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RESEARCH ARTICLE

Phytochemicals, proximate composition, minerals and volatile oil analysis of *Zanthoxylum acanthopodium* DC. fruits

[version 1; peer review: 1 not approved]

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Abstract

Background: The use of herbal plants is adopted as a traditional medicine because of their minimal side effects. Most plants have bioactive ingredients and nutritional content that can potentially be used as treatments. One plant that has the potential to be a source of modern medicine is *Zanthoxylum acanthopodium* DC. Historically the use of traditional medicine as a treatment has enjoyed a good sense of trust among the public. The purpose of this study was to perform a qualitative phytochemical screening and proximate analysis of samples of *Zanthoxylum acanthopodium* DC.

Methods: Used in this study were the phytochemical screening test using the thin-layer chromatography method and the proximate analysis using the AOAC method, which included measuring the ash, water, carbohydrate, total fat, protein, and crude fiber content of the samples. Hydrodistillation was used to isolate volatile oil from the sample, which was then identified using gas chromatography-mass spectrometry. The research method used is experimental.

Results: From the test, it was found that the phytochemical content of *Zanthoxylum acanthopodium* DC is alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and triterpenoids. Proximate analysis obtained ash content of 6.19%, water content of 6.35%, carbohydrates of 35.4%, total fat of 2.46%, protein of 16.2%, and crude fiber of 33.4%. Mineral test results prove that *Zanthoxylum acanthopodium* DC contains Pb (<0.07 mg/Kg), Cd (<0.03 mg/Kg), As (<0.03 mg/Kg), Hg (0.0011 mg/Kg), Mn (43.1 mg/Kg), K (321 mg/Kg), Ca (0.22 mg/Kg), Mg (198 mg/Kg), Fe (52.1 mg/Kg), and Na (23.6 mg/Kg). The highest content of essential oil in *Zanthoxylum acanthopodium* DC is geranyl acetate, with a concentration of 24.26%.

Conclusions: This study concludes that the research findings of *Zanthoxylum acanthopodium* DC indicate that the phytochemical, mineral, and volatile oil content of the sample is strongly related to its potential to be developed as food and medicine.

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Approval Status

1

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Keywords

Zanthoxylum acanthopodium DC, essential oil isolation, essential oil content, proximate analysis, mineral analysis, GS-MS, secondary metabolites, TLC.



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Introduction

Due to their potent antioxidant qualities, lack of side effects, and economic viability, plants have been studied for their medicinal properties in the modern scientific community. In terms of effectiveness and absence of side effects, alternative medicine systems can always be trusted.¹ According to World Health Organization (WHO) statistics, over 50,000 plant species are used in traditional medicines around the world, and about 21,000 plants are employed in alternative medicine. The entire plant or different parts, such as the leaves, stem, bark, root, flower, tuber, and seed, among others, are used to make the drugs.² Over 30% of all plant species have been used for medical purposes at some point in their history. When compared to ethnomedicine's use in practice, conventional medication practice in the treatment of diseases and infections has embraced a more scientific and broad measurement, particularly in developing nations.³

Phytochemicals are just one of the several secondary metabolites that give medicinal plants their therapeutic potential. Recent research on phenolic compounds has highlighted several of their potential medical benefits.⁴ Andaliman's phenolic compounds can help people with diabetes heal burns by making more of a protein called VEGF (vascular endothelial growth factor)⁵ and are able to inhibit the development of free radicals, so many tests are carried out as an antioxidant.⁶ The phenolic mixtures' abilities to scavenge free radicals and lessen lipid peroxidation are linked to their anticarcinogenic, antimutagenic, and cardioprotective effects.⁷

Zanthoxylum acanthopodium DC is also used to treat dysentery. Andaliman has been utilized by Indians to treat leprosy and skin conditions like abscesses and paralysis.⁸ In North Sumatera, notably in North Tapanuli, andaliman has been utilized as a spice.^{7,8} Numerous substances, including phenol hydroquinones, flavonoids, steroids/triterpenoids, tannins, glycosides, volatile oils, alkaloids, coumarins, lignans, amides, and terpenes, are found in plants belonging to the *Zanthoxylum* genus.^{9,10} It has been demonstrated that the andaliman fruit ethylacetate extract (EAF) has cytotoxic effects on the MCF-7 and T47D cell lines. It was discovered that doxorubicin and EAF worked in harmony.¹¹ By a cardioprotective impact and action on T47D resistant cells, EAF was demonstrated to have anticancer activity in mice induced with benzo(a) pyrene.¹¹ Given the aforementioned background, the researchers are interested in performing proximate analyses, secondary metabolite components, and essential oil concentration found in *Zanthoxylum acanthopodium* DC.

Methods

Plant material

The material used in this study was the fresh fruit of *Zanthoxylum acanthopodium* DC. Green-black in color, round in shape, and weighing 5 grams on average, this fruit was collected in Onan Rugu Village, Samosir Regency, North Sumatra Province, Indonesia.

Preparation of simplicia

Andaliman fruit is washed with running water and then dried at room temperature. After drying, it was ground using a blender. The sample was blended until smooth, until it looked like powder, to get *Zanthoxylum acanthopodium* DC powder.⁹

Qualitative phytochemical screening

The identification of secondary metabolites was carried out using the thin-layer chromatography (TLC) method. A total of 100 mg of extract was dissolved in 1 ml ethanol and then smeared on the stationary phase. The stationary phase was a plate coated with silica gel 60 F254 (Merck, Germany) measuring 10x5 cm. The scale was the inserted into a chamber that has been saturated with mobile phase vapor.^{12,13} Phytochemical screening tests can be seen in [Table 1](#).

Isolation of volatile oils

The hydrodistillation method was used to isolate essential oils. The *Zanthoxylum acanthopodium* DC powder was placed in a long-necked flat-bottom flask with distilled water and distilled for 4-5 hours. The essential oil was collected in a separatory funnel, and then the essential oil was separated from the water. 1ml of the anhydrous sodium sulfate was added to the essential oil, shaken, let stand for 1 day, and stored in a dark bottle.¹⁴

Analysis of essential oil components

The determination of essential oil components was carried out using a Gas Chromatograph-Mass Spectrometer (GC-MS; MS). An Rtx-5 MS capillary column (Restek) with a column length of 30 meters, a column diameter of 0.25 mm, an injector temperature of 270 C, and a flow rate of 1.16 ml/minute were used in the analysis. The column temperature was programmed (temperature programming) with an initial temperature of 60 C for 5 minutes, then increased slowly at an increase rate of 5.0 C/minute until the final temperature was 280 C, which was maintained for 30 minutes with the ionizing electron impact (EI) type.

Table 1. Qualitative phytochemicals analysis with TLC.

Compound	Mobile phase	Solvent comparison	Detected	Result
Alkaloids	Chloroform: methanol: ammonia	(85:15:1)	Dragendorff's	Orange stain
Flavonoids	Ethylacetate-methanol-water	(100:13,5:10)	AlCl ₃ 10%	Yellowish-orange or greenish-yellow stains
Saponins	Chloroform-acetic acid-methanol-water	(11:6:2:1)	Methanol, sulfuric acid and vanillin	Yellow-brown stain
Tannins	Chloroform-ethylacetate-n-butanol-water	(5:2:2:1)	FeCl ₃ 10%	Dark blue stain
Glycosides	Ethylacetate-methanol-water	(16:2:2)	H ₂ SO ₄ 50%	Dark green stain
Steroids/Triterpenoid	n-hexane-ethylacetate	(8:2)	Lieberman Burchard reagent	The stain is purplish red

The way to identify essential oil components is to compare the mass spectra of the essential oil components obtained with the mass spectra in the data library that have the highest similarity index.¹⁰

Proximate analysis

The AOAC method was used to identify to quantify the amounts of ash, water, carbs, total fat, protein, and crude fiber.¹⁵ used toy

Water content

The gravimetric method was used to calculate how much water was in the sample. The sample was weighed until the weight stayed the same, which meant that all the water in the sample had evaporated. The procedure for analyzing the water content is that the cup to be used is dried beforehand at a temperature of 100–105°C for 30 minutes. Then it was dried in an oven at 100–150 C for 6 hours and cooled again in a desiccator for 30 minutes.¹⁶

Analysis of ash

The ashing method was used to determine the ash content. The principle of this analysis is to burn the material or incinerate it at a high temperature (about 350 °C) and weighing the substance that remains after the ashing.¹⁷

Protein content

Protein content was determined using 0.1 g of powder placed in a 100 ml Kjeldhal flask. 2 ml of H₂SO₄ 98% was added and 0.9 g selenium as a catalyst, and allowed to digest for 60 minutes, after which a sample was taken. Distillation was then carried out. The distillate obtained was put in an Erlenmeyer flask containing 15 ml of acid solution, borate 4%, and a mixed indicator (2-3 drops of methyl red and methyl blue). The mixed distillate is titrated with a standard solution in the form of 0.02 N HCl, until a light purple color is obtained.¹⁸

Lipid content

A total of 5 g of sample was put in a lead filter, then covered with fat-free cotton wool. Lead containing samples were mounted on a soxhlet extraction unit. The weight is filled with sufficient petroleum benzene (30 ml), then installed on the extraction tool. After the extraction is complete (5 hours), the flask is heated in the oven. It is then put in desicator until all of benzene has evaporated.¹⁹

Carbohydrate content

An analysis of the carbohydrate content was carried out using a different method in the calculated proximate analysis. Using the formula = 100% -(moisture content + ash content + fat content + protein content), so that the carbohydrate content of the material is obtained.²⁰

Mineral analysis

NaCl and KCl were used as standards for the mineral analysis, which was done using a flame photometer (Model 405 Corning, UK), after which the sample's mineral content was checked. For all other metals in the sample, an atomic absorption spectrophotometer (Pekin-Elmar Model 403, Norwalk, CT, USA) was used. The determination of each

mineral was done twice. All compounds are of analytical grade (BDH, London). Limits for metal detection have previously been established using Techtron. The optimal analytical range has a variable coefficient of 0.87 to 2.20% and is between 0.1 and 0.5 absorbance units. All estimated values are provided as a percentage, even though minerals are reported as milligrams per 100 grams.²¹

Results

Phytochemical composition of *Zanthoxylum acanthopodium* DC

The retention factor (Rf) value is the value or size determined based on the position of the stain on the dissolved substance in thin-layer chromatography. The result of the stain produced by the TLC plate is then computed as the Rf.^{22,23} Table 2 below shows the findings of the phytochemical analysis of *Zanthoxylum acanthopodium* DC and the resulting Rf values. According to the analyzed metabolites, stains with different colors were produced by identifying phytochemical substances

Alkaloid compound identification by the TLC method employing chloroform's mobile phase

After spraying with dragendroff, methanol: ammonia (85:15:1) appears as a green stain and yields three stains with Rf values of 0.12, 0.2, and 0.72. The utilized mobile phase has a unique polarity. As a result of chloroform having a higher ratio than methanol and ammonia, the mobile phase is more likely to be non-polar. While substances with higher Rf values are more disseminated in the mobile phase, those with lower Rf values are more distributed in the stationary phase. Because they are more strongly held in the stationary phase compared to compounds with higher Rf values, compounds with lower Rf values are more polar and have more significant distribution coefficients.^{24,25}

Using ethyl acetate to identify flavonoid compounds

One yellow-orange stain with an Rf value of 0.506 was formed by the mobile phase of ethyl acetate: methanol: water (100:13,5:10). Flavonoids are chemicals that are soluble in polar solvents, and when they are separated using mobile phases with semi-polar and polar solubility qualities, the presence of flavonoid molecules in the sample is increasingly evident²⁶

Identification of saponin compounds by TLC using the mobile phase Chloroform: acetic acid; methanol: water (11:6:2:1). The results showed 2 brownish yellow spots with Rf values of 0.41 and 0.49 after being sprayed with methanol: sulfuric acid reagent: vanilla.¹²

Identification of tannin compounds using the mobile phase of Chloroform-Ethylacetate-n-butanol-Water (11:6:2:1) and 10% FeCl₃ stains gave the results of 2 blackish green spots with Rf values of 0.74 and 0.9. This proves that the mobile phase used has the same polarity as tannin, so tannin can be used as an eluent to separate tannin compounds^{27,28}

Identifying glycoside compounds using the TLC method depends on the mobile phase of ethyl acetate: methanol: water (16: 2: 2) produces 2 stains with Rf 0.2 and 0.62 after spraying with 50% sulfuric acid, giving a brown color.¹²

Identification of steroid/triterpenoid compounds by TLC using the mobile phase n-hexane: ethylacetate (8:2) resulted in 2 stains with a purplish-red color, confirming the presence of steroid/triterpenoid compounds.²⁹

Table 2. *Zanthoxylum acanthopodium* DC secondary metabolite screening results.

Compound	Result	Rf
Alkaloids	+	Rf ₁ : 0.12 Rf ₂ : 0.20 Rf ₃ : 0.72
Flavonoids	+	Rf ₁ : 0.51
Saponins	+	Rf ₁ : 0.41 Rf ₂ : 0.49
Tannins	+	Rf ₁ : 0.74 Rf ₂ : 0.96
Glycosides	+	Rf ₁ : 0.74 Rf ₂ : 0.96
Steroids/Triterpenoid	+	Rf ₁ : 0.38 Rf ₂ : 0.45

Essential oil analysis

The results of the analysis of essential oils obtained from the gas chromatography–mass spectrometry (GS-MS) chromatogram contained 57 peaks, and six main components were taken based on the highest concentration. The chromatogram results can be seen in [Figure 1](#). The results of the GS-MS analysis showed six main components, namely: geranyl acetate (24.26%, $C_{12}H_{20}O_2$, m/z 196.29), 1-limonene (20.79%, $C_{10}H_{16}$, m/z 136.24), citronellol (9.72%, $C_{10}H_{20}$, m/z 156.27), geraniol (7.32%, $C_{10}H_{18}O$, m/z 154.25), 1,3,6-octatriene (2.67%, C_8H_{12} , m/z 108.18), and beta-ocimene (2.92%, $C_{10}H_{16}$, m/z 136.23). The physiology of the entire plant is so intertwined with essential oil products that it is reliant on metabolic conditions and established developmental differentiation programs of the produced tissues.³⁰ The essential oil content contained in *Zanthoxylum acanthopodium* DC can be seen in [Table 3](#).

Proximate analysis

Measurements of the ash content, water content, carbs, total fat, and protein were made using proximate analysis. Proximate measures are used to determine the nutritional value of a food product, food ingredient, or food. Analyzing the nutritional value of *Zanthoxylum acanthopodium* DC will help determine whether it has the potential to be used as a food additive and a therapy. [Table 4](#) displays the findings of the close examination of *Zanthoxylum acanthopodium* DC. Substance impacts the texture and nutritional formation of the food generated. Bacteria, molds, and yeast can easily thrive in high water content environments and produce changes in the sample's physical or chemical composition.

Mineral analysis

The results of the mineral analysis test contained in the *Zanthoxylum acanthopodium* DC sample can be seen in [Table 5](#) below. The table shows that the mineral content in the *Zanthoxylum acanthopodium* DC sample is Lead (Pb), Cadmium (Cd), Arsenic (As), Mercury (Hg), Manganese (Mn), Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe) and Sodium (Na).

Discussion

The fact that *Zanthoxylum acanthopodium* DC was found to contain phytochemicals such alkaloids, tannins, steroids/ triterpenoids, flavonoids, saponins, and glycosides supports its usage as a medicinal plant. These secondary plant metabolites' therapeutic capabilities have also been described by a number of writers.^{31–34} There is evidence that alkaloids can stimulate the central nervous system.³⁵ The antiviral, antifungal, antibacterial, and anticancer effects of tannins are well documented. They offer a variety of pharmacological qualities that have been linked to wound healing,

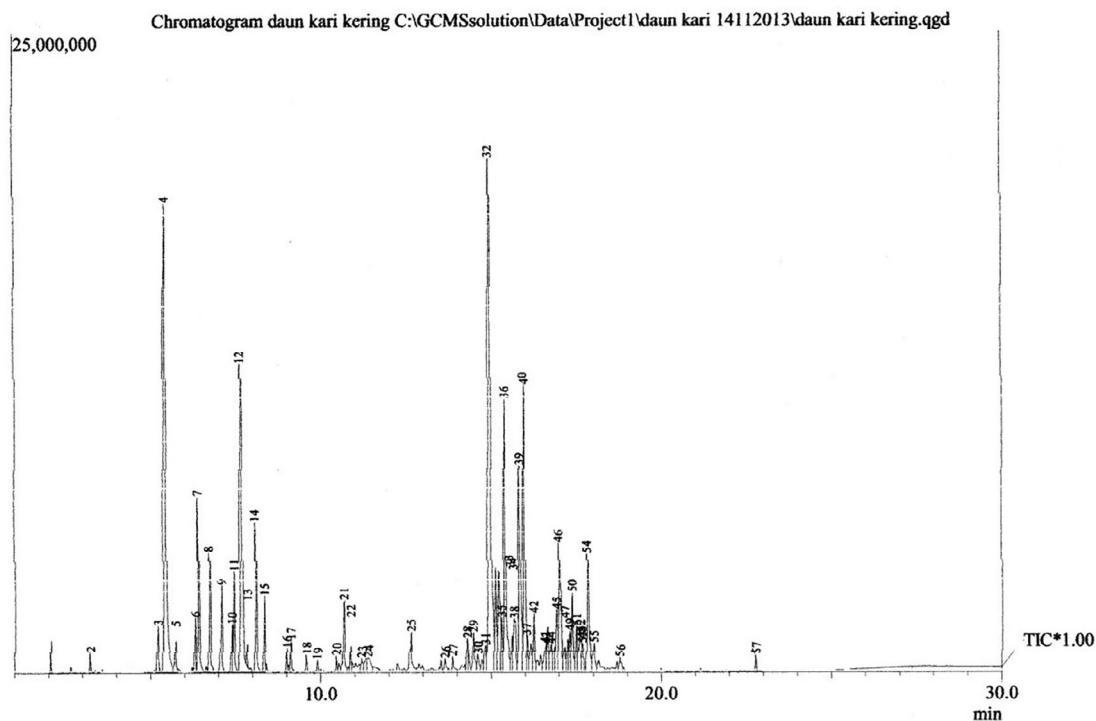


Figure 1. Chromatogram of essential oil of *Zanthoxylum acanthopodium* DC.

Table 3. Essential oil content in *Zanthoxylum acanthopodium* DC.

Compound	Rate (%)
Geranyl acetate	24.26
1-limonene	20.79
Citronellol	9.72
Geraniol	7.32
Beta-citronellol	2.92
1,3,6-octatriene, beta ocimene	2.67
Citral	2.65
Linalool	2.42
Citronellyl acetate	2.41
1-dodecanol	2.14
Alpha-pinene	2.13
2-propenoic acid	2.11
2,6-octadienal	1.86
Dodecyl ester	1.62
Germacrene	1.54
Caryophyllene	1.22
Beta-myrcene	1.14
Spiro[5,5] undec-2-ene	0.87
Farnesyl acetate	0.85
6-octenal	0.70
Spathulenol	0.68
D:A-friedooleanan	0.66
Tridecanal	0.66
Oxirane	0.63
1,6,10-dodecatriene	0.63
Isopulegol	0.60
9,19-cyclolanost-24-en-3-ol	0.55
Alpha terpineol	0.53
Alpha cadinol	0.51
2,6-octadienoic acid	0.49
2-beta-pinene	0.43
Dodecanoic acid	0.42
(-)-caryophyllen oxide	0.42
Delta-cadinene	0,41
1-tetradecanol	0.38
1,6-octadiene-3-ol	0.37
p-menthan-8-ol	0.3

including anti-inflammatory, analgesic, and antioxidant activities.^{34,36} The antibacterial properties of tannins are applied through breakdown of membranes, protein binding, adhesion, and inhibition of enzymes.³⁷ According to reports, detected steroids and triterpenoids exhibit anti-bacterial, antiviral, antimalarial, and anti-cholesterol synthesis inhibiting activities.^{35–37} Antibacterial activities of steroids and triterpenoids have been documented.^{38,39} They have long been used medicinally, and one of its common biologic characteristics is cytotoxicity.⁴⁰ Flavonoid content in *Zanthoxylum*

Table 4. Proximate analysis data of *Zanthoxylum acanthopodium* DC.

Parameter	Result (%)
Ash content	6.19
Water content	6.35
Carbohydrate	35.4
Total fat	2.46
Protein	16.2
Coarse fiber	33.4

Table 5. *Zanthoxylum acanthopodium* DC mineral analysis data.

Parameter	Result
Lead (Pb) (mg/Kg)	<0.07
Cadmium (Cd) (mg/Kg)	<0.03
Arsenic (As) (mg/Kg)	<0.03
Mercury (Hg) (mg/Kg)	<0.0011
Manganese (Mn) (mg/Kg)	43.1
Potassium (K) (mg/Kg)	321
Calcium (Ca) (%)	0.22
Magnesium (Mg) (mg/Kg)	198
Iron (Fe) (mg/Kg)	52.1
Sodium (Na) (mg/Kg)	<0.07

acanthopodium DC has the potential as an antioxidant. Flavonoid can act as antioxidants with the mechanism of chelating or scavenging.^{41,42} Saponins also detected were known to have the properties of precipitating and coagulating red blood cells.⁴¹ Some characteristics of saponins include foam formation in aqueous solutions, hemolytic activity, cholesterol-binding properties and bitterness.^{43,44} Sodipo *et al.* (2000) reported that saponins lower cholesterol levels, act as immune boosters and are anti-carcinogenic.⁴³ However, it was also reported that high levels of saponins could cause gastroenteritis.⁴⁵ There is a low amount of saponins in *Zanthoxylum acanthopodium* DC, which confirms that its consumption will not harm the human body. In addition, according to many reports, *Zanthoxylum acanthopodium* DC fruits contain glycosides known to lower blood pressure.⁴⁶

In addition to the metabolite content, the results of this study also showed that *Zanthoxylum acanthopodium* DC contains essential oils. Essential oils are part of a group of volatile compounds and give off a characteristic odor. The most significant ingredients contained in *Zanthoxylum acanthopodium* DC are essential oils of geranyl acetate (24.26%) and 1-limonene (20.79%). The compounds -myrcene, (z)- β -ocimene, linalool, -citronellol, neral, geraniol, geranyl acetate and sesquiterpenoids contribute to the distinctive aroma of *Zanthoxylum acanthopodium* DC. *Zanthoxylum acanthopodium* DC essential oil has potential in vitro cytotoxic effects on T47D breast cancer cells.^{9,10} Essential oil compounds in *Zanthoxylum acanthopodium* DC can also inhibit the growth of bacteria.⁴⁷

The results of the proximate analysis also show that *Zanthoxylum acanthopodium* DC has a sample moisture content (of 6.35%). The water content value of this sample still meets the Standard National Indonesia (SNI) No 01-2891-1992 criteria even though it is lower than the range of 15-24% allowed by SNI.^{48,49} The ash content of the sample is 6.19%, a measure used to determine the number of minerals present in the sample.^{50,51} Ash content is a metric that indicates the purity of a sample and is affected by its composition and mineral content. The total fat value is 2.46%, the protein value is 16.2%, and the carbohydrate value is 35.4%. A good source of protein was defined as having a protein content that exceeded 12% of its caloric value, and a crude fiber content of 33.4% sample was high enough to meet the recommended daily allowance of fiber.^{52,53} Carbohydrates and crude fiber, such as lignin and pectin, are the two substances in the samples of *Zanthoxylum acanthopodium* DC in the highest concentrations. Total fat is the least abundant element in *Zanthoxylum acanthopodium* DC.

Every herbal plant provides health benefits because it has nutritional value. *Zanthoxylum acanthopodium* DC is detected to contain minerals such as Lead (Pb), Cadmium (Cd), Arsenic (As), Mercury (Hg), Manganese (Mn), Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe) and Sodium (Na). Minerals are essential for the proper functioning of tissues and act as secondary messengers in several biochemical cascade mechanisms.^{54,55} For example, calcium is an important mineral content found in plants because of its many roles in the life of living things. The content of magnesium plays a role in the activity of manganese (Mn), which is considered an antioxidant nutrient and breaks down bile, thereby reducing the risk of cholesterol. Lack of calcium in the body can cause several disorders, for example, inhibiting growth.^{1,10,53} Potassium in *Zanthoxylum acanthopodium* DC plays an essential role in diuretics and regulates cardiac function, water, and ion balance in blood and tissues.^{56,57} Calcium content is well known for the growth and maintenance of bones, teeth and muscles.^{58,59} In addition, calcium is helpful in the formation of blood and intracellular and extracellular fluids inside and outside the body's cells.⁶⁰ The iron content is also essential. Iron plays a vital role in erythropoiesis and many intracellular oxygen transport reactions. It facilitates the oxidation of carbohydrates, proteins and fats.^{61–63} Sodium and potassium are needed to maintain the osmotic balance of body fluids and body pH, regulate muscle and nerve irritability and control glucose uptake.⁶³

Conclusions

The study's findings by *Zanthoxylum acanthopodium* DC demonstrated that the sample's phytochemical, mineral, and essential oil content is strongly associated with its potential to be developed as food and medicine. Every plant must include the biological substances required to serve as metabolites for creating novel medications and foods, so that more comprehensive studies on the effectiveness and compound interactions of these compounds' contents might be developed.

Data availability

Underlying data

Zenodo: The dataset of 'Phytochemicals, Proximate Composition, Minerals and Volatile Oil Analysis of *Zanthoxylum acanthopodium* DC. Fruits'. <https://doi.org/10.5281/zenodo.7355506>.⁶⁴

This project contains the following underlying data:

- Table 1. Qualitative Phytochemicals analysis with TLC.xlsx
- Table 2. *Zanthoxylum acanthopodium* DC secondary metabolite screening results.xlsx
- Table 3. Volatile oil data of *Zanthoxylum acanthopodium* DC.xlsx
- Table 4. Essential oil content in *Zanthoxylum acanthopodium* DC.xlsx
- Table 5. Proximate analysis data of *Zanthoxylum acanthopodium* DC.xlsx

Zenodo: Picture of TLC result of *Zanthoxylum acanthopodium* DC secondary metabolite and Chromatogram of essential oil of *Zanthoxylum acanthopodium* DC. <https://doi.org/10.5281/zenodo.7355669>.⁶⁵

This project contains the following underlying data:

- Figure 1. Picture of TLC result of *Zanthoxylum acanthopodium* DC secondary metabolite.png
- Figure 2. Chromatogram of essential oil of *Zanthoxylum acanthopodium* DC.jpg

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

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[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
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Thanks for inviting me to review the manuscript "Phytochemicals, proximate composition, minerals and volatile oil analysis of *Zanthoxylum acanthopodium* DC. Fruits"

The idea of the manuscript is fine but its not suitable to be indexed in the current form for international readers. The manuscript needs complete overhauling for English grammar and language. The sentences are written very poorly. The arrangement of the paragraphs is not up to the mark. Here are few examples given below, just for guidance, to improve the manuscript:

1. Rewrite the background. Give background about *Z. acanthopodium* traditional use.
2. Author starts the method section in abstract with 'Used'. Please write as 'The method used...'
3. Please proofread and rewrite the whole abstract again.

Keywords: "Zanthoxylum acanthopodium DC, essential oil isolation, essential oil content, proximate analysis, mineral analysis, GS-MS, secondary metabolites, TLC" - Don't repeat words in keywords like essential oil. GSMS? Rewrite it.

No voucher specimen of plant is mentioned.

Methodologies are written incomplete like the section 'lipid content.'

Write in methodologies under 'proximate analysis' how you measured the contents. Like proteins, lipids, carbohydrates etc.

What is GSMS?

What is Rate% in table 3?

Decision: Major revision required.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pharmacognosy, pharmacy, natural products, Quality control of herbal drugs

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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