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Chemical Composition, Antibacterial and Antioxidant Activities of Extracts from Dry Leaves and Ash-Dry Leaves of *Luffa cylindrica* (L.) Roem Cultivated in Vietnam

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Luffa cylindrica (L.) Roem was traditionally used to treat stomachaches, as antihyperlipidemic and antioxidant, particularly for atherosclerosis therapy, as a suppository to cure constipation and spleenopathy, as an anthelmintic, carminative, emmenagogue, galactagogue, and as an antiseptic. Therefore, the aim of this study was to study the chemical composition, antibacterial and antioxidant properties of an extract from dry leaves (LuL) compared to ash-dry leaves (LuA) of *Luffa cylindrica* (L.) Roem.

Our results showed the physical-chemical and phytochemical properties, antioxidant activity, antibacterial activity and the metal ion content of both extracts. The comparison between the extract from dry leaves (LuL) and ash-dry leaves (LuA) of *Luffa cylindrica* (L.) Roem, showed difference in quantitative phytochemical determination of cardiac glycosides, alkaloids, phenolics, flavonoids, and triterpenoids. This finding may be related to the LuA sample being burned out incompletely into ash. Furthermore, this study showed that the activity of extracts from dry leaves (LuL) and ash-dry leaves (LuA) contained both bacteriostatic and bactericidal effects. The antioxidant properties observed may be related to , the flavonoids content. The presence of metal ions in both extracts, which may contribute to the known wound healing effects, deserves further study.

Keywords: Luffa cylindrica (L); inorganic herbal metal ions; antibacterial; antioxidant.

1. INTRODUCTION

Luffa cylindrica (L.) Roem belongs to the family *Cucurbitaceae* [1]. The origin of *Luffa cylindrica* is believed to be in South America [2]. *Luffa cylindrica* is commonly grown in Guinea, Ivory Coast, the Philippines, India, and China [3]. The flowers, buds, and young leaves can be used as food [4]. When the fruit is old and dry, cleared of its epidermis and seeds, it gives an excellent sponge called "vegetable sponge," which can be used as a body scrub, pot, or appliance. It is also used as a heavy metal absorber for dehydration [5], [6]. The seed oil is edible. In America, oil is used as an ingredient in soapmaking [7], [8]. The traditional use has been reported in Africa, China, Vietnam, Cambodia, Thailand, Laos, and the Philippines [9]–[11]. The fruit is used as a galactagogue, the roots as a hydragogue and purgative [12], and the root and the whole plant as a suppository to cure constipation [13]. Seed acts as an anthelmintic drug, an inducing vomiting drug, and a laxative [9], [14], [15].

The leaves are prescribed for skin diseases, treat wounds, reduce swelling, treat stomachaches, antihyperlipidemic and antioxidant, particularly for atherosclerosis therapy [16]. Freshly crushed leaves act as emmenagogues, blood detoxifiers, and are used to treat papules and swelling skin [17]. A decoction of leaves is used as a diuretic [18]. Past research has found that leaf extract contains saponin, flavonoids, alkaloids, and cardiac glycosides, and the extract can inhibit *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus,* and *Salmonella typhi* [19]. Aqueous extracts also have an oxytocic activity [13], [20].

The present knowledge of the wound healing process comprises coagulation, inflammation, proliferation, formation and accumulation of fibrous tissues, collagen deposition, epithelialization, contraction of the wound with the formation of granulation tissues, remodeling, and maturation [17], [21]. The constituents of the plant extracts modulate one or more of the above stages.

It was the endeavor to identify the active constituents responsible for antimicrobial activity, free radical scavenging properties, stimulators of enhanced collagen production, and/or angiogenesis promoters through the identification of lead scaffold chemical structures [20], [21].

Some studies have shown that *Luffa Cylindrica* is able to affect wound healing, which is a wisdom of folk medicine in many countries [22], but in Vietnam it is used in a different way by using only the leaves [23] to make the wound heal faster. Minerals in organics are known to have an effect on wound healing, such as zinc and chromium shots, which speed up wound healing [24]. However some report already showed on the trend of using *Luffa* leaves for wound healing suggest that this may be a product that helps with diabetes [22], [25]. In our previous research, the trace of traditional use of *Luffa* leaves was done in Hai Duong province, which is located in the center of the Red River Delta with a total area of 1,668.28 km^2 and a population of

more than 1.9 million people. The province has good conditions for agriculture, transportation, and industrial production and plays an important role in the social and economic development of the country. A total of six traditional medicine practitioners were interviewed for this survey. Informed consent was obtained from all, and the survey was explained to them in detail, including the information that the survey results may be published internationally. Findings showed that *Luffa* leaf was used long ago by both traditional medicine doctors and the old generation themselves to treat open wounds that were affected long-term by bacteria or fungi. The conservative burned ash from *luffa leaf* was pound into dried powder and then applied to the acne, boils, pressure ulcers, and fungal infection areas. The treatment was very effective in many cases of pressure ulcers and fungal infections in the area between the toes during flood season. The wounds were quickly healed and recovered. The aim of this study was to study the chemical composition, antibacterial and antioxidant effects of extracts from dry leaves (LuL) compared to ash-dry leaves (LuA) of *Luffa cylindrica* (L.) Roem. as a preliminary study of sample extracts obtained from Vietnam with possible properties that may contribute to wound healing.

2. MATERIALS AND METHODS

2.1 Plant Material, Extraction, and Chemicals

Luffa leaves were collected from a Vietnamese farm in Hai Doung Province, Vietnam. Voucher specimen No. 0023302 was identified and kept at the Herbarium of the Faculty of Pharmacy, Chiang Mai University, Thailand. The chemical ingredients and solvent used for extraction of the leaves and ash were of pharmaceutical grade and were purchased from Union Sciences Co. Ltd., Thailand. The leaf was dried in a hot air oven at 60 °C and ground to powder (LuL). *Luffa* ash (LuA) was prepared by burning the *Luffa* dried leaves at a normal temperature in open air until the blackish-grey ash was obtained in an uncompleted burning condition. This process was done by the local people.

2.2 The Pharmacogenetic Evaluation of the Raw Material of Crude Dried Leaves

A microscopic examination of powdered LuL was studied. The Thin Layer Chromatography, moisture content, and extractive value were done according to Thai Herbal Pharmacopoeia V.I. in order to prove the scientific database for further use. Two systems of developing solvents for TLC plates were used: hexane: ethyl acetate (6:4) and dichloromethane: ethyl acetate (9:1). TLC patterns were determined under UV light at 254 and 366 nm detectors. The plate was sprayed with a freshly prepared anisaldehyde-sulfuric acid reagent (AS).

2.3 Sample Extraction

Samples of LuL and LuA were ground to 60–80 mesh size with an electric grinder. Each sample was extracted with 95% ethanol in a ratio of 1:10. Sonication was done under an ultrasonic device for 1 hour, separated the clear parts, repeated three times, and then evaporated under pressure.

2.4 Quantitative Phytochemical Determination

1) Determination of total phenolic content **(**TPC**)**: The total phenolic content of the sample was examined by the Folin-Ciocalteu colorimetric method modification [26]. The sample solutions (1 mL) were mixed with 5 mL of the Folin-Ciocalteau reagent (diluted with distilled water in a ratio of 1:10). After 8 min, a sodium carbonate solution (4 mL, 7.5% w/v) was added and incubated in the dark at room temperature for 2 hrs. Finally, the absorbance of the test samples was measured at 765 nm by a Milton Roy Spectronic 21D spectrophotometer. The gallic acid equivalent values (GAE mg/100g) were calculated and compared with the standard curve of gallic acid. All tests were done in triplicate.

2) Determination of Total Flavonoids Content: The extract solution (1 mg/mL in ethanol: water 1:1, 1 ml) was mixed with a 2% $AlCl_3$ solution (1 ml) and kept in the dark at ambient temperature for 25 minutes. The absorbance was determined at 415 nm compared with rutin.

3) Determination of Total Alkaloids Content: The extract solution (0.1 µg /ml in purified water, 1 ml) was mixed with phosphate buffer solution (pH 4.7, 2 ml). The bromocresol green solution (2 ml) was added to the mixture and then extracted with 1, 2, and 2 ml of chloroform. The absorbance was determined at 415 nm by using berberine chloride as a standard.

4) Determination of Total Triterpenoids Content: The extract solution (1 mg/ml in glacial acetic acid, 200 µl) was mixed with a 5% vanillin-acetic acid solution (1 ml) and sulfuric acid (1.8 ml). The sample solutions were allowed to stand at 70°C for 30 minutes and then cooled down to room temperature before adding glacial acetic acid (2 ml). The absorbance of sample solutions was measured at 573 nm by using ursolic acid (Tokyo Chemical, Japan) as a standard.

5) Determination of Total Cardiac Glycoside Content: The extract (1 mg/ml in 50% aqueous ethanol, 1 ml) was mixed with 1 ml of freshly prepared Baljet's reagent (95 mL of 1% picric acid and 5 ml of 10% sodium hydroxide solution). The reaction mixture was incubated for 1 h, then diluted with 2 ml of purified water. The absorbance was quantitatively determined at 495 nm by using digoxin as a standard.

2.5 Biological Activities of LuL and LuA Extract

2.5.1 Determination of antioxidant activity

The *Diphenylpicryl-hydrazyl* (DPPH) radical scavenging assay was used for determination using the method described by Wu et al., 2005 [27]. The solution of DPPH radicals was prepared in methanol (81.2 mM in methanol). The sample solution (1 mL) was mixed with 5 mL of DPPH solution. The mixtures were vigorously shaken and left for 30 minutes in the dark. The absorbance was measured at 517 nm using methanol as a blank. 5 mL of DPPH solution in 5 mL of methanol were used as controls.

Percent inhibition = [(A control-A sample)/A control] x 100. Where A control is the absorbance of only DPPH radical solution, A sample is the absorbance of a sample mixed with DPPH radical solution. The sample concentration providing 50% inhibition (IC50) was calculated from the graph of inhibition percentage against sample concentration.

Antibacterial activity: The antibacterial activity of LuA extact and LuL extact were determined at minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) using the broth dilution method (CLSI, 2018). A single colony of tested bacteria wee inoculated in Mueller-Hinton broth (MHB) and incubated at 37°C for 18 to 24 hours. The extracts were dissolved with dimethyl sulfoxide (DMSO) at a concentration of 500 mg/mL as a stock solution. The MHB (0.1 mL) was dispensed in a sterile 96-well plate. The stock solution of the extract (0.1 mL) was added to the first test well and the extract was serial two-fold diluted in each well in the 96-well plate. The bacterial culture (McFarland standards No. 0.5) was added to all wells. The mixture of extract and bacterial culture were incubated at 37^{o}C for 18 to 24 hours. The MIC was determined from the lowest concentration of the extract not showing any bacterial growth. Therefore, MBC value was determined from each well showing no visible bacteria growth of as compared with the bacterial growth control. The culture broth not displaying bacterial growth was streaked on Mueller-Hinton agar (MHA), and bacteria colonies were determined after incubating. The MBC value was the lowest concentration inhibiting bacterial growth by 99.9% [28].

2.5.2 Identification of the metal ions in the samples

The LuL and LuA dried samples were sent for checking for metal ions at the Central Laboratories (Thailand) Co., Ltd. The analysis was performed based on the EPA 3052 method (to prepare the samples) and the analysis performed using Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES).

3. RESULTS

3.1 Microscopic Identification

The Microscopic characteristics of *Luffa leaves* are shown in Figs. 1-3.

The diagnostic characters are:

1. In surface view, the fragments of the lamina in the upper polygonal epidermis and lower epidermis were wavy in outline. Anomocytic stomata were also present on both surfaces.
2. Palisade mesophyll was usually found in surface view; it is composed of cells with thin walls, circular in outline, containing abundant chloroplasts.
3. The fragments of spongy mesophyll show thin-walled parenchyma containing moderately large chloroplasts with large intercellular spaces and air chambers.
4. The vascular strand was found in various sizes and views, some of which are associated with spongy mesophyll.

5. The fragments of spiral and reticulated vessels in longitudinal view were not very frequent.
6. The occasional fibers could be found in groups or solitary.
7. The occasional glandular trichome appeared as whole trichomes with stalk and head, or fragments of them.
8. The very occasional tracheid fragments in longitudinal view.
9. Starch grains were seldom found and accumulate in parenchymatous tissue.

3.2 Physico-chemical Identification

The samples were tested as in the Thai Herbal Pharmacopeia. The physico-chemical examinations were as follows: Loss on drying, total ash, ethanol-soluble extractive value, and chloroform water extractive value The mean values are presented in Table 1. TLC is shown as in Fig. 4, Fig. 5, Table 2, and Table 3. From the results, the optimum system used in quality control of raw materials, LuL, should be: Solvent system: Hexane: ethyl acetate (6:4), with a UV 366 detector.

3.3 Quantitative Phytochemical Determination

Phytochemical screening of LuL and LuA demonstrated the presence of cardiac glycosides, alkaloids flavonoids, phenolics and triterpenoids as shown in Table 4.

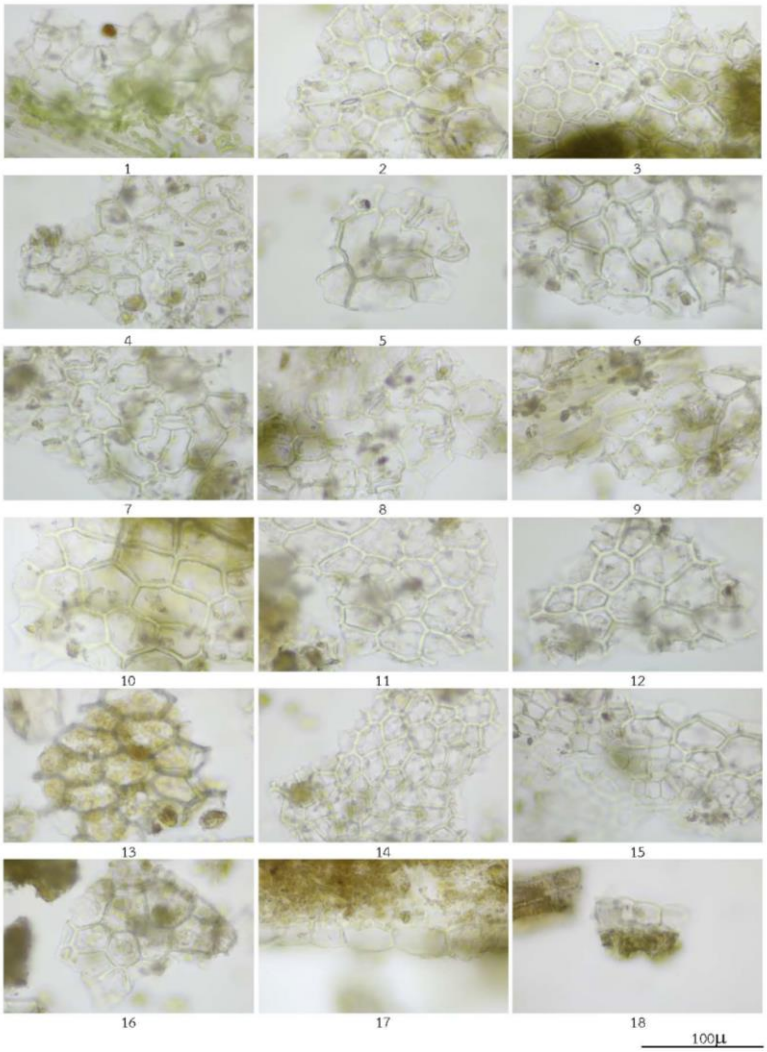

Fig. 1. Powder drug of *Luffa* cylindrica leaf; 1-6 upper epidermis showing stoma, 7-8 lower epidermis showing stoma and wavy epidermis, 10 upper epidermis over vein with stoma, 11-16 upper epidermis with palisade underneath, 17-18 epidermis in sectional view

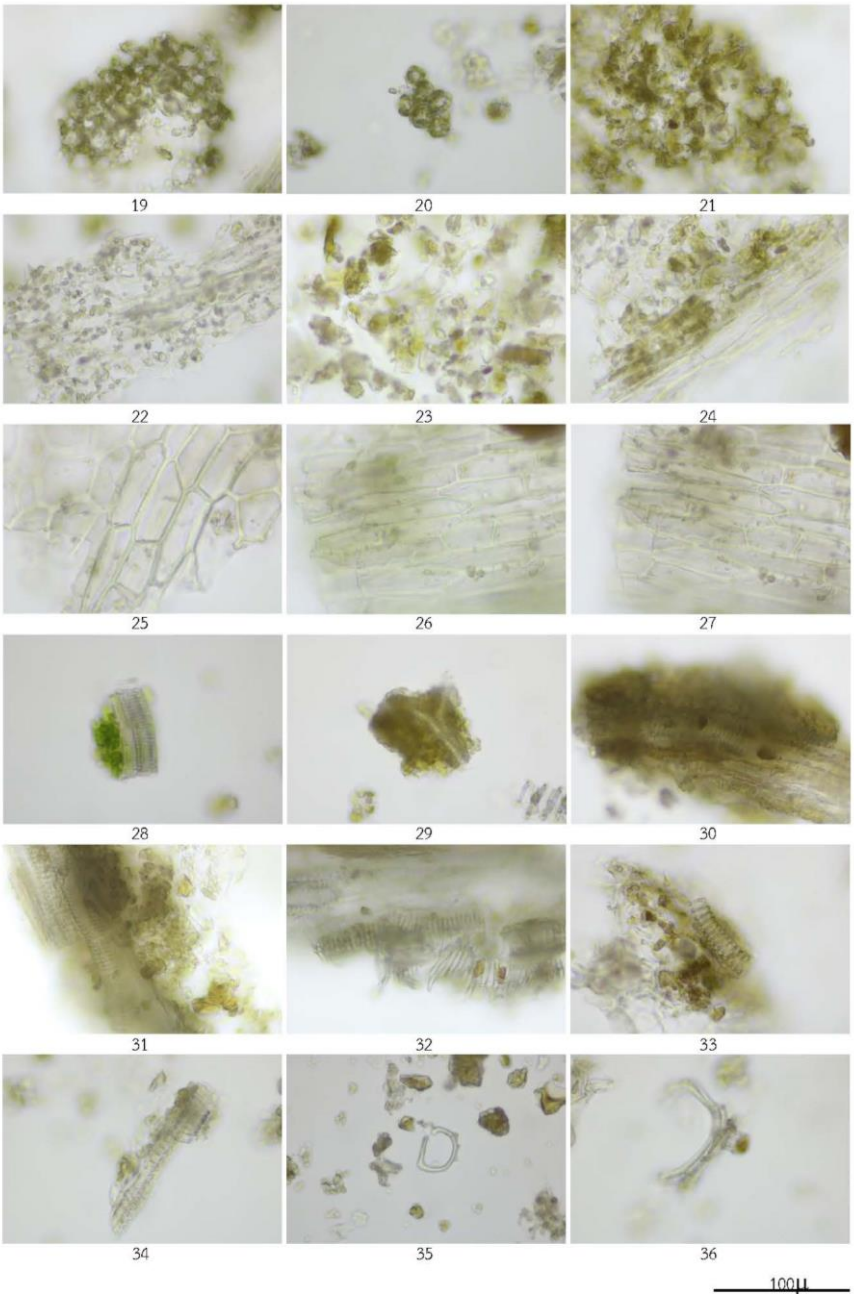

Fig. 2. Powder drug of *Luff* sp. Leaf (continueue); 19-21 palisade in surface view, 22-24 spongy mesophyll, 25-27 polygonal epidermis, 28-29 vascular strand of mesophyll, 30-32 vascular bundle, 33-34 spiral vessel associated with chlorenchyma, 35-36 fragments of vessel

Table 1. Pharmacogenetic characteristic of LuL

Specification	Content (%)
Loss on drying	13.01±0.20 (W/W)
Total ash	21.9144±0.59 (W/W)
Ethanol-soluble extractive value	11.7900±0.17 (W/W)
Chloroform water extractive value	18.3633±0.22 (W/W)

The data represent mean values of three replicates ± SD.
*Thai Herbal Pharmacopoeia 1995 Volume 1 pp.*123, *1*26

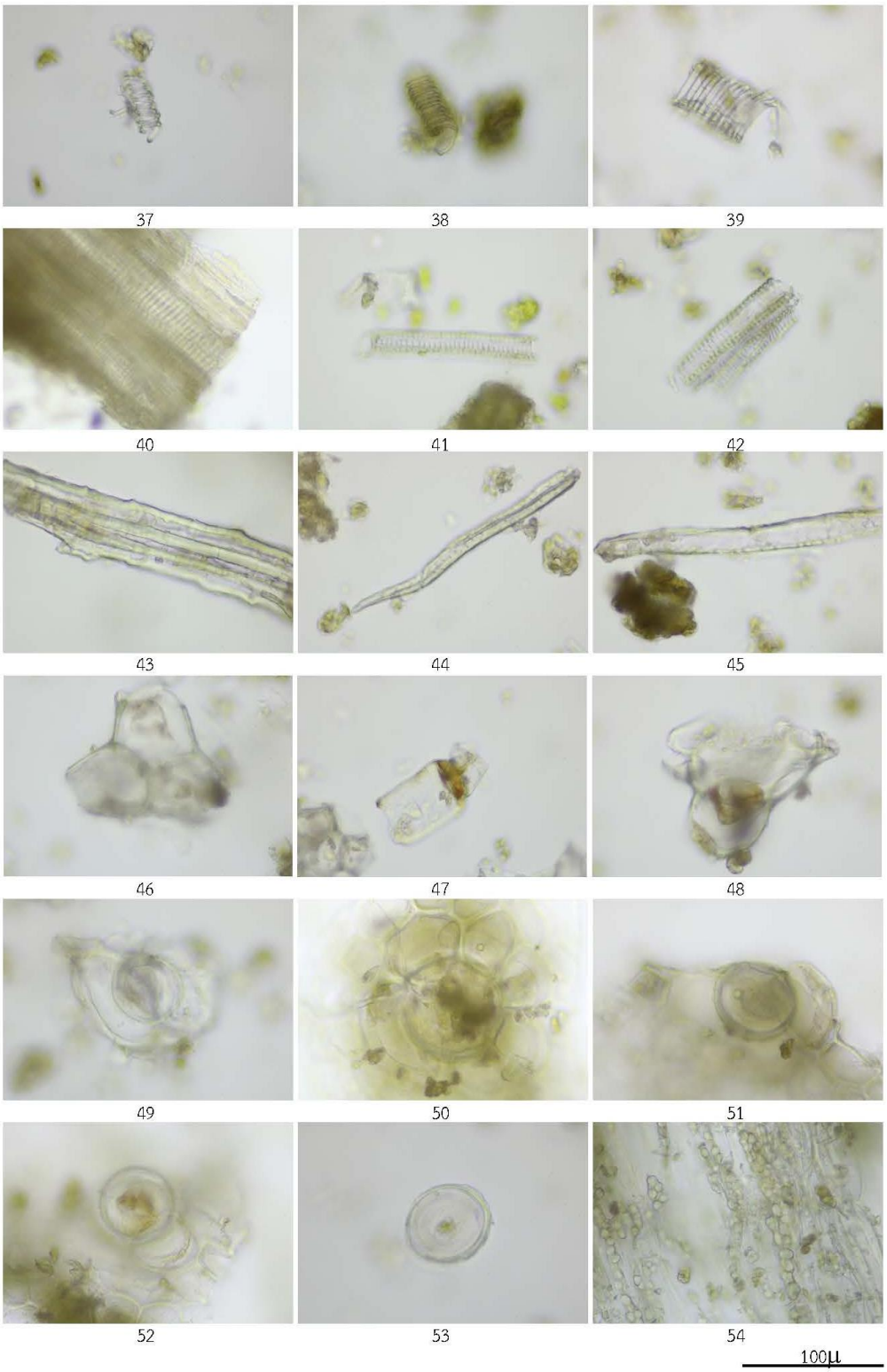

Fig. 3. Powder drug of *Luffa* cylindrica Leaf (continueue); 37-38 spiral vessel, 39 reticulated vessel, 40 xylem element showing reticulated vessel and xylem fiber, 41-42 tracheid, 43 group of fiber, 44-45 solitary fiber, 46-48 trichome stalk, 49-52 capitate stalked trichome in surface view, 53 capitate stalked trichome head, 54 starch grains

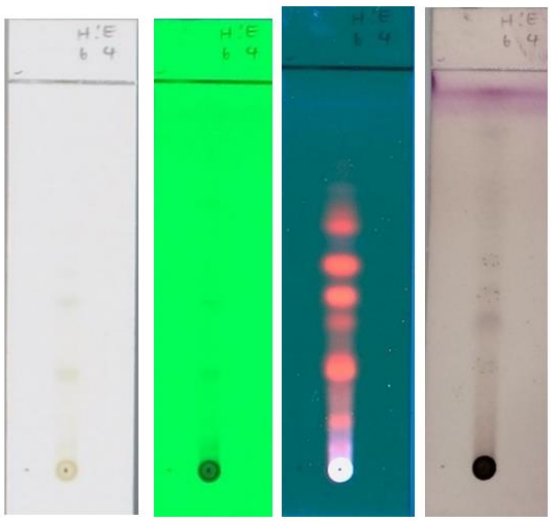

Fig. 4. Sample before (1) and after (2) detected with UV 254 detector, with UV 366 detector (3) and after spraying with Anisaldehyde – sulfuric acid (AS) (4). Solvent system: Hexane: Ethyl acetate (6:4)

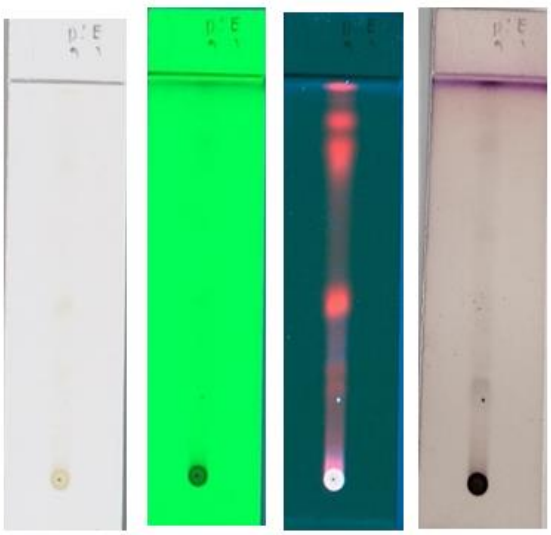

Fig. 5. Sample before (1) and after (2) detected with UV 254 detector, with UV 366 detector (3) and after spraying with Anisaldehyde – sulfuric acid (AS) (4). Solvent system: Dichloromethane: Ethyl acetate (9:1)

Table 2. TLC of LuL extract in Hexane: Ethyl acetate (6:4)

Rf value	Visual inspection	Spot color UV254	Spot color UV366	Spraying with Anisaldehyde – sulfuric acid
0.14	-	-	orange	-
026	opaque	opaque	orange	-
0.36	-	-	orange	purple-gray
0.48	opaque	opaque	orange	-
0.50	opaque	opaque	orange	-
0.60	-	-	orange	-

Table 3. TLC of LuL-extract in Dichloromethane: Ethyl acetate (9:1)

Rf value	Visual inspection	Spot color UV254	Spot color UV254	Spraying with Anisaldehyde – sulfuric acid
0.26	-	-	orange	purple-gray
0.30	-	-	orange	-
0.44	opaque	opaque	orange	-
0.84	-	-	orange	-
0.90	-	-	orange	-
0.98	-	-	orange	-

Table 4. Contents of bioactive compounds referenced to their standards

		Cardiac glycosides		Alkaloids		Phenolics		Flavonoids		Triterpenoids	
Extract No.	Name of the extract	mg digoxin /g extract		mg berberine /g extract		mg gallic acid /g extract		mg Rutin /g extract		mg Ursolic acid/g extract	
		average	SD	average	SD	average	SD	average	SD	average	SD
1	LuA	22.3	0.17	10.7	0.59	16.7	0.24	20.3	1.02	6.9	0.68
2	LuL	63.7	0.75	20.1	0.95	59.0	0.98	47.8	0.31	46.1	0.34

Table 5. MIC and MBC values of LuL extract and LuA extract against pathogenic bacteria

sample	MIC and MBC (mg/ml)					
	S. aureus		*Ps. aeruginosa*		*E. coli*	
	MIC	MBC	MIC	MBC	MIC	MBC
LuL extract	125± 0.0	125± 0.0	125± 0.0	125± 0.0	250± 0.0	250± 0.0
LuA	125± 0.0	125± 0.0	125± 0.0	125± 0.0	125± 0.0	125± 0.0

Data represents mean values of three replicates ± SD

LuA contained secondary metabolites as follows: cardiac glycosides 22.3±0.17 (mg digoxin/g extract), flavonoids 20.3±1.02 (mg rutin/g extract), phenolics 16.7±0.24 (mg gallic acid/g extract), alkaloids 10.7±0.59 (mg berberine/g extract), terpenoids 6.9±0.68 (mg ursolic acid/g extract). LuL contained secondary metabolites as follows: cardiac glycosides 63.7±0.75 (mg digoxin/g extract), phenolics 59.0±0.98 (mg gallic acid/g extract), flavonoids 47.8±0.31 (mg rutin/g extract), triterpenoids 46.1±0.34 (mg ursolic acid/g extract), alkaloids 20.1±0.95 mg (berberine/g extract). The results show that LuA still contained all active compounds but in a lower quantity than sample LuL. This could be explained by the fact that the sample was burned out into ash incompletely.

3.4 Antioxidant Activity of LuA and LuL Extract

The DPPH radical scavenging activities of LuL extract showed an IC_{50} value of 87.63 ± 10.00 mg/ml. or LuL 87.63 mg/ml can remove 50 % of free radical, DPPH. Whereas LuA presented % inhibition 3.17±0.69 (mg/mL) or LuA at a concentration of 1 mg/ml reduce can remove 3.7 % of DPPH ($p < 0.05$).

This phenomenon showed the importance of phenolic compounds in DPPH's radical scavenging activities.

3.5 Antibacterial Activity of *Luffa* Leaf (LuL) Extract and *Luffa* Ash (LuA) Extract

In this study, the inhibitory activities of leaf extract were investigated for the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against pathogenic bacteria, *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Escherichia coli.* by broth dilution method. The result demonstrated that LuL extract revealed an inhibitory effect on all tested bacteria*.* Moreover, LuL extract showed the lowest MIC and MBC values of 125 mg/ml against *Staphylococcus aureus and Pseudomonas aeruginosa,* followed by *Escherichia coli* with MIC and MBC values of 250 mg/ml (Table 5). But LuA extract showed the lowest MIC and MBC values of 125 mg/ml against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. This revealed that LuA extract possessed more effective antibacterial effect on *E. coli* than LuL.

Therefore, LuL and LuA extracts showed strong antimicrobial activity against pathogenic bacteria, which are usually present on the skin. In addition, the extract demonstrated a high content of phenolics and flavonoids that served the antimicrobial activity. Flavonoids are effective both in directly damaging the envelope of Gram-negative and Gram-positive bacteria [29]. Thus, this study implied that the LuL and LuA extracts contained both bacteriostatic and bactericidal effects.

3.6 Metal ion in the LuA and LuL

The results of the metals in the sample were as presented in Table 6. The minerals that were higher in LuL were high in iron, zinc, and copper, but also they were in larger quantities in LuA. Interestingly, a high-level supplement of trace metal not only improved growth performance but also reduced footpad lesions by improving the wound healing process via promotion of collagen synthesis, decomposition and organization, cell migration, matrix remodeling, angiogenesis, and regulation of inflammation [30]. The role of each mineral in wound healing should be the subject of future studies.

Fig. 6. LuA extract at 500 mg/ml in DMSO

Fig. 7. LuL extract at 500 mg/ml dissolve in DMSO

Table 6. Metals in LuL and LuA by Inhouse Method Based on EPA 3052, by ICP-OES Technique

Test Item	Result		Unit
	LuL	LuA	
Arsenic (As)	0.9	2.04	mg/kg
Cadmium (Cd)	0.15	0.29	mg/kg
Copper (Cu)	7.25	25.34	mg/kg
Iron (Fe)	148	1332	mg/kg
Lead (Pb)	1.56	8.83	mg/kg
Mercury (Hg)	Not detected	Not detected	mg/kg
Zinc (Zn)	43.28	189	mg/kg

4. DISCUSSION

Herbal medicines have become a popular form of therapy in developing countries. They are believed to be nontoxic, with little side effects compared to modern drugs. This is in accordance with the common use of *Luffa cylindrica (L.) Roem* in several countries worldwide for the traditional management of diseases. There was evidence from a few pharmacological investigations that *Luffa cylindrica* possessed anti-inflammatory, analgesic, antipyretic, hypoglycemic, antibacterial, antifungal, antiviral, anthelmintic, antioxidant, anticancer, hepatoprotective, antiemetic, wound healing, immunological, bronchodilation, reproductive effect, and in the treatment of cataract [13], [20], [22], [25].

The aim of the present study was to to compare the chemical composition, antibacterial, antioxidant, and wound healing activity of extract from dry leaves (LuL) and the ash dry leaves (LuA) of *Luffa cylindrica* (L.) Roem,through the determination of the physicoal-chemical effect, phytochemical effect, antioxidant activity, antibacterial activity, and metal ion contents.

The present study show that LaH and LuL can be candidated for therapeutic treating of pathogenic skin infection and antioxidation. The determination of the antibacterial activity of *Luffa* Leaf (LuL) extract and *Luffa* ash (LuA) extract revealed that LuA extract was more effective in antibacterial activity than LuL. This result was in accordance with studies by [17], [19], [21], and may be explained by the high content of phenolics and flavonoids of the extract that served the antimicrobial activity. Flavonoids and phenolic compound presented in this study, are component in a variety of medicinal, pharmaceutical, nutraceutical and cosmetic applications. This is attributed to their antioxidative, anti inflammatory, antimutagenic, anticarcinogenic properties and capacity to modulate key cellular enzyme functions [31].

In LuA, more metal ion items were detected, such as arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), mercury (Hg), and zinc (Zn). This is in accordance with the preview study by [24], [30], [32-36] The role of each mineral in wound healing should be the subject of future studies.

5. CONCLUSION

The results of the present study clearly indicated that the crude methanol extract of *Luffa cylindrica* did produce strong antimicrobial activity against pathogenic bacteria on the skin. In addition, the extract demonstrated a high content of phenolics and flavonoids that may serve as antimicrobial agents. Thus, this study implied that the activity of extracts from dry leaves (LuL) and ash-dry leaves (LuA) contained both bacteriostatic and bactericidal effects.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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