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Unveiling Nature's Treasure: Investigating the Antioxidant and Anticancer Activities of Mas Banana (*Musa acuminata colla*) Bracts from Lampung, Indonesia

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ABSTRACT

Over the past decade, there has been a global focus on herbal medicines derived from plants. Mas Banana bracts have great potential to be transformed into natural medicine and nutraceuticals to improve the global health, in addition to being a local food supply. This study explores the phytochemical content, antioxidant, and anticancer activity of Mas Banana bracts grown in Lampung, Indonesia. Mas Banana bracts were fractionated with ethyl acetate. Using LC-MS/MS QTOF (Liquid Chromatography-Mass Spectrometry/Tandem Mass Spectrometry Quadrupole Time-of-Flight), the phytochemical composition of the ethyl acetate fraction of mas banana bracts was investigated. The analysis revealed the presence of Afzelechin and Moracenin D. The antioxidant potential was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, yielding a value of 810.79 mg/100 g. The anticancer activity tested against the HeLa cell line using standard procedures was seen from the cells viability percentage that reached 51.28%. The ethyl acetate fraction of Mas Banana bracts exhibited significant antioxidant and anticancer activities with greater activity against the HeLa cell line. The results show that the ethyl acetate fraction of Mas Banana bracts possesses anticancer and antioxidant properties and it can be used as a natural food preservative and as an anticancer agent for the treatment of cancer.

***Keywords*:** Mas Banana bract, ethyl acetate, phytochemical, antioxidant activity, anticancer activity

Introduction

The banana, scientifically known as *Musa spp*., has gained immense popularity and is now in high demand worldwide, with an annual production exceeding 125 million tons. Indonesia was ranked sixth in banana production worldwide, following India, China, the Philippines, Ecuador, and Brazil, contributing approximately 7 million tons each year.[1] Indonesia plays a crucial role in the cultivation of bananas (*Musa spp*.) in Asia. The primary banana cultivation hubs are situated in Sumatra, Java, and Bali.[2,3] In 2019, banana production in the Lampung Province reached 1,202,789.6 tons, accounting for 16.52% of the national banana production. This production volume in Lampung was ranked third, following East Java and West Java provinces. Tanggamus regency is designated as a national agricultural area and one of the national banana development regions based on the Minister of Agriculture's Decision No. 472/Kpts/Rc.040/6/2018, in which one of the banana varieties produced is the Mas Banana (*Musa acuminata colla*).[4,5]

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The banana (*Musa spp*.) is an essential food crop that is extensively grown and consumed due to its delightful flavor, abundant nutritional value, and positive impact on health. Banana plant parts have historically been used to treat a wide range of conditions such as menorrhagia, diarrhea, oxidants, dysentery, diabetes, renal lithiasis, tumor, mutagenesis, bacteria, fungi, liver disorder, hypocholesterolemic, hemorrhage, parasitic infections, ulceration, hair growth promoter, wound, inflammation, pain, and snakebite.[6–8] Meanwhile, banana bracts are often neglected, considered agricultural waste, and utilized as animal feed.[8,9] However, in Asia, especially Indonesia, banana bracts are extensively used in culinary and food preparations.

Banana bract, also known as the "flower" of the banana, consists of male flowers surrounded by reddish-purple leaves.[10,11] In large-scale banana cultivation, banana bracts are typically discarded to promote fruit development. As a result, a banana bract becomes a significant waste in banana cultivation. Banana bract is often considered economic waste, but its utilization in traditional medicine is a common practice across various cultures.[6,12]

Banana bract has a variety of bioactive compounds with significant potential. Recent research indicates the biological benefits of banana bracts, such as becoming an antioxidant, antidiabetic, anti-inflammatory, and anticholesterolemic agents.[13–15] The secondary compounds found in banana bracts include polyphenols, triterpenes, and sterols.[15] Research on the phytochemical compounds of Mas Banana bract is rapidly advancing. Considering the importance of banana bracts as a local food source and their significant potential to be developed into nutraceuticals and herbal medicines beneficial to global health, this study aims to investigate the phytochemical composition and antioxidant activity of mas banana bracts, which are plentiful but neglected in Lampung. The current research novelty lies in the

comprehensive exploration of the phytochemical profile and antioxidant properties of these overlooked bracts, shedding light on their potential value for both local and global contexts.

Materials and Methods

Plant Collection and Identification

Mas Banana Bractea was obtained from the Tanggamus region in the Lampung Province of Indonesia (104°18'-105°12' East Longitude and 5°05'-5°56' South Latitude) and was harvested from December 2023 to January 2024. When it was 2 weeks old after the plants showed the flowers. The identification of the banana bract was carried out by Dra. Yulianty, M.Si as botany expert from the University of Lampung. The outer purple-colored part of the Mas Banana bract was then collected, cleaned, cut into small pieces, and subsequently dried in an oven (Memmert UN30 Universal Oven, manufactured by Memmert GmbH + Co. KG, Germany) at a temperature of 50ºC. The dried raw material was then finely ground and sifted.

The ethyl acetate fraction of Mas Banana bract

The extraction procedure entailed preparing an extract from the powdered Mas Banana bracts through maceration. The pulverized primary substance was soaked in ethanol for 72 hours. After 3 days of maceration, it was filtered using a vacuum Büchner funnel to separate the powder and the macerate. The macerate obtained was subjected to evaporation using a rotary evaporator to yield the initial semi-thick extract, and the ethanol remaining from the evaporation process was used to re-macerate the powder for another 3 days. After the second maceration period, it was filtered using a vacuum Büchner funnel to separate the powder and the macerate. The macerate obtained was subsequently evaporated once more using a rotary evaporator to achieve the second extract with a semi-thick consistency, and the remaining ethanol from the evaporation process was used for a third maceration of the powder for 3 days. After the third maceration, it was filtered using a vacuum Büchner funnel to separate the powder and the macerate. The resulting macerate was evaporated with a rotary evaporator (RV 10 digital V-C manufacturer by IKA Works GmbH & Co. KG, Germany) to obtain the third semi-thick extract.

The initial, subsequent, and third moderately dense extracts were amalgamated in a porcelain dish and subjected to evaporation in a water bath to yield a concentrated extract. The concentrated extract was then dissolved in distilled water, extracted with N-hexane, and separated using a separating funnel. The fraction insoluble in N-hexane was fractionated with ethyl acetate and then separated using a separating funnel. The extract soluble in ethyl acetate was evaporated with a rotary evaporator (RV 10 digital V-C manufacturer by IKA Works GmbH & Co. KG, Germany) to obtain a semi-thick fraction. The semi-thick fraction was evaporated in a water bath to obtain the ethyl acetate fraction of Mas Banana bract extract.

LCMS/MS QTOF

The process of screening active compounds from natural materials using LCMS/MS QTOF (Xevo G2-XS QTof manufactured by Waters Corporation, United States) was conducted at the Saraswati Indo Genetech (SIG) Laboratory, utilizing the UNIFI software equipped with a spectrum library of active compound mass spectra from the Waters database. The mass spectra of the chemicals in the sample were detected by the UNIFI software, and these spectra were compared to the mass spectra that were already in the library. The LC settings included a C18 column, with a column temperature set at 40°C, an autosampler temperature of 15°C, an injection vol

ume of 10 µL, a flow rate of 0.6 mL/min, and mobile phases A and B comprising of 0.1% formic acid in acetonitrile and 0.1% formic acid in water, respectively. Operated in the Tos MSE mode, ESI+ ionization, and an acquisition range of 50–1200 were all part of the MS parameters. For the sample of ethyl acetate fraction from Mas Banana bract extract (1 g) was weighed into a 10 mL volumetric flask. Methanol or a suitable solvent was subjected to sonication for a duration of 30 min. The solution was filtered using methanol or an appropriate solvent, followed by homogenization. Subsequently, the solution was filtered using a 0.22 µm GHP/PTFE membrane and was subsequently introduced into the UPLC system by injection.

Antioxidant Activity

The antioxidant activity test of Diphenyl-1-picrylhydrazyl (DPPH) was conducted at the Saraswati Indo Genetech (SIG) Laboratory. The procedure for the DPPH antioxidant activity test began with the preparation of a reagent blank in the form of a DPPH solution dissolved in a solvent. If the antioxidant activity was to be expressed as AEAC and/or TEAC values, a minimum of 6 points of standard concentration series were created for vitamin C and/or trolox. Five grams of the test portion was weighed and put into a 50 mL amber flask/coated with aluminum foil. It was dissolved in methanol and water (1:1) and then sonicated in a sonic bath at room temperature for 10 minutes. It was then filtered with methanol and water (1:1) and homogenized. If the sample solution was turbid, centrifugation and filtration were performed using a 0.22 µm syringe filter. The next procedure was creating a concentration series from the sample solution in a 10 mL amber flask. The solution was then filtered with methanol and water (1:1) and homogenized. After which, 2 mL of each standard was pipetted into a covered amber tube and DPPH solution (50 mg/L) was added and vortexed. The sample was thereafter placed in a dark environment and allowed to incubate for 30 min. Following incubation, the absorbance of both the test and reference solutions was measured using a spectrophotometer (UV-1800 manufactured by Shimadzu Corporation, Japan) at λ519 nm. The ethyl acetate fraction of Mas Banana bracts and vitamin C (used as the standard medication) were evaluated for antioxidant activity or DPPH inhibition percentage using the formula provided in equation (1).

$$DPPH\ \%\ Inhibition = \frac{\text{Absrobance of Control} - \text{Absorbance of Sampel}}{\text{Absorbance of Control}} \times 100\% \quad -----(1)$$

A linear regression curve was created by graphing the DPPH inhibition percentage data against the amounts of the ethyl acetate fraction in Mas Banana bracts and the content of vitamin C. The IC50 value, serving as an indicator for antioxidant activity, was determined for both the ethyl acetate fraction of Mas Banana bract and vitamin C. This calculation was performed utilizing the regression equation derived from the obtained linear regression curve.[16]

In vitro Anticancer Assay

HeLa cells, which were 80-90% confluent for harvest, were taken from a CO2 incubator. Furthermore, the number of cells was counted and diluted with a complete medium. A total of 10^4 µL cells, each with a specific density, were transferred into the wells. The condition of the samples was observed under an inverted microscope (CKX41 manufactured by Olympus Corporation, Japan) to assess their distribution.

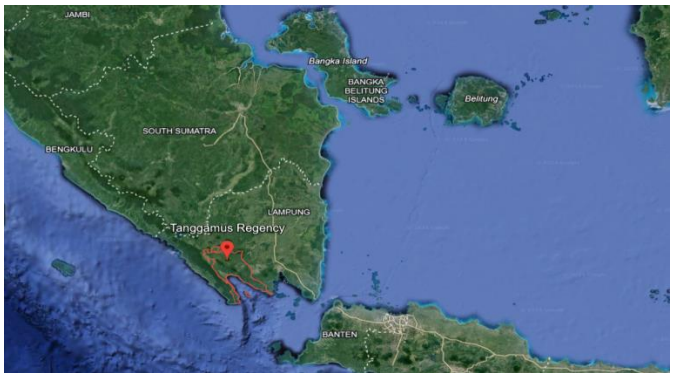

Figure 1: Location of Mas Banana (*Musa acuminata colla*) samples in Lampung, Indonesia.

Following an overnight incubation period in a CO_2 incubator (Series 8000 DH manufactured by Thermo Fisher Scientific, United States), the

samples were allowed to return to normal for one day. Following this, a sample concentration series (ranging from 1,000 to 15.65 µg/mL) was created for the treatment, which included media and cell control. After removing the plate holding the cells from the CO2 incubator, the medium was thrown away. Sample concentrations ranging from 104 µL were placed in triplicate wells and incubated for a full day in a CO2 incubator. The MTT reagent was added to PBS (0.5 mg/ml) and diluted to 10.0 ml with the whole media. Each well received 100 µL of MTT reagent after the media was removed. An inverted microscope was used to observe the cells after they had been cultured in a CO2 incubator for 4 hours. After the production of formazan, 100 µL of DMSO was introduced. The plate was then stirred, covered with aluminum foil, and left to incubate for 15 minutes at room temperature in a dark environment. The plate reader measured the absorbance of each well at a wavelength of 595 nm.

Results and Discussion

Phytochemical composition of ethyl acetate fraction in Mas Banana bracts

Secondary metabolites, including phenolics and flavonoids, play a crucial role in exerting antioxidant and anticancer effects. Flavonoid compounds, identified as secondary metabolites prevalent in plants, are recognized as a plentiful category of phytochemicals associated with various health advantages.[17] The results of the LC-MS/MS QTOF (Liquid Chromatography-Mass Spectrometry/Tandem Mass Spectrometry Quadrupole Time-of-Flight) analysis are shown in Table 1 and Figure 2. The chromatograms of the LC-MS/MS QTOF analysis revealed the presence of afzelechin and moracenin D. Afzelechin and moracin D are flavonoids, a group of plant metabolites that are responsible for the vivid colors in fruits, vegetables, and flowers. They are renowned for their diverse health advantages, which encompass antioxidant and anti-inflammatory characteristics.[18,19] Afzelechin is a flavan-3-ol, a type of flavonoid found in plants, while moracin D is a flavonol, another subclass of flavonoids.[18,20] These compounds are being studied for their potential pharmacological activities and health-promoting effects.

Afzelechin and moracenin D are secondary metabolites that have been associated with many health advantages, such as antioxidant and possibly anticancer properties. Afzelechin, a flavanol present in plants like *Camellia sinensis var. assamica*, is linked to diverse health advantages, encompassing antioxidant properties and potential anticancer activities. Flavonoids, including afzelechin, are acknowledged for their antioxidant, anti-inflammatory, and potential anticancer effects.[21] The antioxidant properties of flavonoids, such as afzelechin, are well-documented and have been linked to their potential role in preventing various diseases, including cancer.[22]

Moracenin D, identified as a prenylated arylbenzofuran derivative present in *Morus* plants, has been linked to diverse health advantages, encompassing antioxidant properties and potential anticancer activities. Investigations have indicated that *Morus* plants, containing moracenin D, showcase antioxidant, anti-inflammatory, and antiproliferative properties.[23] Additionally, the broader group of compounds found in the *Morus* plant has been reported to possess antitumor and anticancer effects, making them promising candidates for the treatment of various types of cancer, including lung, breast, cervical, and hepatocellular carcinoma.[24] In in vitro studies, Moracenin D has been tested against melanoma cancer cells. Moracenin D has been shown to suppress TRP-1 expression in B16 cells. Tyrosinase-related protein 1 (TRP-1) is a key enzyme in melanin biosynthesis with important implications for pigmentation, melanoma, and therapeutic development.[25] Thus, while Moracenin D may possess limited chemical properties, it does not necessarily render it ineffectual in the realm of anticancer applications. Additional investigation is required to gain a comprehensive understanding of the activity, mechanism of action, and pharmacological properties of this molecule to determine its potential and limitations as an anticancer agent.

Table 1: Compounds identified from the ethyl acetate fraction of Mas Banana bract

Component Name	Formula	Observed RT (min)	Mass error (ppm)	Molecular Weight	Isotope Match Intensity RMS Percent
Afzelechin	C15H14O5	12.53	-2.0	275	6.48
Moracenin D	C40H38O12	15.57	-2.5	711	5.17

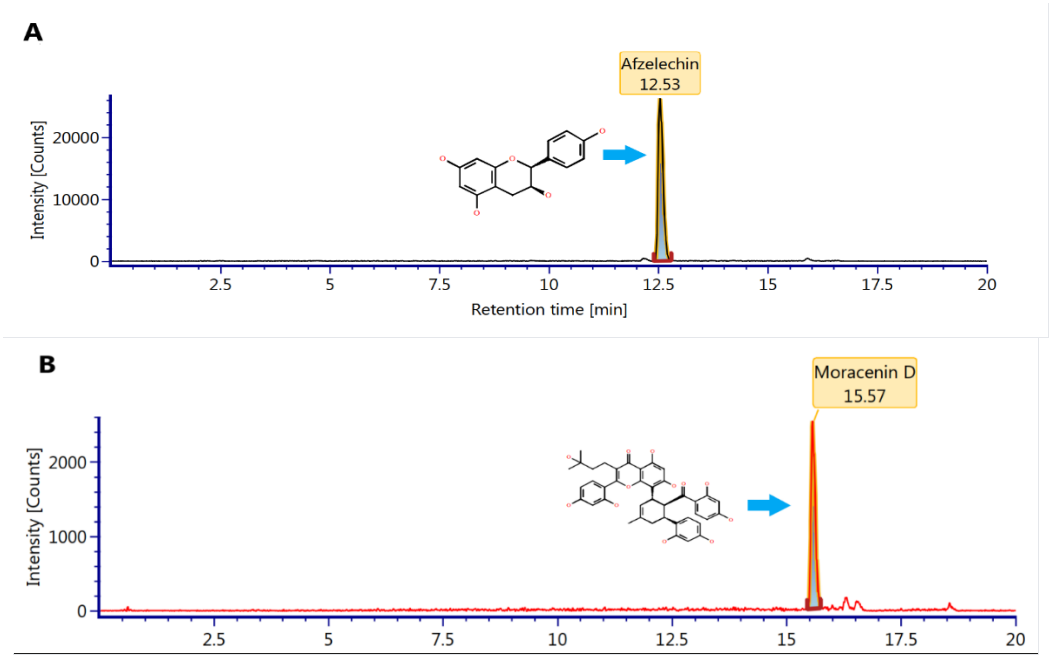

Figure 2: The chromatograms of the LC-MS/MS QTOF: A. The retention time (RT) of Afzelechin is 12.53 minutes; B. The retention time (RT) of Moracenin D is 15.57 minutes.

Table 2: The percentage of DPPH inhibition of ethyl acetate fraction of Mas Banana bracts and Vitamin C at various concentrations

Sample	Absorbance (519 nm)					Percentage of DPPH inhibition				
	40 ppm	80 ppm	160 ppm	240 ppm	320 ppm	40 ppm	80 ppm	160 ppm	240 ppm	320 ppm
Ethyl acetate fraction in Mas Banana bracts	1.23	1.21	1.19	1.16	1.14	0.94	2.02	4.08	6.06	7.88
	Absorbance (519 nm)					**Percentage of DPPH inhibition**				
	2 ppm	4 ppm	6 ppm	8 ppm	10 ppm	2 ppm	4 ppm	6 ppm	8 ppm	10 ppm
Vitamin C	1.16	1.09	1.01	0.92	0.86	6.25	11.98	18.44	25.03	30.46

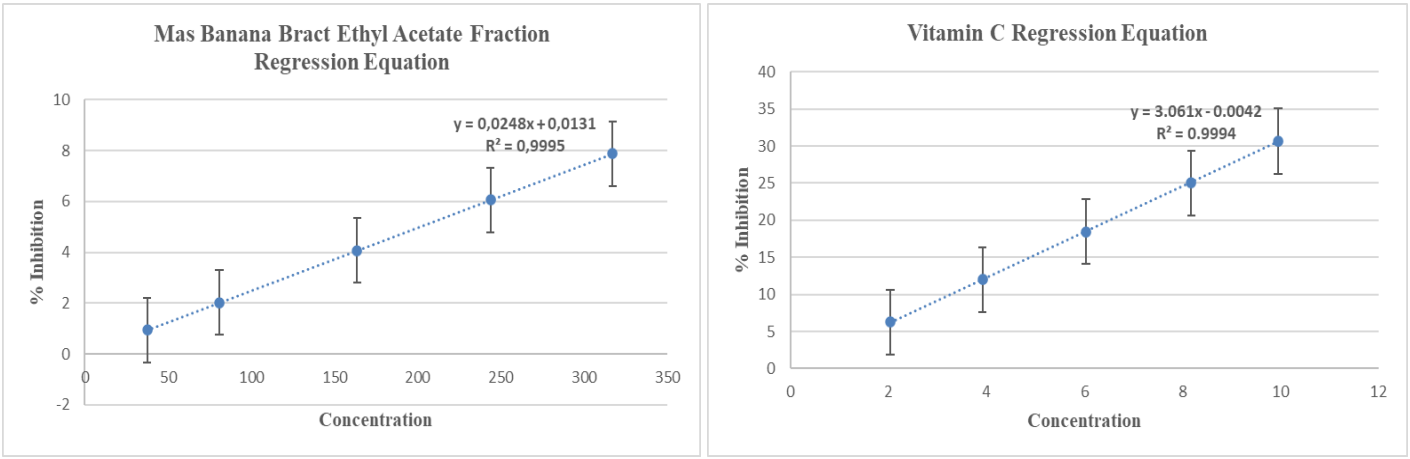

Figure 3: The graph of linear regression equation of ethyl acetate fraction in Mas Banana bracts and Vitamin C

Table 3: The IC_{50} values of ethyl acetate fraction of Mas Banana bracts and Vitamin C by linear regression equation

Sample	Regression Equation	IC50 Value
Ethyl acetate fraction in Mas Banana bract	y = 0.0248x + 0.0131	2014.78
Vitamin C	y = 3.061x – 0.0042	16.33

Antioxidant Activity of Ethyl Acetate Fraction of Mas Banana Bract.
The DPPH technique was employed in this investigation to evaluate the antioxidant activity of vitamin C (a standard medication) and the ethyl acetate portion of Mas Banana bract. The degree of DPPH inhibition was then plotted against the amounts of vitamin C and the ethyl acetate component in the banana bracts (Figure 3). This research has quantified the antioxidant activity in terms of equivalents of a standard antioxidant per gram of the sample. The antioxidant activity of the ethyl acetate fraction in Mas Banana bracts is measured using ascorbic acid as the reference antioxidant, with a value of 810.79 mg/100 g. The values were computed using the formula provided in equation (2).

$$\text{Ascorbic Acid Equivalent Antioxidant Capacity (AEAC)} = \frac{\text{IC50 Vit C}}{\text{IC50 Sampel}} x\ 10^5 \quad -----\quad (2)$$

Table 2 and Table 3 display the findings of the IC_{50} values computation for the standard medication, vitamin C, and the ethyl acetate component in Mas Banana bracts. These examples demonstrate the common practice of quantifying antioxidant activity in terms of equivalents of a standard antioxidant per gram of the sample, using standard assays and expressing the results in equivalent units. To quantify the antioxidant activity in terms of equivalents of a standard antioxidant per gram of the sample, standard assays such as the Ascorbic Acid Equivalent Antioxidant Capacity (AEAC) are employed. The AEAC is a measure of the antioxidant capacity of a sample relative to ascorbic acid, which is a well-known antioxidant.[26]

Anticancer Activity (MTT Assay)
HeLa cells, derived from cervical cancer, have long served as a reliable and widely used in vitro model due to their rapid proliferation and consistent behavior. This current study seeks to exploit these attributes to comprehensively understand how the ethyl acetate fraction may influence the intricate biological processes within HeLa cells. Cell viability and cytotoxicity assessments are crucial in vitro research to evaluate the effects of various compounds and treatments on cell growth and survival. In the context of HeLa cells, these assessments have been conducted using established assays such as the MTT assay. The MTT assay, which uses the tetrazolium salt 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide, is a widely used method to determine cell viability and cytotoxicity in vitro.[27,28]
The MTT assay functions on living mitochondria cells that are mediated by macrophages and assesses cell viability and proliferation.[29] Figure 4 shows a cytotoxic effect of the test HeLa cells. Based on the displayed graph, it is evident that at a concentration of 1000 µg/mL of the ethyl acetate fraction in Mas Banana bracts, the cell viability percentage reached 51.28%.
The MTT method, employed for assessing the cytotoxic potential of a chemical compound, involves utilizing MTT, a pale-yellow substrate. Active mitochondria play a crucial role in the process of live cell division when MTT is converted into a dark blue formazan product. This test is utilized to characterize proliferation and complement-mediated cytotoxicity assays.[27] The underlying mechanism of the reaction involves the activity of succinate dehydrogenase. In this process, the tetrazolium salt undergoes conversion to a purple formazan, which is subsequently quantified using spectrophotometry.[30] The objective of this study was to ascertain the ethyl acetate portion of cytotoxicity (IC_{50}) in Mas Banana bracts on HeLa cervical cancer cells. Cytotoxic activity was determined based on the IC_{50} value, and smaller values indicate stronger cytotoxic. The determination of the IC_{50} value relied on the percentage inhibition derived from the test extract. As observed in Figure 4, the concentration in the cell viability test rises. There is a corresponding decrease, suggesting a potential correlation with DNA damage.[31] These results indicated that the ethyl acetate fraction of Mas Banana bracts could inhibit the growth of HeLa cervical cancer, and the secondary metabolite content of ethyl acetate fraction in Mas Banana bracts can be an anticancer agent.

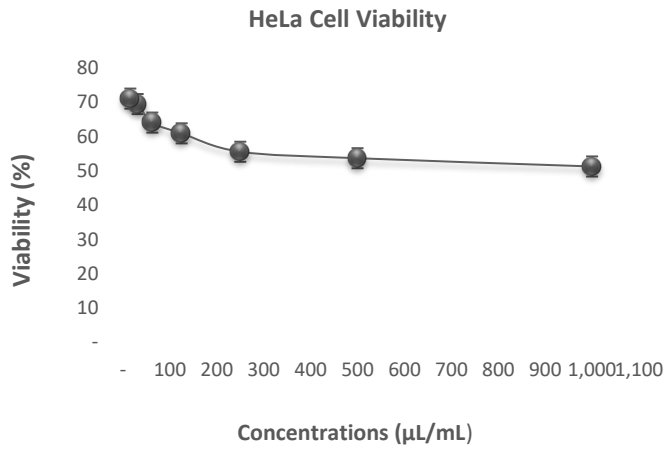

Figure 4: Percentage of viability cells against HeLa cells

Conclusion

The ethyl acetate fraction of Mas Banana bracts exhibited chemical compounds that contribute to the reduction of HeLa cell survival. These findings could aid in the development of anti-cervical cancer drugs. As recommendations for future research, some measures that should be done include understanding compound mechanisms, conducting more trials for human safety and efficacy, and exploring additional compounds for cervical cancer therapy.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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