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Original Article

# Potential of Ethanolic Extract from Ripe *Musa balbisiana* Colla Fruit Using Ultrasound-Assisted Extraction as An Antioxidant and Anti-Gout

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**ABSTRACT**

Plant extracts or their secondary metabolites have functioned as antioxidants in phytotherapy drugs which function as protection against various diseases related to oxidative stress and free radicals. Free radicals play an important role in the initiation and development of various diseases, one of which is uric acid. Hhis study aims to obtain ethanolic extract from the ripe fruit of *Musa balbisiana* Colla using the UAE method and obtain information about secondary metabolites and their bioactivity as antioxidants and anti-gout. The results showed that antioxidant activity test using the DPPH and FRAP methods gave $IC_{50}$ values of 150.24 ± 0.0348 mg/L and 227.80 ± 0.0986 mg / L, respectively. The total phenolic content value of 625.64 ± 0.36 mg GAE/g ethanolic extract is thought to have a role in high antioxidant activity. In addition, ethanol extract with a concentration of 50 mg / L has activity in reducing uric acid levels by around 9%. It can be concluded that the ethanolic extract produced by UAE has potential as a source of anti-oxidants and anti-gout.

**Key words**: Anti-gout, Antioxidant, *Musa balbisiana* Colla, Phenolic content, UAE.

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## INTRODUCTION

Plant extracts or their secondary metabolites have functioned as antioxidants in phytotherapy drugs which function as protection against various diseases related to oxidative stress and free radicals [1]. Free radicals mainly include reactive oxygen species (ROS) such as hydroxyl radicals, peroxyl radicals, super oxide radicals, hydrogen peroxide, singlet oxygen, and various lipid peroxides [2]. ROS is also capable of reacting with membrane lipids, proteins, nucleic acids, various metabolic enzymes, and small molecules from living systems. Free radicals play an important role in the initiation and development of various diseases such as atherosclerosis, cardiovascular disease, aging, respiratory disease, cancer, and gout [3].

Gout is a condition in which uric acid levels in the blood increase and become saturated. Gout develops due to the deposition of uric acid in the form of urate monohydrate crystals in the synovial joint during catabolism of purines by xanthine oxidase [4]. The enzyme xanthine oxidase (XO) catalyzes the metabolism of hypoxanthine to xanthine, and xanthine to uric acid, which is responsible for a medical condition that causes painful inflammation called gout [5]. XO also serves as a biological source of oxygen-derived free radicals which contribute to oxidative damage to living tissue which is involved in many pathological processes such as inflammation, atherosclerosis, cancer, and aging. This requires the search for new, safer drugs for humans. In-vitro bioassays are used to check the test material for XO inhibition, because XO inhibitors may be potentially useful for the treatment of gout or other XO-induced diseases [6].

Allopurinol as a specific inhibitor of the xanthin oxidase (XO) enzyme has been shown to be effective in reducing uric acid levels. The side effects of allopurinol are predominantly rash and fever, however allopurinol hypersensitivity syndrome (SHA) can be life threatening, which occurs in about 0.1% of SHA cases [7].

Research on the inhibitor of xanthine oxidase activity has been carried out on various medicinal plants that have the potential as an anti-gout drug. Research on methanol extracts of *Cinnamomum cassia, Rysanthemum indicum*, and *Lycopus opaeus* had xanthine oxidase inhibiting activity greater than 50% [8]. The 6-aminopurin compound derived from wheat leaves has a strong inhibitory power with an IC value of 10.89 µM [9]. The ethyl acetate extract of the inner seed coat of *Archidendron bubalinium* (Jack) IC Nielsen has high potential as anti-gout [10].

Indonesia has a large number of native and exotic fruit species that have not been exploited, such as the kluthuk banana (*Musa balbisiana* Colla). It contains flavonoids, polyphenols, tannins, monoterpenoids and sesquiterpenoids, quinones, and saponins. Kusuma *et al* [11]., reported that the antibacterial activity of the ethanol extracts of *Musa balbisiana* Colla against *S. Dysentery* because of the antibacterial content of secondary metabolites, especially flavonoids. The fresh ripe pulp of *Musa balbisiana* Colla fruit has anti-oxidative and antioxidant properties that can prevent oxidative stress-related diseases. Revadigar *et al* [12]., studied that the ethanolic extract of the inflorescence of *Musa balbisiana* Colla possesses moderate antioxidant activity.

The antioxidant activity found in *Musa balbisiana* Colla may be related to anti-gout activity, where

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there is a role for antioxidants in the inhibition of xanthine oxidase activity [13]. However, there has been no research on the antioxidant and anti-gout activity of the ripe *Musa balbisiana* Colla fruit, it is necessary to conduct a research on the anti-gout activity test of the ethanolic extract of the ripe *Musa balbisiana* Colla fruit obtained using the Probe Type Ultrasound-Assisted Extraction (UAE) method.

Common extraction procedures for the isolation of organic compounds from natural materials are hydrodistillation, maceration, and low pressure solvent extraction (LPSE). However, this technique usually requires a long extraction time and has low efficiency. Recently, the UAE which is the "Clean Technology" in the food industry, has been the subject of a lot of research and development. This new technology also considers sustainable environmental aspects (environmentally friendly). UAE technology is an effective method for the extraction of chemical constituents from plant materials. Extraction can be done in a shorter time than other extraction techniques, costs less and suggests that this method could potentially be used in the extraction of thermally sensitive materials used in food, health products, cosmetics and pharmaceuticals [14].

Thus far, no reports were found regarding the extraction of secondary metabolites from the ripe fruit of *Musa Balbisiana* Colla using the UAE method. Therefore, this study aims to obtain ethanolic extract from the ripe fruit of *Musa Balbisiana* Colla using the UAE method and obtain information about secondary metabolites and their bioactivity as antioxidants and anti-gout.

# METHODS

## Simplicia Set Up

The part of the plant used for the study was the ripe fruit of *Musa Balbisiana* Colla. Ripe fruit was peeled, then the pulp was separated from the seeds. The pulp was mashed and then dried in an oven at 45°C. Simplicia powder was stored separately in dry, closed, identified containers, and protected from direct sunlight until extraction was carried out.

## Extraction of Simplicia

The simplicia powder of rock banana fruit was weighed as much as 20 g, then added 150 mL of ethanol pa solvent. The mixture was sonicated using a vibrating ultrasonic probe for 30 minutes at room temperature with an amplitude of 0.6 m.

## Phytochemical Screening

Phytochemical screening using the Ciulei method [15] was carried out on the crude ethanolic extract. Phytochemical screening tests carried out included tests for alkaloids, flavonoids, phenols, saponins, tannins, glycosides, and sterols-triterpenoids.

## Total Phenolic

The total phenolic content of the extract was determined by the Folin–Ciocalteu method. Briefly 400 µL of crude extract (1 mg/mL) that was made up to 6 mL with distilled water, mixed thoroughly with 1 mL of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2,5 mL of 10% ( w/v) sodium carbonate, measured with distilled water in a 10 mL measuring flask, then homogenized. The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve of gallic acid (concentration 0, 2, 4, 6, 8 mg/L), and the results were expressed as mg of gallic acid equivalent per g dry weight ([16], methodes with modification).

## DPPH Method Antioxidant Activity Test

Amount of 5 mg of the crude extract was dissolved with methanol pa in a 5 mL measuring flask, resulting in a sample solution with a concentration of 1,000 mg/L. Solution pipette 40 µL; 80 µL; 160 µL; 320 µL; 640 µL, then each was put into five 5 mL measuring flasks, then added 1 mL of DPPH 39 mg / L solution, then measured with methanol pa, and homogenized (sample concentrations 8, 16, 32, 64, 128, and 256 mg / L). The solution was incubated for 30 minutes at room temperature (25°C), then the absorption of the solution was measured using a visible light spectrophotometer at a wavelength of 516 nm. The process was carried out in two repetitions. The same operation was carried out on the BHT comparators by pipetting 10 µL; 20 µL; 40 µL; 80 µL; 160 µL BHT solution 1,000 mg / L (BHT concentrations 2, 4, 8, 16, and 32 mg / L).

Antioxidant activity was measured as a decrease in DPPH solution uptake due to the addition of sample. The absorption value of the DPPH solution on the sample is called the percent inhibition (% inhibition) with the following equation:

$$\%\ Inhiibition = \frac{\left(A_{blank} - A_{sample}\right)}{A_{blank}} x100\%$$

Explanation:

$A_{blank}$ = Absorbance without sample

$A_{Sample}$ = Absorbance of sample at t minutes

The calculated value is entered into a linear equation (Y = aX + b) with the concentration ppm (mg / L) as the abscissa (X axis) and the% inhibition value as the ordinate (Y axis). The $IC_{50}$ value is obtained from the calculation when the % inhibition is 50% ([17], methods with modification).

## Antioxidant Activity Test of the FRAP Method

A total of 5 mg of the extract was dissolved with methanol pa in a 5 mL measuring flask, resulting in a sample solution with a concentration of 1,000 mg / L. Each of solution pipette of 40 µL; 80 µL; 160 µL; 320 µL; 640 µL; 1280 µL, was put into five 5 mL measuring flasks, then added 0.4 mL of 0.001 M citric acid; 0.2 mL of $Fe^{3+}$ 0.002 M solution; 0.4 mL o-phenanthroline 0.2%, then filtered with distilled water, and homogenized (sample concentrations 8, 16, 32, 64, 128, and 256 mg / L). The solution was incubated for 35 minutes at 37°C, then the solution absorption was measured using a visible light spectrophotometer at a wavelength of 510 nm. The process was carried out in two repetitions. The same operation was carried out with a comparator of gallic acid with a concentration of 0.5; 1.0; 1.5 mg / L).

Reducing activity can be calculated with the following equation:

$$\%\ \text{Reducing Power} = \frac{A_{Sample} - A_{Blank}}{A_{Sample}} \text{ x } 100\%$$

Explanation:

$A_{blank}$ = Absorbance without sample

$A_{Sample}$ = Absorbance of sample at t minutes

The calculated value is entered into a linear equation (Y = aX + b) with the concentration ppm (mg / L) as the abscissa (X axis) and the% value of the reduction as the ordinate (Y axis). The $IC_{50}$ value is obtained from the calculation when the% reduction is 50% ([18], methods with modification).

### Uric Acid Test

A total of 5 mg of extract was dissolved with methanol pa in a 5 mL measuring flask, resulting in a sample solution with a concentration of 1,000 mg/L. The solution was piped 40 µL, then put into a 5 ml measuring flask, added 40 µL standard of uric acid 6 mg/dL and let stand for 5 minutes. The solution was added with 0.25 mL of reagent 1 and allowed to stand for 5 minutes, then 62.5 mL of reagent 2 was added, allowed to stand for 30 minutes at 20-25 °C. The solution was measured using a visible spectrophotometer at 513 nm absorption. The same treatment was carried out on uric acid standards, allupurinol as a positive control, and blanks ([10], methods with modification).

Uric acid (mg / dL) = Sample absorption x standard concentration (mg / dL)

Standard absorption

Uric acid activity = Standard Concentration - Uric Acid Concentration (the rest of reaction)

## RESULT AND DISCUSSION

### Ultrasonic-Assissted Extraction

Ultrasonic-Assissted Extraction (UAE) has been applied in various food processing technologies to extract bioactive compounds from plant materials. Ultrasonic, with a rate of more than 20 kHz, is used to destroy plant cell walls, which help to increase the ability of solvents to penetrate cells and obtain higher extraction yields. In the process, UEA can use low temperatures and maintain the quality of the compounds in the extract [19]. In this study, ethanol was used as a solvent. Table 1 shows the crude extract yield of 6.0300 g with a yield of 30.15%. In the UAE process, the yield value is higher than the maceration process commonly used.

Corrales *et al*., proved that the UAE process can break down plant tissue and work well in the process and release of active compounds into solvents with high efficiency. The extraction of phenolic compounds with the UAE in recent years has grown due to its role in reducing the amount of solvent and energy used, so the application of this green technology is needed to protect the environment from toxic substances [20].

In another study, Mulinacci *et al*., compared the extraction times of phenolic compounds from strawberries with other extraction methods such as solid-liquid, subcritical water, and MAE methods. These results confirm that the UAE is the most effective method because it reduces the degradation of phenolic compounds [21-23].

**Table 1: Weight of ethanol extract and yield resulting from UAE process.**

| | Weight (g) | Yield (%) |
|---|---|---|
| Dry sample of Ripe *Musa Balbisiana* Colla | 20,000 | 30.15 |
| Ethanolic Extract | 6.0300 | |

**Table 2: Phytochemical screening results of ethanolic extract of Musa Balbisiana Colla.**

| Secondary Metabolite | Test Results |
|---|---|
| Alkaloids: | |
| Dragendrof | +++ |
| Meyer | +++ |
| Flavanoids | - |
| Phenolic | + |
| Saponin | + |
| Tannin | ++++ |
| Steroid Glycosides | + |
| Triterpenes Sterol | - |

### Phytochemical Screening from Ethanolic Extract of Musa balbisiana Colla

Phytochemical tests were carried out on ethanolic extracts that had been prepared using the UAE method to confirm the presence of bioactive compounds in the extract. Phytochemical testing is widely recognized as a preliminary method that has successfully demonstrated various phytochemical contents in plant parts, such as alkaloids, flavonoids, tannins, phenols, saponins and terpenoids [24-26]. The results of phytochemical tests on ethanolic extract of *Musa Balbisiana* Colla using the UAE method can be seen in Table 2.

Table 2 shows the positive results of the Meyer and Dragendorf tests which prove that there are alkaloids in the ethanolic extract of *Musa balbisiana* Colla. Positive results were also obtained for phenolic tests, saponins, tannins and steroid glycosides. The content of tannins and alkaloids gave stronger results than other compounds. Meanwhile, flavonoids and sterol triterpenes showed negative test results.

Alkaloids have various pharmacological activities including anticancer and antibacterial activities and are therefore widely used as natural healing drugs [27]. Tannins were found to be responsible for high immunomodulatory activity in previous studies. Phenolic compounds in plants are also very important because their hydroxyl groups provide the ability to suppress free radicals. Phenolic-rich plant materials are increasingly being used in the food industry because they can slow down the oxidative degradation of lipids and improve the quality and nutritional value of food [28].

### Phenolic Content of Ethanolic Extract

Phenolic compounds are a class of antioxidant agents that act as free radical terminators and their bioactivity may be related to the ability of antioxidants to chelate metals, inhibit lipoxygenase, and extinguish free radicals [29]. Antioxidants play an important role in chelating metal ions, deactivating lipid chains of free radicals, and preventing the conversion of hydro-peroxide to reactive oxygen radicals.

In this study, gallic acid was used as a standard compound, the standard curve (figure 1) and total phenol were expressed as mg gallic acid equivalent /g extract using the standard curve of the equation: $y = 0.0977x + 0.0628$. Based on the standard curve of gallic acid that was used as a standard, the total phenolic content value was 625.64 ± 0.36 mg GAE/g ethanolic extract. In a previous study conducted by Loganayaki *et al*.,[30] The total phenol content of banana extract was reported to be as high as 1.4 g GAE / 100 g when it was extracted with methanol. Total phenol content of *Musa* sp. was known to be higher than some tropical plants that are commonly consumed [31]. The phenol content in plants can directly contribute to antioxidant activity [32].

### Antioxidant Activity Against DPPH

The diversity of properties and complexities of phytochemical compounds from plant extracts supports the development of methods for evaluating antioxidant activity and estimating the effectiveness of these substances. Most of these methods are based on changing the reagent color in the reaction medium. Antioxidants can be classified into two groups: tests used in food and biological systems to evaluate lipid peroxidation while measuring levels of oxidation inhibition and tests used to measure the suppression ability of free radical activity [33].

The DPPH test method is based on the DPPH reduction reaction, which is a stable free radical. DPPH free radicals with odd electrons give the maximum absorption at a wavelength of 517 nm (purple color). The effective concentration of the sample required to extinguish DPPH radical activity of 50% ($IC_{50}$ Value) was obtained by linear regression analysis of the dose-response curve between percentage inhibition and concentration (Figure 2). Butylated hydroxytoluene (BHT) was used as

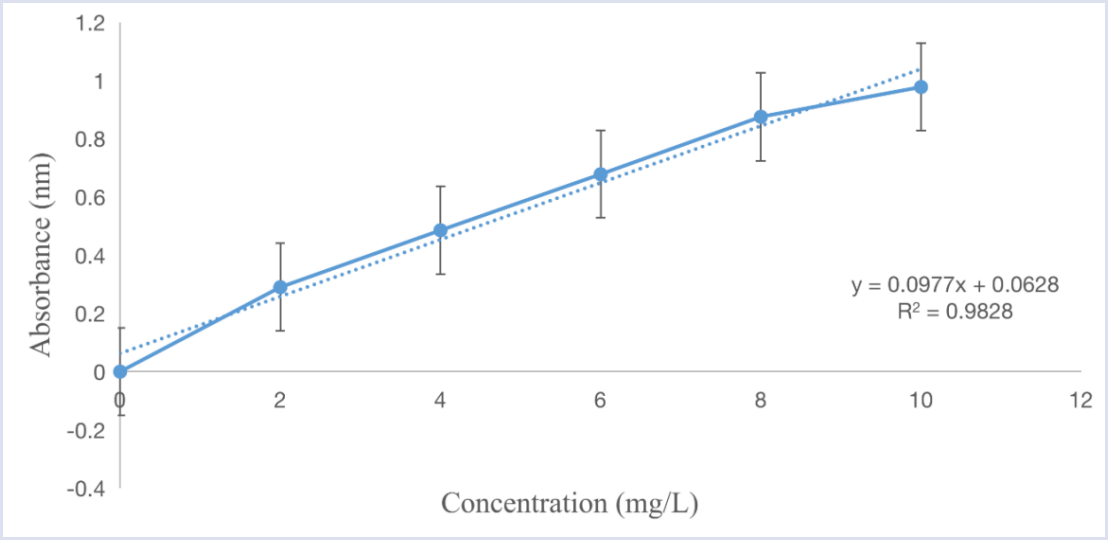
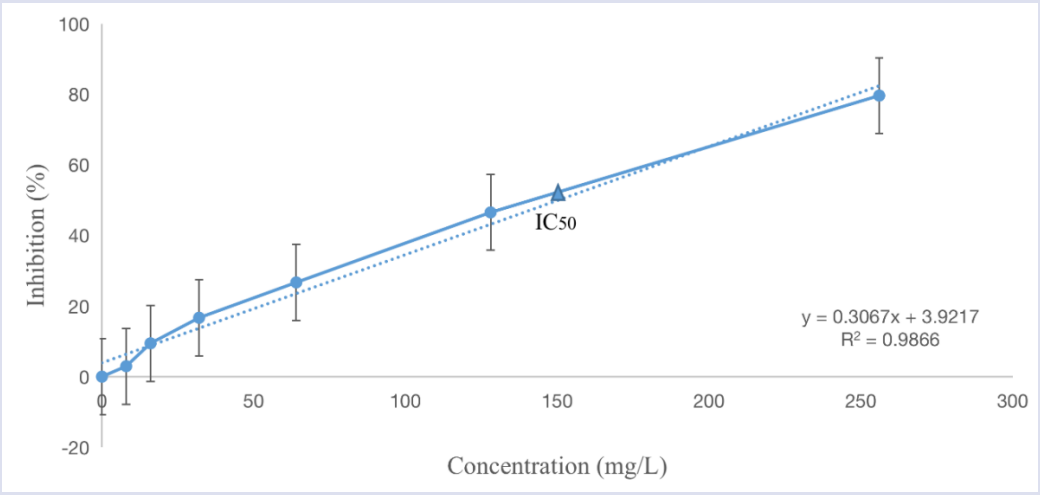
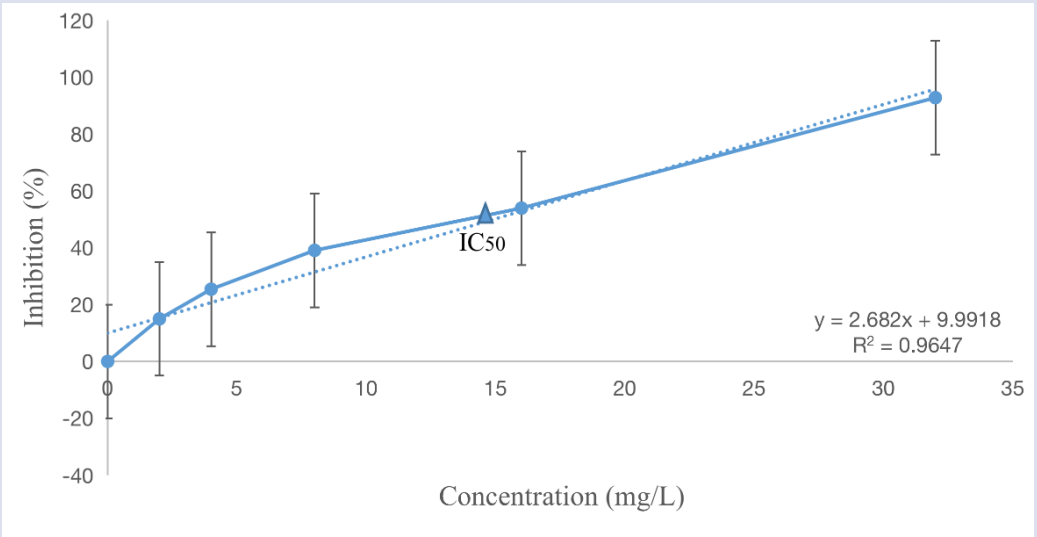



**Figure 1:** Standard curve for gallic acid used as a standard.




(a)




(b)

**Figure 2:** DPPH radical scavenging activities (%) of (a) ethanolic extracts of Musa balbisiana Colla and (b) BHT.

a positive control in this study because it is the most commonly used antioxidant and is known to be safe for use in fat-containing foods, pharmaceuticals, petroleum products, rubber and the oil industry. In Figure 2, the $IC_{50}$ values of *Musa Balbisiana* Colla ethanolic extract and BHT were 150.24 ± 0.0348 mg/L and 14.92 ± 0.0013 mg/L, respectively. The results show that the ethanolic extract of *Musa Balbisiana* Colla has potential as an antioxidant and can be used as an alternative source of natural antioxidants. With this, the polarity of the solvent indirectly plays an important role in the extraction process because it will increase the solubility of antioxidant compounds.

The antioxidative effect is mainly due to phenolic components, such as phenolic acids and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or breaking down peroxides [34].

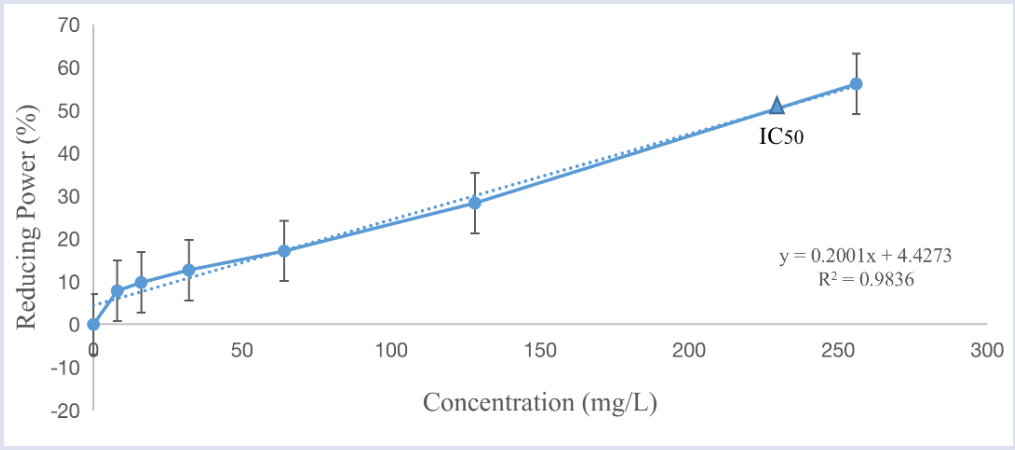
Research conducted by Kanazawa and Sakakibara (2000) [35] states that bananas are classified as a source of antioxidants. This tropical fruit has a strong ability to protect itself from oxidative stress caused by intense sunlight and high temperatures by increasing its antioxidant levels. bananas are known as a weak source of primary antioxidants but a strong source of secondary antioxidants [36-38].

## Ferric Reducing Antioxidant Power (FRAP) Assay

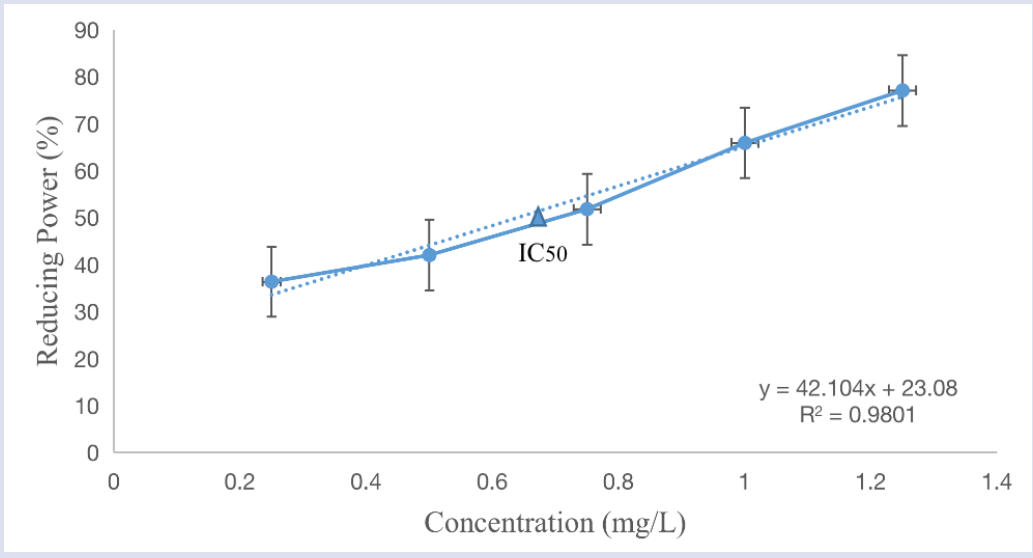
The FRAP test is widely used to determine the efficiency of antioxidant compounds in plants to compete with FRAP reagents and reduce ferric to ferrous. Antioxidant compounds capable of functioning in this approach are categorized as secondary antioxidants where they suppress radical formation and prevent oxidative damage. In addition, secondary antioxidants are also active in metal chelating and oxygen quenching. Reduction of iron in the FRAP reagent will lead to the formation of a blue complex, ferrous-2, 4, 6-tris (2-pyridyl) -s-triazine (TPTZ).

In the FRAP test (Figure 3), the $IC_{50}$ value of ethanolic extracts of *Musa Balbisiana* Colla and gallic acid were 227.80 ± 0.0986 mg/L and 0.63 ± 0.0001 mg/L, indicating the ethanolic extract concentration needed to reduce ferric by 50%. Testing for total phenol relies on the mechanisms involving oxidation and reduction reactions such as the FRAP test. This mechanism can be correlated with the redox properties of antioxidant compounds in plants. Antioxidant compounds will react with Folin-Ciocalteu reagent and thus measure the concentration of phenolic groups [39].

The $IC_{50}$ value of the FRAP method gives smaller results than the DPPH method, because the FRAP method is only limited to water-




(a)




(b)

**Figure 3:** Ferric reducing ability of (a) ethanolic extracts of Musa Balbisiana Colla and (b) gallic acid.

**Table 3: The anti-gout activity of Ethanolic extract and Allopurinol.**

| Sample | Concentration (mg/L) | Adsorbance (average) | Reducing Uric acid Level (%) |
|---|---|---|---|
| Uric Acid Standard | 0.5 | 0.0601 | - |
| Allopurinol | 0.5 | 0.0584 | 2.83 |
| Ethanolic Extract | 50 | 0.0547 | 8.99 |

soluble antioxidants, it cannot be used for compounds containing thiol guus or carotenoids. This is because carotenoids do not have the ability to reduce ferrics [40].

## Anti-gout Potential of Ethanolic Extract

The xanthine oxidase inhibitory activity test of ethanolic extract of *Musa Balbisiana* Colla was carried out by calculating the percentage of inhibition of the xanthine oxidase enzyme which would then be compared with the standard xanthine oxidase enzyme inhibitor, namely allopurinol. Allopurinol was chosen as a positive control, because this compound is able to reduce uric acid through xanthin oxidase inhibition. The decrease in uric acid levels by ethanolic extracts and allopurinol can be seen in Table 3, showing the values (%) of 8.99% and 2.83%, respectively, proving the potential for ethanolic extract as a source of anti-gout compounds.

Uric acid is involved in complex reactions with several oxidants and may have several protective effects under certain conditions. On the other hand, uric acid cannot extinguish the activity of all free radicals. Uric acid is an antioxidant only in a hydrophilic environment, which may be the major limitation of uric acid's antioxidant function. The reaction of uric acid with oxidants can also produce other radicals that can spread radical chain reactions and oxidative damage to cells [41]. For that we need antioxidants to overcome the formation of free radicals.

The decrease in uric acid levels given by ethanolic extract *Musa Balbisiana* Colla is thought to have a relationship with its antioxidant activity. The antioxidants in the extract also play a role in inhibiting the xantin oxidase enzyme.

# CONCLUSION

The crude ethanol extract of *Musa Balbisiana* Colla obtained from the UAE process was 6.0300 g with a yield of 30.15%. From the results of phytochemical tests on ethanolic extract, it was found that alkaloids, phenols, tannins, saponins and steroid glycosides were found. The antioxidant activity test using the DPPH and FRAP methods gave $IC_{50}$ values of 150.24 ± 0.0348 mg/L and 227.80 ± 0.0986 mg / L, respectively. The total phenolic content value of 625.64 ± 0.36 mg GAE/g ethanolic extract is thought to have a role in high antioxidant activity. In addition, ethanol extract with a concentration of 50 mg / L has activity in reducing uric acid levels by around 9%. It can be concluded that the ethanolic extract produced by UAE has potential as a source of anti-oxidants and anti-gout.

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# CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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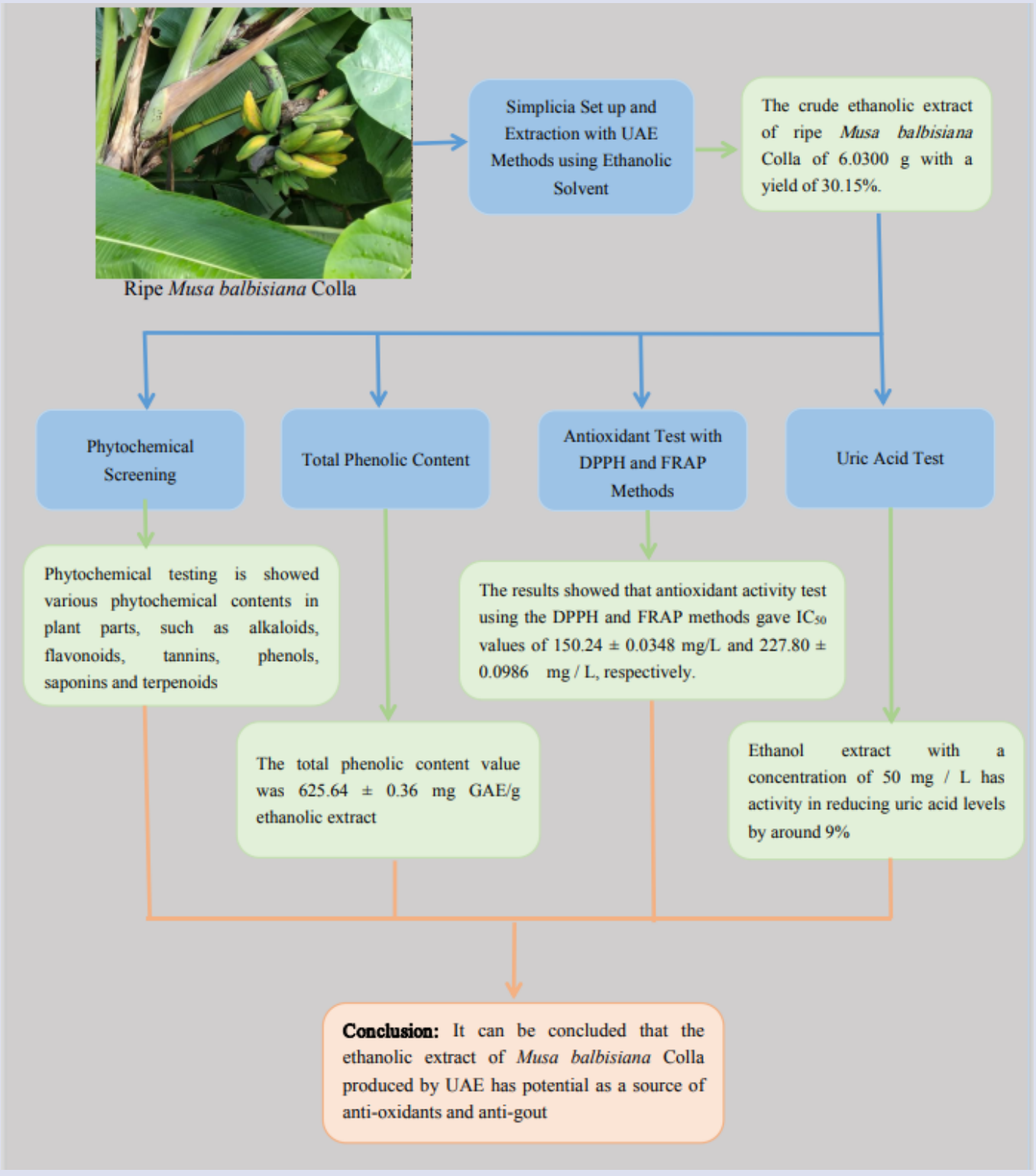
## GRAPHICAL ABSTRACT



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