



## CHEMICAL AND BIOLOGICAL ASSESSMENT OF *Calophyllum inophyllum* (LINN) SEED OIL

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### Abstract

The extraction of *Calophyllum inophyllum* (Linn) seed showed that the oil content is about half the weight of the seed. The phytochemical analysis indicated the presence of tannin as the major secondary metabolites in hexane, ethylacetate and methanol fractions. Tannin account principally for the pharmacological activities of the oil. Brine Shrimps cytotoxicity test revealed that the entire fractions were toxic, with hexane fraction having the lowest LC<sub>50</sub> value of 37.473 µg/ml and ethylacetate and methanol fractions having 93.476 µg/ml and 62.27 µg/ml respectively. The antibacterial activities of the test extracts at 10000, 1000, 100 and 10 µg/ml were fairly moderate but lesser than that of gentamycin. The HPLC analysis of vitamin A and E in hexane fraction showed preponderance of both vitamins to a justifiable level that approve its use topically.

Keywords: *Calophyllum inophyllum*, cytotoxicity, phytochemical, antibacterial, vitamin

## Introduction

*Calophyllum inophyllum* belongs to the family Guttiferae. It is commonly known as Tamanu plant. This plant occurs in the folklore medicine of more than one continent. It is cultivated in all tropical regions of the world, including several Pacific islands and Tropical Asia. It grows primarily on coral sands and on the sea shore. It is best known as an ornamental tree because of its decorative leaves, fragrant flowers and spreading crown [1].

Seed oil of the fruit is commonly referred to as Tamanu Oil. It has been shown to greatly aid cicatrization of wounds, including severe cut and burns. It is also found to possess effective germicidal activities [2]. The seed oil is used for cosmetic and topical application on burns and skin diseases. It is used for washing face, to cure nappy rash after shaving, combat head lice, dandruff and to relieve sore throats and strained muscles [3].

The bark is medicinal in Asia for orchitis, vaginal discharge and gonorrhoea. The aqueous extract of the leaves serves as an eye-lotion in Indonesia and as an astringent for piles in Philippines. In Java, the tree is believed to have diuretic properties, whereas in Samoa every part of the plant is considered a virulent poison with the milky juice causing blindness, the sap once introduced into the blood circulation causes death, it is therefore used as an arrow poison. The gum obtained from the plant is emetic useful for the treatment of wounds and ulcer. The bark is astringent and also useful for orchitis, vaginal discharge and hemorrhages [4-6]. The root also found uses in dressing ulcers and heatstroke. In Cambodia, the leaves are prescribed as an inhalation for migraine and vertigo and the oil for scabies and the mature fruit is sufficiently poisonous to be ground and used as rat bait [7]. The plant contains antibacterial principle and novel inhibitors of HIV-I reverse transcriptase [8].

Tannin was found to be majorly present in the aqueous extract of the leave, bark and root but the phytochemical composition of the seed oil is yet to be reported.

The oil is reportedly poisonous on oral ingestion [9]. Short-term toxicological of *Calophyllum inophyllum* seed oil on rats revealed that 5% of the oil in normal rat feed caused a moderate myocardioc necrosis, significant change in plasma cholesterol level and glomerulonephrotic changes in the kidney.

Fatty acid analysis of the oil showed high level of unsaturation with preponderance of linoleic and oleic acids [10].

## Materials and methods

### Plant Material

Mature and ripe fruits of *Calophyllum inophyllum* were collected from the premises of Bowen University, Iwo, Osun State in the month of December, 2008 and identified at the Herbarium Unit of Forestry Research Institute of Nigeria (FRIN) at Ibadan, Oyo State.

### Extraction

The air-dried ground seed sample (800 g) was sequentially extracted with hexane, ethylacetate and methanol for a period of 72 h. The extracts were filtered and concentrated on water bath.

### Phytochemical analysis

The phytochemical screening was carried using methods described by Trease and Evans, and Harbone [11-12]. The presence of anthraquinone, alkaloid, phenol, sterol, flavanol, tannin, glycoside and saponin were tested for through these methods

### Test Organisms

Two gram positive bacteria *Bacillus subtilis* (ATCC14579) and *Bacillus aureus* (ATCC33923) and two gram negative bacteria *Pseudomonas aeruginosa* (ATCC25179) and *Salmonella typhi* (ATCC25179). They were cultured on Nutrient agar slants and

twenty-four hours old pure cultures were prepared for use.

### Preparation of test samples

20 mg of hexane and ethylacetate extracts were dissolved in 2 ml of Dimethylsulfoxide (DMSO) separately while the extract of methanol was dissolved in distilled water to give 10,000 µg/ml stock solution. Serial dilutions were made to prepare concentration of 1000,100, and 10 µg/ml. These concentrations were prepared in triplicate for each of the extract.

### Antibacterial Sensitivity testing

The antibacterial sensitivity testing was determined by disc diffusion method. Solutions of concentration 10,100,1000,10000 µg/ml were used [13]. Suspension of micro-organisms was made in sterile peptone water and adjusted  $1.5 \times 10^6$  using optical density of 0.1 at 600 nm (Jenway 6305 model) [12]. The microorganisms were inoculated on the prepared Nutrient agar plates using sterile cotton swabs. The sterile filter paper discs of 6 mm diameter were impregnated with test extracts and placed aseptically on each plate. The plates were incubated at 37°C for 24 h and the diameter of the inhibition zones were measured in millimeters [14].

Gentamycin, at concentration of 5 µg/ml and DMSO were used as positive and negative control respectively.

### Brine Shrimps cytotoxicity test

Shrimps eggs were added to sea water in a small tank with perforated dividing dam and allowed to hatch within 48 h at room temperature. 10 shrimps were placed in test tubes containing solutions of concentration 10,100 and 100 µg/ml of test extracts. Each test solution was prepared in triplicates

The count of the number of surviving shrimps was taken after 24 h and analysis was done using Finney Computer Programme to determine  $LC_{50}$  value at 95% confidence limit [15-16].

### Determination of Vitamins

AKTA HPLC with UV detector was used for quantification of fat soluble vitamin A and E in the hexane fraction

see Table 1.

see Table 2.

see Table 3.

see Table 4.

At all concentrations in the fractions, the negative control showed no inhibition.

### Result and Discussion

Calophyllum inophyllum seed oil contains an appreciable amount of saturated fatty acid (84%) [17] Table 1 shows highest yield for hexane while methanol fraction was the least. The assay for secondary metabolite indicated the presence of tannin in all the fraction and phenol in the hexane fraction (Table 2). Similar report was made by Burkill. The Brine Shrimps cytotoxicity evaluation revealed that all the fraction were toxic with hexane fraction ranking highest followed by methanol and ethylacetate respectively. This is also in agreement with Burkil's report and short-term toxicological evaluation of the seed oil which claim that the oil is toxic on oral ingestion [18]. Table 3 showed that the hexane fraction which is richest in the oil has the lowest  $LC_{50}$  (37.423 µg/ml) indicating its high toxicity relation to other fractions. However, this finding is at variant with the report of Yimdol *et al.* which claimed that the oil has little to no toxicity [19].

All the fractions were active on *Bacillus subtilis* in a concentration dependent pattern with exception of ethylacetate fraction whose activity was not concentration dependent. Also, ethylacetate fraction showed no detectable activity on *P. aeruginosa* at all concentrations compared to hexane and methanol fraction which have their activities concentration dependent and independent respectively. Gram positive *B. cereus* defied the antibacterial

potency of the entire fractions except for ethylacetate fraction at concentration of 1000 µg/ml and 10 µg/ml

Ethylacetate fraction showed activity on *S. typhi* at all concentration though not concentration dependent, however, methanol fraction showed no detectable activity on the organisms at all concentration. Hexane fraction showed activity at lower concentration.

Reportedly, at a dose of 20 mcg/disc some isolates were found to inhibit the growth of *B. aureus* but not *E. coli* and the yeast *Candida tropicalis* [20].

#### Vitamin A and E

Vitamin A and E were quantified as 4226.376 µg/ml and 935.9 µg/ml retinol equivalent respectively.

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EXTRACT	COLOUR	WEIGHT OF EXTRACT(g)	% YIELD
Hexane	Yellow	409.6	51.2
Ethylacetate	Greenish yellow	143.9	17.33
Methanol	Reddish Yellow	25.4	3.06

Table 1: Extraction

SECONDARY METABOLITE	HEXANE	ETHYLACETATE	METHANOL
Anthraquinone	-	-	-
Alkaloid	-	-	-
Phenol	-	-	+
Sterol	-	-	-
Flavanol	-	-	-
Tannin	+	+	+
Glycoside	-	-	-
Saponin	-	-	-

Table 2: Phytochemical Screening

Present: + Absent: -

Extract	LC <sub>50</sub> (µg/ml)
HEXANE	37.473
ETHYLACETATE	93.476
METHANOL	62.270

Table 3: Brine Shrimps Cytotoxicity

ORGANISM	HEXANE( µg/ml)	EHTYLACETATE ( µg/ml)	METHANOL ( µg/ml)
	10 <sup>4</sup> , 10 <sup>3</sup> , 10 <sup>2</sup> , 10, +ve	10 <sup>4</sup> , 10 <sup>3</sup> , 10 <sup>2</sup> , 10, +ve	10 <sup>4</sup> , 10 <sup>3</sup> , 10 <sup>2</sup> , 10, +ve
<i>S. typhi</i>	ND, ND, 9, 8, 7, 25	12, 11, 12, 21	ND, ND, ND, NA, 25
<i>B. cereus</i>	ND, ND, ND, ND, 14	ND, 10, 25	ND, 9, ND, ND, ND, 23
<i>P. aeruginosa</i>	10, 7.5, 7, 7, 23	ND, ND, ND, ND, 23	10, 7, 15, 7, 20
<i>B. subtilis</i>	12, 12, 10, 10, 20	10, 14, 12, 14, 20	ND, 12, 7, 7, 25

Table 4: Antibacterial activities. Zone of inhibition in millimeter  
ND: Not detected