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**PYTHOCHEMICAL SCREENING OF *Musa acuminata* STEM
WATER EXTRACT**

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ABSTRACT

Background: Regenerative therapy has been progressing into the utilization of mesenchymal stem cell (MSC). Nevertheless, the limited number of MSC has put growth factor as an essential supplement for cell culture media yet relatively unaffordable because highly priced. Alternative compound which cost reasonably is required. Exogenous phytochemical material in herbal plant extract may increase the number of MSC, one of which is mauli banana stem. **Purpose:** To analyze secondary metabolites identified in mauli banana stem water extract. **Method:** Maui banana stem was macerated using water solvent to be analyzed qualitatively for alkaloid, tannin, flavonoid, saponin, terpenoid, diterpen and steroid. Screening was followed by quantitative analysis to determine the total of alkaloid, flavonoid, condensed tannin and hydrolysable tannin. **Result:** Secondary metabolite compounds of mauli banana stem water extract were alkaloid (4.15%), hydrolysable tannin (1.055%), condensed tannin (0.42%) and flavonoid (0.31%). **Conclusion:** Maui banana stem water extract has potential as alternative growth factor to increase the number of MSC in vitro.

Keywords: Phytochemical screening, stem mauli banana, water extract.

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INTRODUCTION

Regenerative therapy has been progressing into the utilization of mesenchymal stem cell (MSC). Mesenchymal stem cell is a precursor of non-hematopoietic tissue which obtainable from bone marrow. Such cell will remain multi-potent after in vitro expansion, showing a relatively low immunogenicity and freezable property. MSC has been implemented as regenerative therapy by considering its ability to migrate to wound area, differentiate as target cells in microenvironment, secrete paracrine factor for regeneration process, modulate inflammation and immune response to reduce the risk of rejection from xeno or allo transplantation therapy.¹

Prior to its application as regenerative therapy, mesenchymal stem cell should be cultured due to the limited number of MSC which is one per 10.000 nucleated cell.² MSC culture is performed by adding growth factor as one of cell culture replenishments so that anticipated number of MSC may be obtained. Growth factor is able to stimulate MSC proliferation because several growth factor receptors are found on MSC surface such as EGFR, β FGFR, IGFR, PDGFR, TGF β RI and RII.³ Growth factors used as supplement for cell culture media are

expensive, thus alternative substance with affordable price is required. Exogenous phytochemical compounds identified in herbal plant extract may be an alternative to enhance MSC number, one of which is found in banana.

Maui banana is a particular variance of banana which originates only from South Kalimantan region.⁴ The extract of mauli banana stem has shown to increase macrophage number on day 3 and intensify NF-kB expression in the healing process of traumatic ulcer.⁵ It is an antioxidant which may reduce reactive oxygen species (ROS).⁶ ROS act as secondary messengers for cell proliferation which is regulated in intracellular signaling pathway.⁷ A study by Carabelly has proven that mauli banana stem methanol extract is toxic towards MSC culture, while mauli banana stem water extract shows no toxicity upon the culture.^{2,8} This reveals that different solvent for plant extraction may result in contrasting variance of secondary metabolites. The objective of this study is to elaborate secondary metabolite components presented in mauli banana stem water extract so that mauli banana stem may be utilized as an alternative growth factor for MSC culture.

MATERIALS AND METHODS

This research has been approved in reference to Ethical Clearance Letter No. 194/KEPKG-FKGULM/EC/VI/2019 from Health Research Ethics Committee of Dental Faculty Universitas Lambung Mangkurat.

Formulation of Mauli Banana Stem Water Extract

Mauli banana stem used as study sample was procured from SMK-PP Banjarbaru. Mauli banana stem was obtained 10 cm from root lump. It was then washed, cut and put into an oven at 60°C temperature. Stem which had been dried were further smoothed using a blender and sifted with a 35-size mesh, and then weighed 50 mg to be poured into a beaker glass. Beaker glass was filled with mauli banana stem extract which was mixed with 1000 ml of distilled water and then heated on a hotplate at 50°C temperature for 5 hours. The mixture was subsequently blended three times using 500 rpm stirrer in the beginning. The extraction result was cooled and filtered and inserted in evaporator at 60°C for 2 x 24 hours. After evaporated, extract was processed on a water bath at 60°C until viscous extract was attained.

Qualitative Screening of Mauli Banana Stem Water Extract

Detection of Alkaloids

Mayer's Test: Dissolution of extracts was performed in diluted hydrochloric acid respectively. The solution was then filtered and filtrates were reacted with Potassium Mercuric Iodide (Mayer's reagent). Yellow-coloured precipitation indicates the presence of alkaloids.

Detection of Saponins

Foam Test: Extract weighed for 0.5 gram was shaken with 2 ml of water. Any persistent foam produced for ten minutes indicates the presence of saponins.

Detection of Tannins

Gelatin Test: 1% sodium chloride-containing gelatin solution was added. Any white-coloured precipitation suggests the presence of tannins.

Detection of Flavonoids

Lead acetate Test: Treatment with few drops of lead acetate solution will illustrate the presence of flavonoids by the formation of yellow-coloured precipitation.

Detection of Terpenoid

Two gram extract was poured into beaker glass and mixed with 10 mL ethanol. The mixture was later heated and filtered to obtain 5 mL extract and added with 2 mL chloroform and 3 mL viscous sulphic acid. Any changes were then observed.

Detection of Diterpenes

Copper acetate Test: After dissolved in water, extracts were mixed with three to four drops of copper acetate solution. Emerald green colour formulated in the mixture indicates the presence of diterpene.

Detection of Steroid

Two gram sample was added into beaker glass and mixed with 20 mL methanol containing 2 mL sulphic acid which was subsequently heated and filtered. Two milliliters anhydric acetic acid was mixed into the solution and any changes should be observed.

Quantitative Analysis of Mauli Banana Stem Water Extract

Identification of Alkaloid

Five milligrams sample were weighed and poured into 250 ml beaker glass to be mixed with 200 ml acetic acid at 10% concentration in ethanol. Beaker glass was later covered and stored for 4 hours. The mixture was then filtered and evaporated using water heater until a quarter of initial volume was obtained. Further, viscous ammonium hydroxide was added drop by drop into extract resulting in complete sedimentation. The whole mixture was dwelled and sedimentation was collected to be washed with ammonium hydroxide. Sedimentation was subsequently filtered and the residues identified as alkaloid was dried and weighed. Alkaloid was lastly identified using Harborne method (1973).

Identification of Alkaloid

Ten milligrams of sample were extracted multiple times using 100 ml of 80% methanol at room temperature. The liquid was then filtered using Whatman paper No 42. The filtrate immediately inserted into porcelain crus and evaporated using water heater. The weight was measured until stable rate was attained. Boham and Kocipai-Abyazan (1994) method was applied.

Identification of Tannin

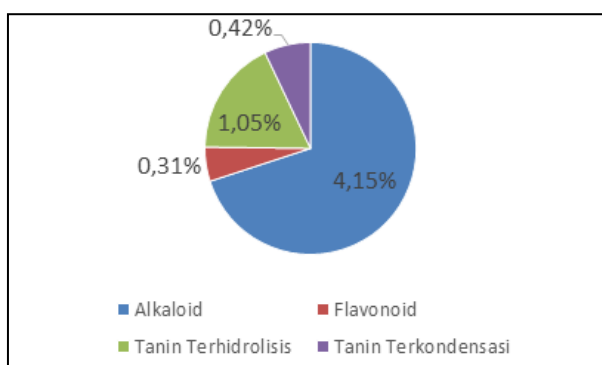
Sample was combined with 3% formaldehyde + HCl 1 N (2:1) for the identification of condensed tannin. Any pinkish residue will result in positive containment of condensed tannin. Filtrate of condensed tannin was identified using FeCl₃ at 1% concentration to determine hydrolysable tannin. The presence of ink blue or black color will represent positive result of hydrolysable tannin in the extract.⁹

RESULTS

The result of this study presents that mauli banana stem water extract contain alkaloid, tannin and flavonoid.

Table 1. Qualitative Analysis of Mauli Banana Stem Water Extract

Components	Observation Result	Qualitative Test
Alkaloid	Presence of sedimentation	+
Tanin	White sedimentation	+
Flavonoid	Dark yellow sedimentation	+
Saponin	Absence of any foam	-
Terpenoid	White colour in solution	-
Diterpen	White colour in solution	-
Steroid	White colour in solution	-

**Figure 1.** Quantitative analysis of Mauli Banana Stem Water Extract

DISCUSSION

This study results in the identification of several secondary metabolites found in mauli banana stem water extract such as alkaloid, phenol, tannin and flavonoid. Mauli banana stem water extract contains alkaloid (4.15%), hydrolysable tannin (1.055%), condensed tannin (0.42%) and flavonoid (0.31%). It is distinguished with bioactive compounds found in mauli banana stem methanol extract, which are 67.59% comprised of tannin, 14.49% of saponin, 0.34% of alkaloid and 0.25% of flavonoid.⁶ Difference in the total of bioactive compound are resulted from different solvent used in extraction process. Most alkaloid, flavonoid, tannin and saponin are semi polar or polar compound.¹⁰ Based on its polarity and solubility, polar compound are easily extracted in polar solvent, while non-polar compound easily solved in non-polar solution. Polar solvent includes ethanol, methanol, buthanol and water.¹¹ Water possesses polar property enabling the solvent to extract saponin, tannin and flavonoid which are non-toxic to the cell. Notwithstanding,

methanol possesses higher polarity than water thus resulting in higher level of tannin, flavonoid, alkaloid and saponin in methanol solvent which amount is inflated than those in water solvent.¹¹⁻¹³

Natural phytochemicals are commonly found in health additives-containing food and nutraceuticals which have been advised to prevent and manage oxidative stress and inflammation-mediated diseases.¹⁴ Alkaloids are verified to stimulate cell proliferation.¹⁵ Berberine alkaloids was corroborated for their role in inducing VEGF and miR-126 expression inside the exosomes of mesenchymal stem cell.¹⁴ β -carboline alkaloids identified in *Banisteriopsis caapi*, the plant of ayahuasca tea, is affirmed to stimulate the proliferation of neural stem cells and their differentiation into mature neurons.¹⁶ Alkaloids, such as sinomenine, brucine and halofuginone, also show their ability to directly regulate proliferation in vitro.¹⁴

As water-soluble polyphenolic metabolites which established naturally, tannins are produced in vast variety of higher plants. Tannins are classified into two main groups based on their structures and characteristics, which are hydrolysable tannin and condensed tannins.¹⁷ Tannins act as antioxidant because of its ability to reduce ROS.¹⁸ Reactive oxygen species (ROS) at low level holds a crucial part as second messenger which may induce MSC proliferation.¹⁹ Meanwhile, condensed tannin demonstrates its role in inducing tyrosine phosphorylation through tyrosin kinase insulin receptors located in the surface of MSCs for the activation of mesenchymal stem cell proliferation.⁸ Gallic acid and farmatan, derivate of hydrolysable tannin, are reported to enhance the proliferation of beonatal porcine cell line JPEC-J2.²⁰

Flavonoids are constructed by a large complex of polyphenolic compounds which have a benzo- γ -pyrone structure and are pervasively present in plants. They are capable for growth factors regulation in plants, such as auxin, and modulation of protein activities involved in cell growth. This will consequently enable flavonoids to act as transcriptional regulators.²¹ Evidence of flavonoids' role on cell growth is portrayed by flavonoids extracted from traditional Chinese medicine Epimedium herb where the epimedium flavonoids effectively promotes neural stem cell proliferation and differentiation in vitro.²² Quercetin which is also one of the principal flavonoids proven to enhance the proliferation of Bone Marrow Stem Cell.²³ Based on elaboration above, it is concluded that mauli banana stem water extract has potential as alternative growth factor to enhance the number of MSC in vitro.

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