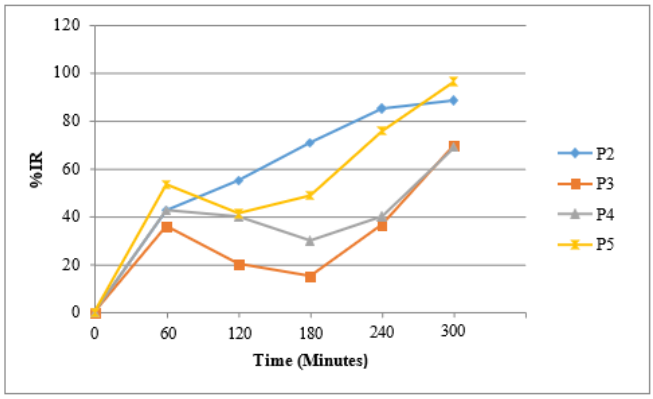

Figure 2. Graph of Percentage Inhibition of Mice's Foot Inflammation

Calculating the AUC (Area Under Curve) is used to calculate an extract's inhibitory power as an active substance against edema formation. The AUC is a value that describes the volume of edema from each treatment group at each time unit. The greater the AUC value, the lower the drug's anti-inflammatory ability; conversely, the lower the AUC value, the higher the drug's anti-inflammatory ability.

AIP (Anti-inflammatory power) indicates the percentage of a compound that provides anti-inflammatory activity. AIP is a parameter that describes the inhibition of inflammatory symptoms by looking at the rate at which a compound provides anti-inflammatory activity. The AUC and AIP values are inversely proportional, where the smaller the AUC value, the greater the AIP value (Priamsari and Krismonikawati, 2020). Table 2 shows that the highest AUC value is in the negative control group at 2.64 and the smallest in the positive control group at 2.34. In EEDB dose of 100 mg/KgBB, the AUC value was greater than the doses of 200 mg/KgBB and 400 mg/KgBB, then the highest % DAI value was in the positive control group (11.48%), followed by EEDB dose of 400 mg/KgBB (8.83%), EEDB dose of 200 mg/KgBB (6.16%), and EEDB dose of 100 mg/KgBB (5.36%).

A related study, Setyopuspito (2017), stated that the percentage of inflammatory power obtained from ethanol extract of soursop leaves using the carrageenan induction method obtained an AIP value of 55.56% at a dose of 100 mg/kgBW. In addition, the percentage value of anti-inflammatory power at a dose of 400 mg/kgBW of purslane ethanol extract was 30.20% after measuring edema volume for 300 minutes (Andayani et al., 2018).

Table 2. Calculation of AUC and AIP

Treatment Group	AUC0-5	AIP (%)
P1	2.64	0
P2	2.34	11.48
P3	2.50	5.36
P4	2.48	6.18
P5	2.41	8.83

There are three phases in anti-inflammatory activity: the first phase is the serotonin and histamine stage from the cell mastocytes, the second phase is the kinin release stage, and the third phase is the prostaglandin release stage, cyclooxygenase, and lipogenase production. Various anti-inflammatory testing studies using carrageenan-induced paw edema models include the ethanol extract of Solanum betaceum fruit peel, Cav. It contains flavonoid compounds that are believed to have anti-inflammatory properties (Priamsari and Krismonikawati, 2019). Polar (alcohol) extracts of Andrographis paniculate, Tectona grandis, and Calotropis gigantea leaves showed anti-inflammatory activity at a dose of 200 mg/kg body weight (Rao, 2021). Ethanol extract of Spatholobus littoralis Hassk stalks at a dose of 2.5 mg/kg body weight provided an anti-inflammatory effect of 19.21% (Rousdy et al., 2022).

The role of secondary metabolites as anti-inflammatory agents is significant. Polyphenols are potential anti-inflammatory compounds. Polyphenolic compounds successfully isolated from C. inophyllum leaves include isocalophylic acid and calophylic acid (Patil et al., 1993); isoinophynone and inophynone (Khan et al., 2002). The polyphenolic compounds dehydrocycloguanandin and calophyllin B isolated from C. inophyllum leaves exhibit anti-inflammatory activity in vivo via the interperitoneal and oral routes in adrenalectomized rats (Gopalakrishnan et al., 1980). Acetone extract of C. inophyllum leaves exhibits anti-inflammatory activity in lipopolysaccharide-induced RAW 264.7 macrophage cells by inhibiting nitric oxide production and reducing NF-kb, iNOS, and COX-2 (Tsai et al., 2012). A flavonoid compound, amentoflavone, has been isolated from C. inophyllum leaves and inhibits 15-lipooxygenase (LOX) (Aminudin et al., 2016). Polyphenol and flavonoid compounds isolated from ethanol extract of C. inophyllum leaves showed good anti-inflammatory activity with an inhibition percentage of 40.98% and 48.62% at a dose of 50 mg/kgBW (Navysari et al., 2021).

CONCLUSION

Ethanol extract of bintangur leaves at doses of 200 mg/kgBW and 400 mg/kgBW can act as an anti-inflammatory agent in mice induced by carrageenan. The 400 mg/kgBW dose had the highest edema-reducing effect, with an average inflammation inhibition percentage of 60.01% at 5 hours and a DAI percentage of 8.83%.

REFERENCES

I. Vittaya, L., Chalad, C., Ratsameepakai, W., and Leesakul, N. 2023. Phytochemical Characterization of Bioactive Compounds Extracted with Different Solvents from Calophyllum inophyllum Flower and Activity Against Pathogenic Bacteria. South African Journal Of Botany. 153 (2023), 346-355

II. Farida, S., Jenie, RI, and Fakhrudin. 2024. Calophyllum inophyllum: A Comprehensive

Analysis of Its Ethnobotanical, Phytochemical, and Pharmacological Properties. Traditional Medicine Journal. 29(2), 121-142

III. Budiarti, M., and Sholihah, IYM 2018. Research Report on the Development of Antimalarial and Anticancer Drug Raw Materials Based on Ristoja's Results. In the Health Research and Development Agency of the Republic of Indonesia, Jakarta.

IV. Chrystomo, LY, Karim, AK, Anatari, NN, Dwa, S., Wona, Y., and Pongtiku, A. 2016. Traditional Papuan Medicinal Plants Based on Local Community Wisdom (eds. I). Nulisbuku Jendela Dunia. Jakarta.

V. Yi, YS 2023. Inflammation, Inflammatory Diseases, and Inflmmasomes. International Journal Of Molecular Sciences. 24(11), 24-26.

VI. Jaikumar, K., Sheik, NMM, Jhon, WW, Deventhiran, M., Babu, A., Anand, D., and Saravanan, P. 2017. In Silico Docking Analysis of Bioactive Compounds from Calophyllum inophyllum L. Ethanol Leaf Extract Against Epidermal Growth Factor Receptor (EGFR) Protein. Asian Journal of Pharmaceutical and Clinical Research. 10(8), 214-219.

VII. Dutta, S., and Ray, S. 2014. Evaluation of Antioxidant Potentials of Leaf Aqueous and Methanolic Extracts of Calophyllum inophyllum in Relay to Total Phenol and Flavonoid Contents. International Journal of Pharma and Bio Sciences. 5(3), 9691511.

VIII. Senduk, TW, Montolalu, LADY, and Dotulong, V. 2020. The Yield of Boiled Water Extract of Mature Leaves of Mangrove Sonneratia Alba. Journal of Tropical Fisheries and Marine Sciences, 11(1), 9.

IX. Hapsari, S., Yohed, I.,ristianta, RA, Jadid, N., Aparamarta, H., W., and Gunawan, S. 2022. Phenolic and Flavonoid Compounds Extraction from Calophyllum inophyllum Leaves. Arabian Journal of Chemistry, 15(3), 1036666.

X. Indrakumar, I., Selvi, V., Gomathi, R., and Karpagam S. 2012. Phytochemical Analysis of Leaf Extracts of Calophyllum inophyllum L. and Cananga odorota (Lam.) Hook.F.&Thomson. IOSR Journal of Pharmacy and Biological Science. 3(2), 35-37.

XI. La, EOJ, Dewi, PIC, and Widyawati, NKY 2023. Antibacterial Activity Test of Ethanol Extract of Croton Leaves (Condiaeum variegatum L.) Against Staphylococcus aureus Bacteria. Proceedings of the National Health Symposium. 2(1), 110-119.

XII. Fajriaty, I., Ih, H., Andreas, and Setyaningrum, R. 2018. Phytochemical Screening and Thin Layer Chromatography Analysis of Ethanol Extract of Bintangur Leaves (Calophyllum soulattri Burm. F.). Journal of Informatics and Science Education, 7(1), 54-67.

XIII. Sa'adah, H., and Nurhasnawati, H. 2017. Comparison of Ethanol and Water Solvents in the Preparation of Tiwai Onion Bulb Extract (Eleutherine Americana Merr) Using the Maceration Method. Manuntung Scientific Journal. 1(2), 149.

XIV. Ferdosh, S. 2024. The Extraction of Bioactive Agents from Calophyllum inophyllum L., ang Their Pharmacological Properties. Sci. Pharm. 92(1), 6.

XV. Cassien, M., Mercier, A., Thetiot-laurent, S., Culcasi, M., Ricquebourg, E., Asteian, A., Herbette, G., Bianchini, J., Raharivelomanana, P., Pietri, S., Cassien, M., Mercier, A., Thétiot-laurent, S., Culcasi, M., Ricquebourg, E., Cassien, M., Mercier, A., Th, S., Culcasi, M., & Ricquebourg, E. (2021). Improving the Antioxidant Properties of Calophyllum inophyllum Seed Oil from French Polynesia: Development and Biological Applications of Resinous Ethanol-Soluble Extracts To cite this version: HAL Id : hal-03130041 Improving the Antioxidant Properties . 0–23.

XVI. Yusuf, M., Sari, I., and Wijaya, A. 2021. Anti-inflammatory Effect of Ethanol Extract of Seagrass Leaves (Enhalus acoroides) on Male Mice (Mus musculus) Induced by Carrageenan. Jurnal Ilmua Manuntung. 7(2), 165-174

XVII. Suwandi, DW, Imaroh S., and Kurniati, NF 2022. Anti-inflammatory Activity of Fresh Garlic and Black Garlic (Allium sativum L.) Infusions in White Rats (Rattus Norvegicus L.) Induced by Carrageenan. Acta Pharmaceutical Indonesia. 47(2), 1-8.

XVIII. Priamsari, MR, and Krismonikawati, RA 2020. Anti-inflammatory Power Test of Ethanolic Extract of Dutch Eggplant (Solanum betaceum, Cav.) Peel on Carrageenan-Induced Male Mice. Journal of Pharmaceutical Sciences and Clinical Pharmacy. 16(2), 86.

XIX. Steyopuspito, PA 2017. Anti-inflammatory Effectiveness Test of Ethanol Extract of Soursop Leaves (Annona squamosa L) Against Paw Edema in Male White Rats of Wistar Strain. Scientific Journal of Pharmacy. 13(1), 8-14.

XX. Andayani, D., Suprihartini, E., and Astuti, M. 2018. Anti-inflammatory Effect of Ethanol Extract of Purslane (Portulaca oleracea, L.) on Carrageenan-Induced Edema in Rats. Journal of Pharmaceutical Science and Clinical Research, 2018(01), 43-49.

XXI. Patil, AD Freyer, AJ, Eggleston, DS, Haltiwanger, RC, Bean, MF, Taylor, PB, Caranfa, MJ, Breen, AL, Bartus, HR, Johnson, RK, Hertzberg, RP, & Westley, JW 1993. The Inophyllums, Novel Inhibitors of HIV-1 Reverse Transcriptase Isolated

from the Malaysian Tree, Calophyllum inophyllum Linn. Journal of Medicinal Chemistry, 36(26), 4131–4138.

XXII. Khan, MR, Kihara, M., and Omoloso, AD 2002. Antimicrobial activity of Calophyllum inophyllum. Phytotherapy. 73(7-8), 741-743.

XXIII. Gopalakrishnan, C., Shankaranarayanan, D., and Nazimudee, SK 1980. Anti-inflammatory and CNS Depressant Activities of Xanthones from Calophyllum inophyllum and Mesua ferrea. Indian Journal of Pharmacology. 12(3), 181-191.

XXIV. Tsai, SC, Liang, YH, Chiang, JH, Liu, FC, Lin, WH, Chang, SJ, Lin, WY, Wu, CH, & Weng, JR (2012). Anti-inflammatory effects of Calophyllum inophyllum L. in RAW264.7 cells. Oncology Reports, 28(3), 1096–1102.