

Ochna kirkii Oliv: Pharmacognostical Evaluation, Phytochemical Screening, and Total Phenolic Content

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ABSTRACT

Background: *Ochna kirkii* Oliv. belongs to family Ochnaceae is commonly known as “mickey mouse plants” in Asia, included in Jakarta. **Objectives:** This research was conducted to study the pharmacognosy and total phenolic content. **Methods:** The pharmacognosy study including the macroscopic, microscopic, fluorescence and phytochemical characteristic, which enable to identification of medicinal plant drug, as well as the total phenolic content using spectrophotometric method. Physicochemical parameters, like loss on drying, ash value, extractive values etc. were also determined following WHO guidelines. **Results:** Physicochemical standard quantified include total ash 5.15% \pm 0.4623, acid insoluble ash 0.32% \pm 0.2614, water soluble ash 10.72% \pm 0.2340, alcohol soluble extractive 12.8% \pm 1.3770, ether soluble extractive 7.30% \pm 1.8341, water content 5.76 % \pm 0.0050, loss on drying 6.91% \pm 1.6133. Preliminary phytochemical screening of the leaves showed the presence of flavonoids, tannins, phenols, saponins and triterpenoid. TLC profile of the *O. kirkii* leaves extracts (*n*-hexane, DCM, ethyl acetate and 70% ethanol) showed 8, 6, 7 and 10 spots respectively; in different solvent system. The total phenolic content was 252.08 mg GAE/g \pm 0.4430. **Conclusions:** The various macroscopic, microscopic, physical and phytochemical analysis of the *O. kirkii* leaf is useful in standardization for quality, purity and sample identification.

Key words: Chromatogram profile, Fluorescence, Macroscopic, Microscopic, *Ochna kirkii*.

INTRODUCTION

Ochna kirkii is an ornamental perennial shrub or small tree, one species among 86 species of evergreen trees in the genus of *Ochna*, belongs to the Ochnaceae family. Its species has synonym *Ochna carvalhi*, *Polythecium carvalhi*, *Polythecium kirkii*, *Ochna thomasi* Engl. & Gilg. Unique flower shaped like a cartoon character of Mickey Mouse is a specific feature of this plant. So, they are generally referred to as ‘Mickey-Mouse plants’ or ‘Ochnas’. Several members of this family are cultivated as decorative plants due to their colorful flowers and unusual fruits.¹ This plant is originally from tropical Africa, but now widely cultivated in tropic countries such as Asia and Madagascar. Traditional remedies of Ochnaceae have been recorded in several Asian and Africans countries. For example, the stem bark of *O. lanceolata* Spreng., a semi-evergreen tree found widely in Central and Peninsular India, is used by the Palliyar tribes as an abortifacient and for treatment of gastric complaints and menstrual disorders.¹ In Tanzania, Washambaas used *O. macrocalyx* Oliv. bark for treatment of dysmenorrhoea, diarrhea, hemorrhoids, and stomach pain.²

Previous phytochemical studies have revealed that the Ochnaceae is a prolific source of complex flavonoids and related phenolic compounds. Reported chemical constituents from the genus *Ochna*, so far in total ca. 111, include flavonoids, anthranoids, triterpenes, steroids, fatty acids, and a few other compounds and biflavonoids are the

predominant constituents within the genus *Ochna*.¹ Vitexin (apigenin-8-C-glucoside) and isovitexin (apigenin-6-C-glucoside), flavonoid compound that has been proved to give multiple pharmacological effect including anti-cancer^{3,4}, anti-oxidant^{5,6}, anti-inflammatory^{7,8}, anti-nociceptive.⁹ Nair *et al.* isolated vitexin from the acetone-insoluble fraction of the EtOH extract of *O. jabotapita* leaves. while isovitexin was obtained from the leaves of *O. squarrosa*.¹

On the other hand, research data on the chemical compounds and the traditional used of *Ochna kirkii* have not been found. Recently, the pharmacognostical studies of *Ochna kirkii* was carried out. In addition, phenolic level as one of essential compound also explore. This study expected to be useful in the initial data for standardization of its species.

MATERIALS AND METHODS

Plant collection and authentication

The leaves, flower and fruits were collected on June 2019 from Duren Sawit area, located in the eastern of Jakarta, Indonesia. The plant was characterized by the Research Centre for Biology, Indonesian Institute of Sciences, (LIPI), Cibinong Bogor Indonesia and voucher specimen was kept in the Pharmacognosy Laboratory, Faculty of Pharmacy and Sciences, UHAMKA University, Jakarta, Indonesia. Plant material were dried at room temperature for 2 weeks in a dry and airy environment. The dried samples were powdered using an electric grinder, then stored in the dark bottle at a dry place until further use.

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Chemical reagent

Folin-Ciocalteu, gallic acid, were purchased from Sigma Aldrich. Sodium carbonate, *n*-Hexane, dichloromethane, ethyl acetate, ethanol was obtained from Merck Chemical Supplies. All chemicals used were analytical grade.

Pharmacognostical study

Macroscopic evaluation

The macroscopic study is the morphological characters/description of the plant parts (leaf, flower, fruit etc.) were observed by naked eye or magnifying lens. Various organoleptic features of *O. kirkii* parts like color, shape, size, odor, taste, surface characteristic and fracture were evaluated.

Microscopic study

Microscopic of transverse section of fresh leaf, stem and seed was perform. Preparing for powder microscopic of the plants part were observed under the microscope and photographed. The procedure for the microscopic study was described in Indonesian Herb Pharmacopoeia¹⁰ and Hanani et al.¹¹

Extract evaluation

Extract preparation

The powdered leaves were extracted using maceration method to determine the phytochemical parameter and total phenolic content. A 500 g of sample was soaked in 5 L of 70% ethanol for 24 hr at room temperature. The mixture was filtered and the residue was extracted again in 3 L the same solvents and repeated the same procedure. All filtrate combined and concentrated under vacuum rotary evaporator N-1200 BS series (EYELA, Shanghai, China) at 40 – 50°C to a yield of 21.25% of plant extract. This extract stored in air-and water proof containers kept in a refrigerator at 4° C. From this stock, fresh preparation was made whenever required.

Physicochemical evaluation

Physicochemical parameters, of the extract was conducted on the determination of moisture content, ash content, acid insoluble ash content, Water-, ethanol- and ether-soluble extractive value. The extract characterization method was performed by standard procedures in Indonesian Herb Pharmacopoeia and WHO guidelines.^{10,12,13} Test samples were run in triplicate.

Phytochemical screening analysis

Phytochemical analysis of this extract was conducted by screening chemical substances and determination of major compounds. Identification of the presence of phenols, alkaloids, flavonoids, tannins, saponins glycosides, terpenoids were carried out according to the procedures in WHO guidelines⁶, Indonesian Herb Pharmacopoeia¹⁰, and Dixon and Jena.¹⁴

Fluorescence character analysis

Ethanol extract of *Ochna kirkii* leaves powder was fractionated further with *n*-hexane, dichloromethane (DCM), ethyl acetate and 70% ethanol. All extracts were concentrated using a rotary evaporator. Leaves powder and all fractions are treated with various chemical reagents. Strong acids, alkalis and organic solvents were added and observed under the daylight and ultraviolet light at 254 nm and 366 nm.^{11,15}

Thin layer chromatography profile

All fractions were analyzed for the chromatographic profile using TLC (silica gel 60 GF₂₅₄) with different solvents. Each fraction was solved with suitable solvents and spotted using the micro-pipette in the silica

plate surface. The plates were developed using a different mobile phase: *n*-hexane-ethyl acetate (9:1), chloroform-methanol (9.5:0.5), methanol - ethyl acetate (1:9), and 100% ethyl acetate. The dried plate was sprayed with 10% sulfuric acid/methanol solution, followed by heating at 105°C for 5 minutes in an oven, and observed under visible light¹⁶. The retention factor value was measured and the color was observed.

Total phenolic content

Total phenol content (TPC) in the ethanol extract of *Ochna kirkii* leaves was determined using the Folin-Ciocalteu method described by Stankovic (2011) with minor modifications.¹⁷ The extract was dissolved in distilled water to a concentration of 50 µg/mL. The calibration curve was established using gallic acid (15; 25; 35; 45; 55 µg/mL). The Dilute extract or gallic acid (1.6 mL) was added to 0.2 mL FC reagent (5-fold diluted with distilled water) and mixed thoroughly for 3 minutes. Sodium carbonate (0.2 mL, 10% w/v) was added to the mixture and allowed to stand for 30 minutes at room temperature. The absorbance of the mixture at 765 nm was measured by spectrophotometer UV-Vis UV-1601 series (Shimadzu, Kyoto, Japan). The concentration of total phenolic compound was determined as mg of gallic acid equivalents/g of the extract by using an equation obtained from the gallic acid calibration curve. The samples were prepared in triplicate and the mean value of absorbance was obtained.

RESULTS

Macroscopic and microscopic analysis

Macroscopic

Ochna kirkii is a big shrub or a small tree, with a height of 3-5 meters tall (Figure 1). The leaves are dark green color, glossy, alternate, oblong-elliptic to narrow-obovate, heart-shape, rounded at the tip, up to 12 cm long (Figure 2A). Flowers are bright yellow color and vibrant red flower-like calyxes (Figure 2B, 2C, 2D, 2E), as well as green immature fruits and ripe black berries (Figure 2F, 2G, 2H). Each flower is about 4 – 5.3 cm in diameter, has 5-petaled single in axillary and terminal clusters and has a cluster of yellow stamens with orange anthers in the center.

Microscopic

The transverse section of the leaf of *Ochna kirkii* (Figure 3A) showed cuticula, upper epidermis, palisade, collenchyma, mesophyll and oxalate crystals; Transverse section of stem of *O. kirkii* (Figure 3B) showed parenchyma, sclerenchyma and calcium oxalate crystals. The

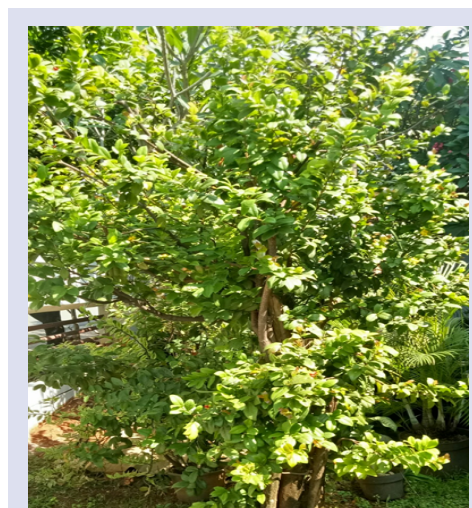


Figure 1: *Ochna kirkii* Oliv.

powder of leaves showed parasitic stomata (Figure 4A); glandular hair (4B); calcium oxalate crystals (Figure 4C); collenchyma cell (Figure 4D) and vascular bundle (Figure 4E). the flower powder showed parenchyma that contains anthocyanin inside which gives a clear red color (Figure 5A), the seed powder showed oil globules (Figure 5C); starch grains (Figure 5D) and endosperm (Figure 5E).

Physicochemical characteristics

The physicochemical characteristics such as loss on drying, ash values, water, alcohol and ether soluble extractive, were given in Table 1.

Phytochemical screening

The preliminary phytochemical screening of the ethanol extract of the leaves of *O. kirkii* were analyzed. Flavonoids, phenols, tannins, saponins, and terpenoids were present in the extract, except the alkaloids.

Fluorescence analysis

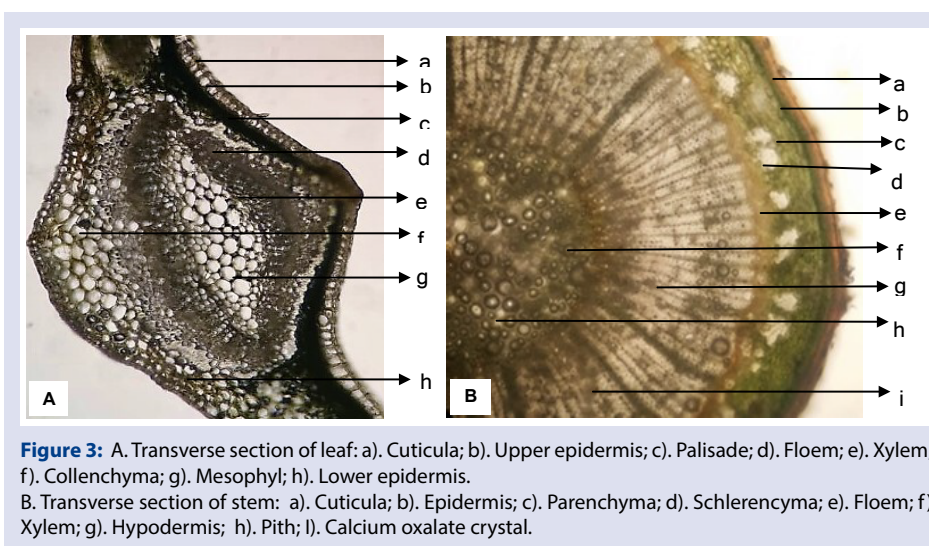
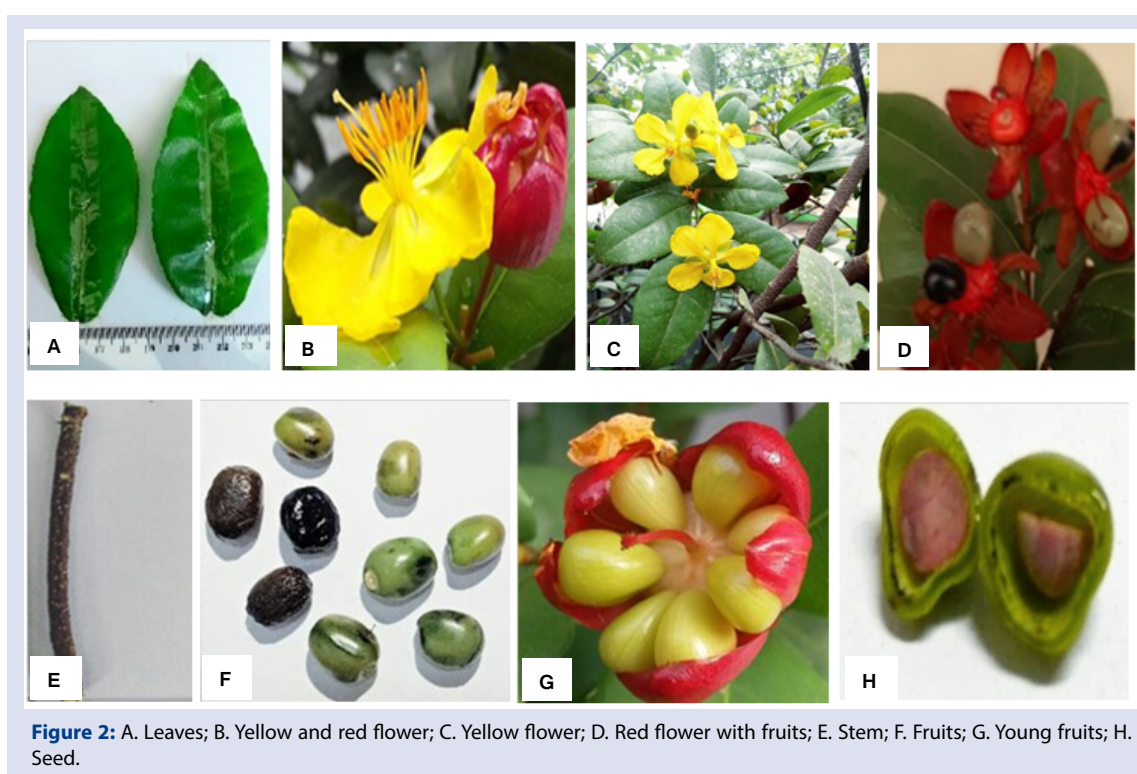
The fluorescence of leaves powder and all fraction were analyzed under daylight, ultraviolet light (254 and 366 nm) by treatment with different chemical reagents. The results were summarized in Table 2.

TLC chromatography

The TLC chromatography results (Table 3) of the *n*-hexane, DCM, ethyl acetate and, 70% ethanol extract showed the presence of 8, 6, 7, and 10 spots respectively, in different mobile phase system.

Determination of total phenolic content

The total phenolic content of the extract was determined by Folin-Ciocalteu methods, and gallic acid was used as the standard. The amount of phenolic content in the extract was presented in Table 4.



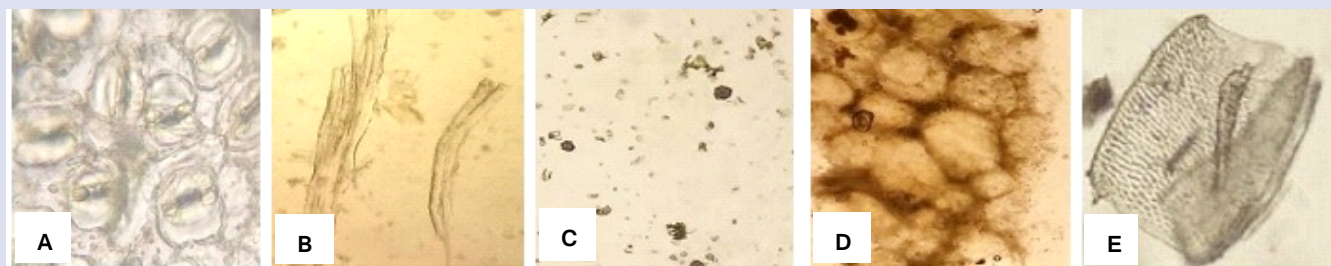


Figure 4: Microscopic of leaves powder: A. Stomata (parasitic type); B. Glandular hairs; C. Calcium oxalate crystals; D. Collenchyma cell; E. Vascular bundle.

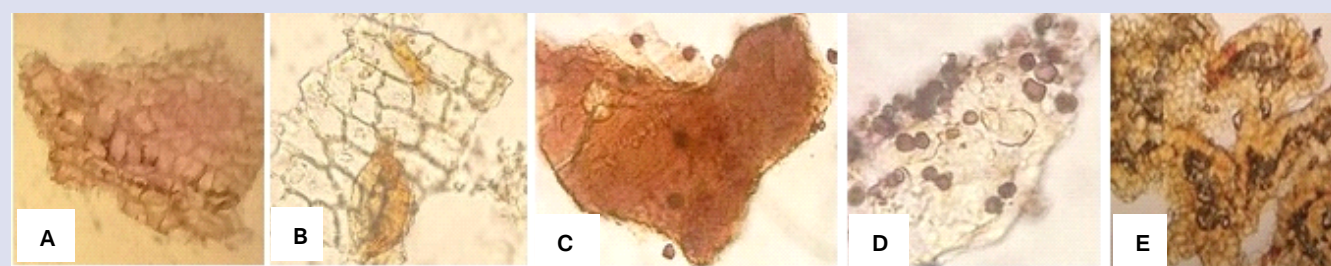


Figure 5: Microscopic of flower powder: A. Parenchyma with anthocyanins inside; B. Epidermis; C. Epidermis with oil gland; D. Starch grains; E. Endosperm.

Table 1: Physicochemical Parameter of Powder of *Ochna kirkii* leaves.

Parameters	Average (%w/w)
Moisture content	
Loss on drying	6.91% \pm 1.6133
Water content	5.76% \pm 0.0350
Ash Value	
a. Total ash	5.15% \pm 0.4623
b. Water soluble ash	10.72% \pm 0.2340
c. Acid insoluble ash	0.32% \pm 0.2614
Extractive values	
a. Alcohol soluble	12.80% \pm 1.3770
b. Water soluble	10.72% \pm 0.9767
c. Ether soluble	7.30% \pm 1.8341

DISCUSSION

The macroscopic and microscopic studies of the plant part will enable to identify the crude drug. The microscopic evaluation is one of the simplest and cheapest methods for establishing the correct identification of the sources of the drug material. The moisture content (loss on drying and water content) were not so high, it means gives to suitable condition and eliminated the proliferation of microorganism.¹⁰ The extractive value of ethanol was found to be highest followed by water and ether. It also helpful in estimation of specific chemical constituents which are soluble in particular solvents.¹⁸ Ash value determination the inorganic substance and other impurities present in the drug. Ash and extractive value can be used as reliable aid for detecting adulteration. Fluorescence is one of the important methods exhibited by various chemical reagents which show different color in daylight and fluorescence in the UV light. Thus, the fluorescence is used for qualitative assessment of crude drug.¹⁹ Fluorescence analysis showed the characteristic of extract reacted to reagent used. Chemical compound in plant extract may be

often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent so that this can be used as one of the important qualitative test parameters in plants.²⁰ Identification results of secondary metabolite of the extract showed the presence of flavonoids, phenols, tannin, saponins, triterpenoids, while the results could not show any positive indication for alkaloids.

The thin layer chromatographic profile of hexane, DCM and 70% ethanol extracts were presented with different mobile phase system to determine how many compounds in the three kinds of extract, and each compound has a different R_f value and color.

Phenols are one class of secondary metabolites in plants. They are known to have antioxidant activity.²¹ Phenolic compounds are known as high-level antioxidants because of their ability to scavenge free radicals and active oxygen species, such as singlet oxygen, superoxide free radicals and hydroxyl radicals.²² The total phenolic compounds are one of an important factor in the consideration of antioxidant activity.²³

Table 2: Fluorescence analysis of the *O. kirkii* extracts.

Sample/Reagents	Visual	254 nm	366 nm
Leaves powder			
10% NaOH	Yellowish red	Green	Green
Dil.NH ₄ OH	Yellowish red	Yellow	Yellow
Conc.H ₂ SO ₄	Dark brown	Black	Light brown
Conc. HCl	Yellowish green	Yellow	Dark green
Conc.HNO ₃	Yellowish red	Yellowish red	Green
n-hexane			
10% NaOH	Yellowish Green	Yellowish red	Yellowish red
Dil.NH ₄ OH	Light brown	Yellowish red	Yellow
Conc.H ₂ SO ₄	Brownish black	Brownish black	Dark black
Conc. HCl	Light green	yellow	Greenish black
Conc.HNO ₃	Yellowish red	Dark green	Light brown
DCM			
10% NaOH	Light green	Dark green	Yellowish red
Dil. NH ₄ OH	Light brown	Light green	Dark green
Conc.H ₂ SO ₄	Dark green	Brownish black	Dark black
Conc. HCl	Light green	Yellow	Yellowish green
Conc. HNO ₃	Yellowish red	Yellowish red	Yellowish green
Ethyl acetate			
10% NaOH	Yellowish brown	Brown	Brown
Dil.NH ₄ OH	Brown	Brown	Brown
Conc.H ₂ SO ₄	Dark brown	Yellow	Brownish black
Conc. HCl	Yellow	Light yellow	Light yellow
Conc.HNO ₃	Red	Dark brown	Light brown
70% Ethanol			
10% NaOH	Light brown	Dark brown	Black
Dil.NH ₄ OH	Brown	Brown	Brown
Conc.H ₂ SO ₄	Dark brown	Brown	Brown
Conc. HCl	Yellowish brown	Light brown	Greenish yellow
Conc.HNO ₃	Greenish brown	Brown	Dark black

Table 3: TLC Profile of different extract of *O.kirkii*.

Extract	Solvent system	No. of spot	Spray reagent (10% H ₂ SO ₄ Methanol)	R _f
<i>n</i> -Hexane	<i>n</i> -Hexane--ethyl acetate (9:1)	8	Brown; Green	0.02 0.15
			Green; Green	0.25 0.49
			Light violet; Grey	0.60 0.82
			Pink; Grey	0.89 0.97
DCM	Chloroform--Methanol (9.5:0.5)	6	Violet; Light violet	0.17 0.30
			Violet; Green	0.47 0.56
			Light blue; Green	0.77 0.90
Ethyl acetate	Ethyl acetate 100%	7	Brown; Violet	0.35 0.43
			Brown; Light brown	0.58 0.70
			Violet; Light yellow	0.76 0.78
			Light green	0.88
70% Ethanol	Methanol--ethyl acetate (1:9)	10	Light brown; Brown	0.17 0.29
			Brown; Light Brown	0.35 0.38
			Brown; Grey	0.44 0.55
			Green; Violet	0.57 0.68
			Green; Violet	0.77 0.85

Table 4: Total phenolic content in the 70% ethanol extract of *O. kirkii*.

Concentration of plant extract (µg/mL)	Absorbance	mg of GA/g extract	Mean ± SD
25.1889	0.356	251.89	252.08 mg GAE/g± 0.4430
25.2711	0.357	252.59	
25.1889	0.356	252.76	

The results in this study have shown in the 70% ethanol extract exist 252.08 mg GAE/g extract of phenolic compound, it can be assumed that the extract have antioxidant activity.

CONCLUSION

Specific characteristics of a plant especially those that are beneficial in medicine are very important to be observed so that the falsification of this plant can be prevented. The pharmacognostical evaluation for the *Ochna kirkii* are laid down for the first time in this study. The present research are helps in setting the standards for proper identification, authentication and also for the standardization of crude drugs material. Herbal manufactures can utilize them for selection and identification of the raw material for drug production. The recent study showed that *Ochna kirkii* Oliv. has a significant phenolic level, it is necessary to evaluate the antioxidant and the specific pharmacological activity for further study.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

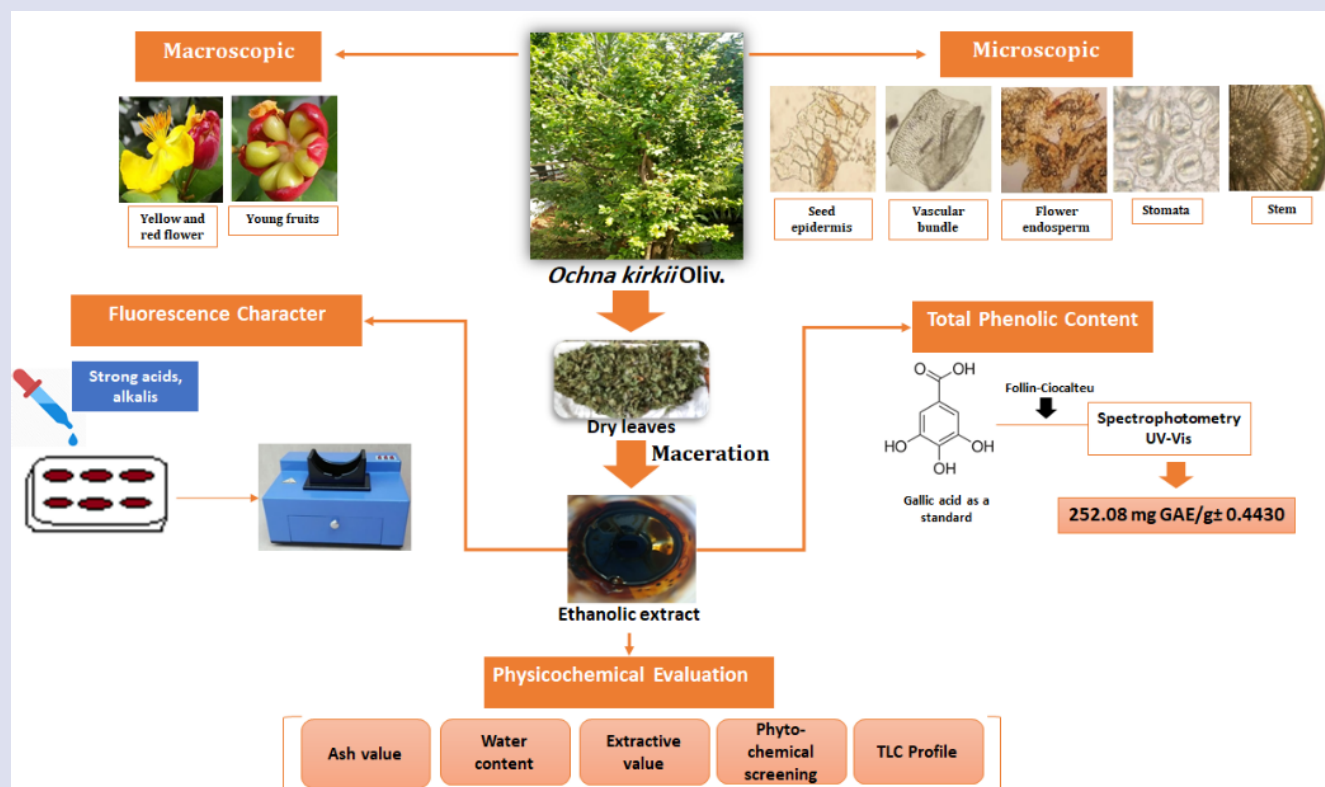
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GRAPHICAL ABSTRACT



SUMMARY

- The present research are helps in setting the standards for proper identification, authentication and also for the standardization of crude drugs material.
- The pharmacognostical evaluation for the *Ochna kirkii* are laid down for the first time in this study.
- *Ochna kirkii* Oliv. has significant phenolic level and it can be assumed that the extract have antioxidant activity.

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