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Identification of phytochemical compounds in *Calophyllum inophyllum* leaves

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ABSTRACT

Objectives: To investigate the proximate composition, mineral content, and phytochemical compounds in *Calophyllum inophyllum* (*C. inophyllum*) leaves. Moreover, isolation and identification of pyrene were also performed.

Methods: *C. inophyllum* leaves were extracted with methanol by percolation methods. The proximate composition of *C. inophyllum* leaves was analyzed by standard methods. Mineral contents in this plant were analyzed by using atomic absorption spectrophotometer. Phytochemical screening and analysis of this plant were performed by spectrophotometric method. Washing method with carbon disulfide was used for isolating dihydropyrene compound from *C. inophyllum* leaves extracts.

Results: The result revealed that *C. inophyllum* leaves contained 11.24% moisture, 4.75% ash, 6.43% crude protein, 23.96% crude fiber, 9.91% carbohydrate, and energy (79.17 kcal/100 g). The leaves also contained 0.007% iron, 1.240% calcium, 0.075% sodium, 0.195% magnesium, 0.100% ppm potassium, and 0.040% phosphorus. Moreover, 11.51% alkaloid, 2.48% triterpenoid, 2.37% flavonoid, 7.68% tannin, 2.16% saponin, 2.53% polyphenol, were identified in the methanolic crude extracts of *C. inophyllum* leaves. It was found that trans-2-[2-(trifluoromethyl)phenyl]-10b, 10c-dimethyl-10b,10c-dihydropyrene was obtained at purity of 79.18% (22.17% yield) from *C. inophyllum* leaves.

Conclusions: *C. inophyllum* leaves may be used as a good source of fiber. It was found that *C. inophyllum* leaves have the potential as herbal drugs due to their phytochemical content. The separation, isolation, and purification of bioactive compounds from this methanolic crude extract and their biological activity are under further investigation.

1. Introduction

Natural products are the chemical substances that produced by living organism, but they are usually referred to as secondary metabolites. Secondary metabolites are generally not important for the growth and reproduction of organisms, but they play an important role in pharmaceutical field [1]. Additionally, natural plants are generally used in cosmetic products and as functional food additives [2,3]. An important part in the research of plant is the identification and analysis of bioactive compound present in plant leading to further biological and pharmacological studies [4,5].

Mangroves contain a number of minerals, vitamins, amino acids that are essential for medical and health progress [6]. The largest areas of mangroves in Southeast Asia are found in Indonesia (almost 66 percent of Southeast Asia total), Malaysia (11.7%), Myanmar (8.8%), Papua New Guinea (8.7%) and Thailand (5.0%) [7]. Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. In order to increase the medical effect of natural plants, bioactive compounds must be extracted, concentrated, separated and purified.

Calophyllum inophyllum (C. inophyllum) is a mangrove species from the family Clusiaceae. It is an evergreen tree with a dense canopy of glossy, elliptical leaves, fragrant white flowers and large round nuts. Its habitat is primarily coastal and adjacent to lowland forests. This tree grows to a height of 8–20 m (25–26 ft), sometimes reaching up to 35 m (115 ft) [8]. It has milky

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white sap. Leaves are opposite, deep glossy green, glabrous, simple, coriaceous with broadly elliptic or obovate-elliptic lamina, 10–20 cm long and 6–9 cm wide, rounded or emarginate apex, rounded or cuneate base, entire margin and distinct parallel lateral veins, perpendicular to mid rib. Flowers are white, fragrant, bisexual, 2.5 cm across and 8–14 mm long on sturdy pedicels and borne in racemose or paniculate, axillary inflorescences of 4–15 flowers. Fruit is globose to subglobose, 2.5–4.0 cm across, indehiscent drupe, light green turning yellow to purplish brown and wrinkled when ripe is found in clusters. The seed is large, brown 2–4 cm across and surrounded by a corky shell and thin pulp [9].

Pyrene is a polycyclic hydrocarbon consisting of four fused aromatic rings. A polycyclic hydrocarbon pyrene can be functionalized by electrophiles, oxidants, reducing agents and transition metal mediated C−H activation to give a few examples [10,11]. Advances in industrial chemistry turned pyrene into a readily available, inexpensive building block for dye industry [12]. Nowadays, pyrene is prepared on a multi-ton scale and utilized in its own right, especially due to its outstanding photophysical properties (*e.g.* as optical brightener Fluorite XMFTM [13], reactive dye C.I. Direct Blue 109 [14] or fluorescent dye Pyranine [15]).

C. inophyllum plants have many properties that can be utilized by humans. The roots, stems, trees and leaves, and the seeds have their own benefits. Generally, *C. inophyllum* plant only known as a plant whose seeds can produce oil that can be used to biodiesel production. The oil, in addition to be used for biodiesel, can also be used as a cure wounds, skin diseases, burns, arthritis, and others [16]. Various parts of this plant have been reported to have traditional medical uses such as for treatment of venereal disease, high blood pressure, rheumatism, inflammation, eye diseases, varicose veins, haemorrhoids, chronic ulcers, skin infections and wounds [17]. The various components of the *C. inophyllum* plant have biological activities, for example, xanthones jacareubin have antiulcer activity [18]; inophyllum, pyranocoumarins, and calophyllolide have antiviral and anti HIV activity [19]; calophyllin, xanthones, jacareubin, calophyllolide, and xanthone have analgesic, antipyretic, anticonvulsant, and anti-inflammatory activity [9].

C. inophyllum is one of the mangroves that has biological activity and has not been used in Indonesia. The identification and utilization of valuable mangrove plants, such as *C. inophyllum*, are important ways to achieve the sustainable development of mangrove areas. Recently, there is an idea to explore *C. inophyllum* leaves as a raw material of herbal drug. However, its proximate, mineral, and phytochemical composition of *C. inophyllum* leaves have not been evaluated. Therefore, the objective of this work was to investigate the proximate composition, mineral content, and phytochemical compounds in *C. inophyllum* leaves. Moreover, isolation and identification of pyrene were also investigated.

2. Material and methods

2.1. Materials

Dried *C. inophyllum* leaves were obtained from the 'Koperasi Jarak Lestari', Cilacap, Central Java, Indonesia. Standards of iron, calcium, sodium, magnesium, potassium, phosphorous were obtained from Sigma Aldrich (St. Louis, MO). All solvents and reagents were obtained from commercial sources.

2.2. Extraction of *C. inophyllum* leaves

The *C. inophyllum* leaves were dried in the sun for 3 d to reduce their water content. The dried *C. inophyllum* leaves (1 000 g) were chopped to a homogeneous size by a mill (about 1 cm diameters) and soaked in 3 L of methanol for 72 h each time. Then, the solutions were filtered through filter paper. The extraction was repeated twice. The filtrate were combined and then evaporated to dryness by distillation at 80 °C to obtain semi solid crude extracts.

2.3. Proximate analysis

2.3.1. Determination of moisture content of *C. inophyllum* leaves

Moisture content was analyzed by procedure described previously [20]. Briefly, a sample (1 g) was weighed accurately in a clean and dried crucible (W). Then, the crucible was placed into oven and held at 105 °C until a constant weight was achieved (W_1). The percent moisture of *C. inophyllum* leaves was calculated as:

$$\text{Moisture content } (\%) = \frac{W - W_1}{W} \times 100 \quad (1)$$

2.3.2. Determination of ash content of *C. inophyllum* leaves

Ash content was determined by procedure described previously [21]. Briefly, a clean and empty evaporating dish was heated in muffle furnace at 600 °C for 30 min, cooled in a desiccator and weighed (W_1). Then sample (2 g) was weighed into evaporating dish (W). The sample was heated in a muffle furnace at 600 °C for 2 h until it was a grayish white ash. This ash indicated that complete oxidation of all organic matters in the sample had occurred. The evaporating dish was cooled and weighed (W_2). The percent ash of *C. inophyllum* leaves was calculated as:

$$\text{Ash content } (\%) = \frac{W_2 - W_1}{W} \times 100 \quad (2)$$

2.3.3. Determination of crude lipid of *C. inophyllum* leaves

Crude lipid content was determined by procedure described previously [22]. A soxhlet extractor, equipped with a condenser system, was employed in this study. The sample (2 g) was wrapped in filter paper and placed inside the Soxhlet extractor (W). Neutral lipids, such as fatty acids and acylglycerols, were extracted from the leaves with hexane (70 mL) as the solvent. The hexane was then put in a 500 mL round-bottom flask and heated. After 6 h, the extraction process was stopped, and the flask that contained the desired extract was removed and replaced immediately by another flask. Then, hexane was removed in the desired extract by distillation and lipid extract was heated in oven at 105 °C. Dried lipid extract was cooled and weighed (W_1). The percent crude lipid of *C. inophyllum* leaves was calculated as:

$$\text{Crude lipid } (\%) = \frac{W_1}{W} \times 100 \quad (3)$$

2.3.4. Determination of crude protein of C. inophyllum leaves

Crude protein content was determined by Kjeldahl method [23]. Total protein was determined by multiplying the amount of nitrogen by the 6.25 correction factor [16]. Briefly, 2 g of dried sample (W) was transferred into a Kjeldahl digestion flask. Then, K_2SO_4 anhydrate (15.00 g), $CuSO_4$ anhydrate (0.45 g), concentrated H_2SO_4 solution (15 mL) was added to the digestion flask. The flask was swirled to mix the contents thoroughly, and then placed on a heater to digest until the mixture became clear. Then, it was cooled and replaced into distillation flask. Distilled water was added to 200 mL and swirled to thoroughly mix the contents. Next, 15 g NaOH/10 mL H_2SO_4 used and Zn granule was added to distillation flask. Erlenmeyer (300 mL) containing 25 mL of HCl solution (0.2 M) was prepared. Then, the solution was distillated until 150 mL distillate volume. Then, a few drops of Methyl-Red indicator were added in distillate. The HCl excess was titrated with NaOH solution (0.2 M). The percent crude protein of *C. inophyllum* leaves was calculated as:

$$\text{Crude protein } (\%) = 6.25 \times \left[(V_1 - V_2) \times N \times 0.014 \times \frac{f}{W}\right] \times 100 \quad (4)$$

where V_1, V_2, N, f, W were the sample titration reading, blank titration reading, HCl normality, sample dilution, and sample weight, respectively. The 0.014 constant was the milli equivalent of nitrogen.

2.3.5. Determination of crude fiber of C. inophyllum leaves

Crude fiber content was determined by gravimetric method [22]. A sample (2 g) was weighed (W) and dried in oven (105 °C). A moisture and lipid free sample was transferred to flask. A total of 200 mL dilution H_2SO_4 (1.25%) was added, and boiled with reflux for 30 min. About 200 mL NaOH solution (3.25%) was added and boiled again for 30 min. Then, filtered in hot conditions with filter paper that has been weighed (W_1). The precipitate was washed repeatedly with hot H_2SO_4 solution (1.25%) and hot distilled water until the filtrate became clear. The precipitate was washed with 96% ethanol. Filter paper was dried in an oven (105 °C), and cooled in a desiccator, and weighed (W_2). The percent crude fiber of *C. inophyllum* leaves was calculated as:

$$\text{Crude fiber } (\%) = \frac{W_2 - W_1}{W} \times 100 \quad (5)$$

2.3.6. Determination of carbohydrate content of C. inophyllum leaves

Carbohydrate content was determined by iodometric method [22]. A sample (5 g) was weighed (W) and placed in the flask. First, carbohydrate was hydrolized by 200 mL of HCl solution (3%) and boiled for 3 h with reflux. The solution was cooled and neutralized with NaOH solution (30%), and a little of CH_3COOH was added to make acid situation in solution. Then, it was filtered. Second, analysis by iodometric method was employed. About 10 mL filtrate was pipetted into the flask. About 25 mL Luff solution, a few grains of boiling stones, and 15 mL distilled water were added respectively. The mixture was heated at a constant temperature and boiled for 3 min, and boiled again for 10 min, then cooled rapidly. After that, 15 mL KI solution (20%) and a H_2SO_4 solution (25%) was added slowly. 0.1 N thiosulfate solution was titrated (0.5% starch indicator solution was added) and a blank titration was also performed. The percent carbohydrate content of *C. inophyllum* leaves was calculated as:

$$\text{Carbohydrate content } (\%) = 0.9 \times \left(\frac{W \times f}{W_1}\right) \times 100 \quad (6)$$

where W, W_1, f were the sample weight (mg), glucose weight (mg), and sample dilution, respectively. Luff solution preparation method was based on SNI 01-2891-1992 [22].

2.3.7. Determination of total energy of C. inophyllum leaves

Energy values were obtained by multiplying the carbohydrate, protein, and fat by the factors of 3.5, 4.0, and 9.0 kcal/g, respectively [24].

2.4. Mineral content analysis

2.4.1. Determination of iron content

Iron content was determined by atomic absorption spectroscopy method. A sample (5 g) was weighed and placed in porcelain dish (W). It was heated in the oven, and cooled in desiccator. Then, 2 mL of demineralized water and 3 mL of HNO_3 (50%) was added and evaporated. Then, it was heated at a lower temperature until all the nitrate was lost. Calibration curve of Fe was created by measuring absorbance values and samples were analyzed by atomic absorption spectrometry (AAS) apparatus. AAS analysis operating conditions for iron content analysis of wavelength is 248.3 nm. Then, iron content in sample (mg/L) was calculated from calibration curve (W_1). The percent iron content of *C. inophyllum* leaves was calculated as:

$$\text{Iron content } = \frac{W_1 \times f}{W} \quad (7)$$

where f was sample dilution.

2.4.2. Determination of calcium, sodium, magnesium, potassium content

Calcium, sodium, magnesium, and potassium content were determined by atomic absorption spectroscopy method. A sample (5 g) was weighed and placed in porcelain dish. Then, dried destruction was done. Samples were heated in muffle furnace at 500 °C overnight. Then, the temperature of the furnace is lowered at room temperature. About 2 mL of HNO_3 (1%) was added and mixed, then it was evaporated on hotplate. Sample was inserted again in furnace at 500 °C for 1 h. Furnace temperature was lowered at room temperature. Ten mL of HNO_3 1% (v/v) was added to the sample, evaporated on hotplate, cooled at room temperature, and transferred into a 25 mL flask. About 0.1% La and 15 mL of 1% HNO_3 (v/v) were added respectively to the line mark of flask. The sample solution was filtered with filter paper Whattman 40, analyzed by AAS

method. Then, calibration curve was created by measuring absorbance values of authentic standard. AAS analysis operating conditions for calcium, sodium, magnesium and potassium content analysis of wavelength were 422.67 nm, 589.00 nm, 285.00 nm and 766.50 nm, respectively. The percent mineral content (calcium, sodium, magnesium and potassium) of *C. inophyllum* leaves was calculated as:

$$\text{Mineral content} = \frac{\text{ppm sample} \times \text{f} \times \text{analyte volume}}{\text{mass of sample (g)}} \quad (8)$$

where f was sample dilution.

2.4.3. Determination of phosphorus content

Phosphorus content was determined by Spectrophotometric method [21]. A sample with the weight of 5 g was placed in porcelain dish, dried in oven at 125 °C, inserted in furnace at 600 °C for 4 h and cooled at room temperature. About 10 mL of HCl (25%) and 3 drops of concentrated HNO_3 was added and boiled and the sample was filtered. Then, 5 mL filtrate was inserted in the 10 mL flask with about 1 mL reagent Molib. Vanadate was added and allowed for 20 min. Distilled water was added to the line mark of flask. The absorbance was measured with Spectrophotometer at a wavelength of 400 nm and calibration curve of P was created by measuring absorbance values. The percent phosphorus content of *C. inophyllum* leaves was calculated as:

$$\text{Phosphorus content} = \frac{\text{ppm sample} \times \text{f} \times \text{analyte volume}}{\text{mass of sample}} \quad (9)$$

where f was sample dilution.

2.5. Phytochemical analysis

Alkaloid, triterpenoid, flavonoid, tannin, saponin, and polyphenol contents were determined by Spectrophotometric UV–Vis method. About 100 mg crude extract was inserted in a 100 mL volumetric flask. Ethanol was added to the flask to the line mark of flask. Then, it was filtered with filter paper Whatmann 40 and the filtrate was diluted with the same solvent when the filtrate was too thick. Standards samples were tested at 215 nm, 280 nm, 350 nm, 225 nm, 245 nm, 315 nm, 220 nm, 228 nm and 205 nm for alkaloid, triterpenoid, flavonoid, tannin, saponin and polyphenol, respectively.

2.6. Separation of pyrene compound from C. inophyllum leaves

In this study, washing method was used to separate the pyrene. Crude extract (3 g) was mixed with CS_2 solvent (30 g) in a flask. The mixture was stirred for 30 min and filtrated by filter paper. This process was repeated until the color of liquid fraction is colorless and the remaining residue was called solid fraction. Each filtrate was collected and signed as liquid fraction.

2.7. Thin layer chromatography (TLC) analysis

TLC was employed to qualitatively analyze the sample, and authentic standards was used as described by research [25]. TLC paper that has been stained by the sample was immersed in a mobile phase of hexane:ethyl acetate:acetic acid at 80:20:1 (v/v/v).

2.8. Gas chromatography (GC) analysis

Chromatographic analysis was performed on a Shimadzu GC-2010 (Kyoto, Japan) gas chromatography equipped with a flame ionization detector. Separations were carried on a DB-5HT (5%-phenyl)-methylpolysiloxane non-polar column (15 000 mm × 0.32 mm *i.d.*; Agilent Tech. Palo Alto, California). Temperatures of the injector and the detector were both set at 310 °C. The temperature of the column was started at 80 °C, increased to 300 °C at rate of 15 °C/min, and maintained at 300 °C for 8 min. The split ratio was 1:50 using nitrogen as carrier gas with a linear velocity of 30 cm/s at 80 °C.

2.9. Gas chromatography mass spectrophotometry (GC–MS) analysis

The GC–MS analysis was conducted by a Agilent 6890 GC system with Agilent 6971 inert mass selective detector (Agilent Tech. Palo Alto, California, USA). Separations were carried out on capillary column, HP5 5% phenylmethyllsiloxane non-polar column (30 000 mm × 0.32 mm *i.d.*, with a layer thickness of 0.25 μm stationary phase. The oven temperature was set at a temperature of 80 °C and maintained for 5 min. Then, the oven temperature was raised 15 °C/min to a temperature of 300 °C and maintained for 2 min. The carrier gas used was helium ultrapure (99.999%). The temperature of injector, interface, and ion source were 310, 280 and 230 °C, respectively. The injection volume was 1 μL, using a model with a 1:10 split inlet, with gas flow rate in the column at 1.3 mL/min.

3. Results

3.1. C. inophyllum leaves

The proximate composition of dried leaves of *C. inophyllum* showed that the dried leaves of *C. inophyllum* contained crude fiber which had the highest content (23.96%), followed by moisture content (11.24%), carbohydrate (9.91%), protein (6.43%), and ash content (4.75%). The caloric value of *C. inophyllum* leaves was 79.17 kcal/100 g.

The mineral composition of some medicinal leaves shows that the dried *C. inophyllum* leaves contained calcium which had the highest concentration (1.240%), followed by magnesium (0.195%), potassium (0.100%), sodium (0.075%), phosphorus (0.040%) and iron (0.007%).

3.2. Crude extract of C. inophyllum leaves

Approximately, 28.87 g (2.88%) crude extract was obtained from 1 000 g *C. inophyllum* leaves after 2 × 72 h of cold percolation (ambient temperature) with methanol. The methanolic crude extract of *C. inophyllum* leaves contained alkaloids which had the highest concentration (11.51%), followed by tannin (7.68%), polyphenols (2.53%), triterpenoids (2.48%) flavonoids (2.37%) and saponins (2.16%).

3.3. Separation of dihydropyrene compound from C. inophyllum leaves crude extract

Figure 1 shows the results of separation components from crude extract by washing method with mass ratio of crude to CS_2

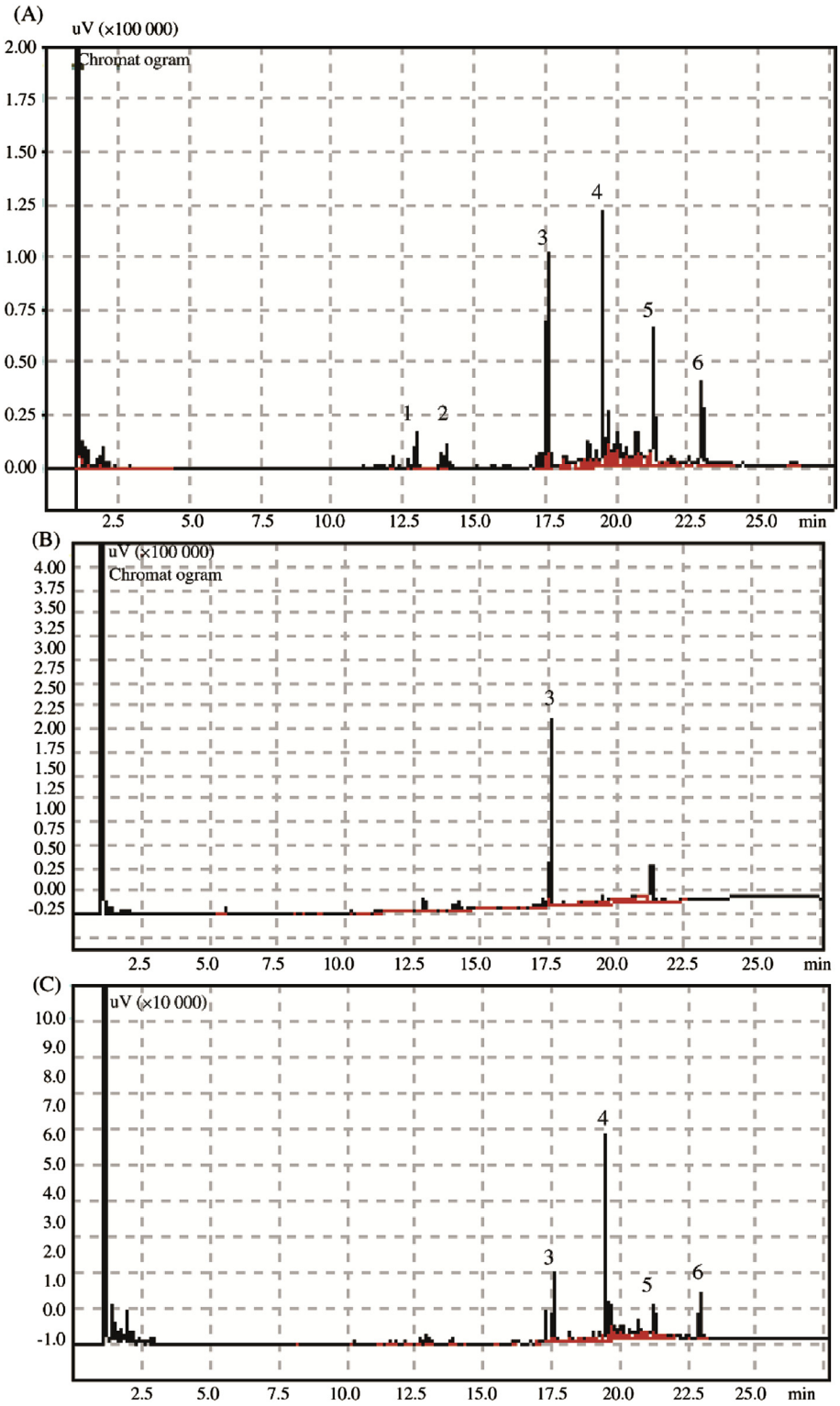

Figure 1. Results of GC analysis of *C. inophyllum* leaves crude extract **(A)**; solid fraction **(B)**; liquid fraction **(C)**.

(1/10 g/g). There were 6 main compounds in the methanolic crude extract of *C. inophyllum* leaves (Figure 1A). Washing method with CS_2 successfully separated crude extract into solid and liquid fractions.

It was found that the component 3 was successfully separated in the solid fraction (Figure 1B), which was a green powder. Identification of component 3 in this solid fraction was done by GC–MS analysis. *Trans*-2-[2-(trifluoromethyl)phenyl]-10b,10c-dimethyl-10b,10c-dihydropyrene was identified at purity of 79.18% (22.17% yield) from *C. inophyllum* leaves.

4. Discussion

Various plants have been reported that their leaves have traditional medical uses, such as *Gongronema latifolium* (*G. latifolium*), *Atheris hispida* (*A. hispida*) and *C. inophyllum*.

C. inophyllum leaves are opposite, deep glossy green, glabrous, simple, coriaceous with broadly elliptic or obovate-elliptic lamina 10–20 cm long by 6–9 cm wide, rounded or emarginate apex, rounded or cuneate base, entire margin and distinct parallel lateral veins, perpendicular to mid rib.

C. inophyllum is a potential source for antiviral, anticancer, antiplatelet, antimicrobial, UV protection, Central Nervous System depressant, antiinflammatory, antiulcer, and molluscicidal [18,19,26–28].

The moisture content of *C. inophyllum* leaves was relatively equal to *G. latifolium* (11.13%) [29], *A. hispida* (11.02%) [30] and *Jatropha curcas* (11.9%) [31]. The moisture content of any food is an index of its water activity and is used as a measure of stability and susceptibility to microbial contamination [32,33]. The high moisture content provides the greater activity of water soluble enzymes and co-enzymes needed for metabolic activities of these leafy vegetables [34]. High amount of moisture in crops makes them vulnerable to microbial attack, hence, spoilage [35]. It is also needed to keep the seeds in cool condition if they would be kept for a long period without spoilage especially in the tropics where wastage of crops is estimated to be around 50% due to high moisture content [36]. The low moisture content may contribute toward roughness of *C. inophyllum* leaves.

Not detected lipid showed that *C. inophyllum* leaves is not a good source of lipids. Accumulation of fats can cause arteriosclerosis and aging [37]. The protein content of *C. inophyllum* leaves was lower than that of *G. latifolium* (33.60%) [29], *A. hispida* (13.78%) [30] and *J. curcas* (26.00%) [31]. Diet is nutritionally satisfactory if it contains high calorie value and a sufficient amount of protein [38].

Crude fiber is not digested by human but the normal functioning of intestinal tracts depends upon the presence of adequate fiber. Fiber helps to maintain human health and has been known to reduce cholesterol level [39]. A low fiber diet has been associated with heart disease, cancer of colon and rectum, varicose veins, phlebitis, obesity, appendicitis, diabetes, and even constipation [40,41]. Hence, *C. inophyllum* leaves could be recommended as a veritable crude fiber source in the diet as a result of its relatively high fiber content even when compared with that of *G. latifolium* (4.20%) [29], *A. hispida* (10.25%) [30] and *J. curcas* (17.67%) [31]. Non-starchy vegetables are the richest sources of dietary fiber and are employed in the treatment of diseases, such as obesity, diabetes, cancer and gastrointestinal disorders [40,42].

The carbohydrate content of *C. inophyllum* leaves was lower than that of *G. latifolium* (38.55%) [29], *A. hispida* (48.48%) [30] and *J. curcas* (36.33%) [31]. As a result, total energy of *C. inophyllum* leaves was lower than that of *G. latifolium* (327.44 kcal/100 g) [29] and *J. curcas* (363.54 kcal/100 g) [31]. The high carbohydrate content suggests that it can be considered as a potential source of energy.

The proportion of ash content is a reflection of the mineral contents present in the food materials [43]. The ash content of *C. inophyllum* leaves was lower than those of *G. latifolium* (9.11%) [29], *A. hispida* (10.32%) [30] and *J. curcas* (14.10%) [31]. Therefore, these results suggest a low deposit of mineral elements in *C. inophyllum* leaves.

Generally, minerals from plants are less-bioavailable than those from animal sources [44]. Minerals are important for the overall development of mental and physics, and are important constituents of bones, teeth, tissues, muscles, blood and nerve cells [45].

Iron is an important trace element in human body, which plays crucial roles in haemopoiesis, control of infection and cell mediated immunity [46]. The iron content of *C. inophyllum* leaves was lower than that of *J. curcas* (0.070%) [31], *Hibiscus sabdariffa* (*H. sabdariffa*) (0.022%) [50] and *G. latifolium* (0.018%) [50]. Iron is an essential trace element for hemoglobin formation, normal functioning of central nervous system and in the oxidation of carbohydrate, protein and fat [47].

Calcium and phosphorus are associated with each other for growth and maintenance of bones, teeth and muscles [48,49]. Phosphorus is an essential component of bone mineral. Deficiency of phosphorus-calcium balance results in osteoporosis, arthritis and tooth decay. The calcium and phosphorus contents of *C. inophyllum* leaves were higher than those of *J. curcas* (0.065% and 0.004%, respectively) [31], *H. sabdariffa* (0.110% and 0.037, respectively) [50] and *G. latifolium* (0.073% and 0.029, respectively) [50].

Sodium and potassium are important as intracellular and extracellular cations, respectively. Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction [51]. Magnesium is a component of chlorophyll and it is an important content in connection with ischemic heart disease and calcium metabolism in bones [52]. The sodium, potassium, and magnesium content of *C. inophyllum* leaves were higher than those of *J. curcas* (0.047%, 0.002% and 0.127%, respectively) [31], *H. sabdariffa* (0.047%, 0.084% and 0.120%, respectively) [50], and *G. latifolium* (0.033%, 0.099% and 0.093%, respectively) [50]. It shows that *C. inophyllum* leaves contain more minerals than *J. curcas*, *H. sabdariffa* and *G. latifolium*.

C. inophyllum leaves were extracted from methanol. Methanol was selected as a solvent because the yield generated with methanol is more than other solvents (ethanol, hexane and petroleum ether). Extraction of the *C. inophyllum* leaves gave dark brown crude extract in 2.88% yield by methanol in this research. The extraction of the air dry heartwood of *C. inophyllum* gave dark brown extracts in 3.89% (ethanol), 4.29% (methanol) and 3.31% (petroleum ether) [53]. The percent yield extract of *C. inophyllum* leaves with methanol (74.44%) was higher than petroleum ether (23.20%) and chloroform (13.60%) by soxhlet extraction [54]. The methanolic extract of some medicinal plants of North Coastal Andhra Pradesh contains all of the bioactive compounds, such as phenols, flavonoids, alkaloids, terpenoids, glycosides, phytosterols, proteins, and exhibits significant antimicrobial and antioxidant activities when compared with the other solvent (chloroform, hexane, and water) [55]. Methanol extract of *Annona squamosa* Linn. leaves exhibit higher antioxidant activity than that of the chloroform and aqueous extract [56].

Phytochemical analysis suggests possible medicinal applications of *C. inophyllum*. Thus, the preliminary screening analysis is helpful in the detection of bioactive compounds and leads to the discovery of new drugs [57]. In the previous study, by qualitative analysis, the methanol extract of *C. inophyllum* contains alkaloids, carbohydrates, glycosides, saponin, phenols, tannins, flavonoids, proteins, amino acids, steroid and coumarin, while phytosterols, diterpenes, anthroquinione and phlobatannins were not identified [54].

Alkaloids are heterogeneous group compounds which have one or more nitrogen atoms in acyclic system. The alkaloids content of *C. inophyllum* leaves extract was much higher than that of *Psidium guajava* (*P. guajava*) (0.04%) [58], *G. latifolium* (0.12%) [58], and *J. curcas* (1.58%) [30], commonly found to have antimicrobial activity due to the ability to intercalate with DNA [58]. Alkaloids have many medicinal effects, such as analgesic, anti-inflammatory, and developing resistance against

diseases and endurance against stress [59]. This shows that methanolic *C. inophyllum* leaves crude extract has very high antimicrobial activity. The high degree of alkaloid precipitation was found in the methanol extract of *Cocculus hirsutus* [60].

Triterpenoid compounds include cardiac glycosides, sterols, saponins, and triterpenes. Triterpenoids compounds isolated from *C. inophyllum* leaves are 3β, 23-epoxy-friedelan-28-oic acid, friedelin, 3-oxofrieddelin-28-oic acid, canophyllal, canophyllol, canophyllic acid, oleanolic acid, and epifriedelanol [30]. Friedelin isolated from *Azima tetracantha* leaves possesses potent anti-inflammatory, analgesic, and antipyretic activities [61].

Phenolics is a phytochemical possessing one or more aromatic rings with one or more hydroxil groups, and generally include phenolic acids, flavonoids, tannins, coumarins, and stillbenes [62]. Phenols are compounds that have activities such as antitumour agents and exhibit antioxidant properties [63]. Their antioxidative effects were shown by various mechanisms, including the ability to scavenge free radicals or activate antioxidant enzymes and inhibit oxidizes [64].

The flavonoids content of *C. inophyllum* leaves extract was higher than that of *P. guajava* (0.40%) [58], but lower than those of *G. latifolium* (11.13%) [58], and *J. curcas* (7.22%) [30]. Flavonoids are structurally derived from the substance flavone, and certain conjugated aromatic systems [65], which have antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activities. The potent antioxidant activity of flavonoids is their ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals [66]. They had been isolated from *C. inophyllum* leaves such as biflavonoid, neoflavonoid, quercetin-3-O-α-L-rhamnoside and amentoflavone [67]. Some flavonoids, such as quercetin and rutin, have medicinal function in human health by serving anti-inflammatory, antihistaminic, and antiviral agents [68]. Glycyrrhizin and chrysin have against HIV activity [69].

Tannin is one compound of anti-nutrients (cyanide, phytate and tannin). Tannin is plant polyphenols which have ability to form complexes with metal ions and with macro-molecules, such as proteins and polysaccharides [70,71]. Tannin has also been claimed to have adverse effect on protein digestibility [72] and its content in *C. inophyllum* leaves was considered lower when compared with the *G. latifolium* (16.23%) [58], but much higher than that of *P. guajava* (0.55%) [58] and *J. curcas* (0.15%) [30].

Saponins have activities of anti-hypercholesterol, anti-inflammatory, cardiac depressant property and also appear to inhibit cancer cells without killing the normal cells in the process [73]. Saponins content in *C. inophyllum* leaves was considered lower when compared with that of *G. latifolium* (18.11%) [58]. They were also detected in *Moringa oleifera* leaves and they have been shown to have cholesterol lowering properties [74]. Saponins also show tumor inhibiting activity on animals [75].

Washing method is the simplest method for separating pyrene compound. This separation was based on the degree of components polarities. Washing method with mass ratio of crude to solvent 1/10 (g/g) was chosen for its better performace of separation compared with other ratios (1/30 and 1/50) (Data not shown). The less amount of solvent used, the more cycle of washing.

The retention time, compositions, and mass spectral data (molecular and fragment ions) of the solid fraction were tested. Based on GC–MS result, separated component was *trans*-2-[2-(trifluoromethyl)phenyl]-10b,10c-dimethyl-10b,10c-dihydropyrene. The structure was supported by molecular ion observed at *m/z* 376 and a base peak at *m/z* 361, while has fragmentation at *m/z* 203, corresponding to pyrene in its mass spectrum.

Isolation and identification of proximate, mineral, and bioactive compounds in the plant serves as the basis in determining the possible health benefits of the plant leading to further biological and pharmacologic studies. So these might be utilized for developing herbal drug and further investigation needs to isolate novel bioactive compounds from the medicinal plants which may create a new way to treat many incurable diseases including cancer and HIV.

The proximate, mineral, and phytochemical compositions of dried *C. inophyllum* leaves were reported. The mineral identified that the amount of calcium was the highest, and that of iron was the lowest. The phytochemical identified that the amount of alkaloid and tannin was the highest, and that the amount of triterpenoid, flavonoid, and saponin was almost equal. The high phytochemical content and proximate composition in this leaves presents that *C. inophyllum* leaves are potential for medicinal use. *Trans*-2-[2-(trifluoromethyl)phenyl]-10b,10c-dimethyl-10b,10c-dihydropyrene has been separated from *C. inophyllum* leaves with 22.17% yield (79.18% purity).

Conflict of interest statement

We declare that we have no conflict of interest.

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