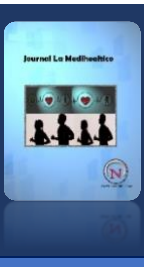

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Comparison of Antibacterial Activity of Ethanol Extract and Nanoparticles of Crassocephallum Crepidioides S.Moore Against Staphylococcus Aureus

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Abstract

This research seeks to examine the width of the inhibitory zone of extracts and nanoparticles of Crassocephalum crepidioides leaf simplisia, which have many advantages such as wound therapy, diarrhea relief, anti-inflammatory properties, and antibacterial effects. The antibacterial activity of Crassochepallum crepidioides (Benth) was evaluated by using the ethanol extract and nanoparticles. S. Moore departs. The agar diffusion technique was used to test it on paper plates. 0.1 ml of bacterial inoculum was introduced into a sterile petri dish, followed by the addition of 15 ml of Mueller Hinton agar media at a temperature range of 45-50ºC. Subsequently, multiple paper plates were immersed in a test solution containing a strong leaf ethanol extract and nanoparticles with different concentrations. Characterization reveals that Crassocephalum crepidioides is composed of many bioactive components, including alkaloids, flavonoids, saponins, tannins, and steroids. The analysis of the extract revealed that it contained 5.92% water, 12.86% total ash, 1.55% acid-insoluble ash, 14.33% water-soluble essence, and 9.34% ethanol-soluble essence. The nanoparticles had a size of 64.37 nm and exhibited antibacterial activity of extract ethanol at a concentration of 50% with a diameter of 12,00 mm. The minor concentration of the extract had a diameter of 3,166 mm, while the nanoparticles showed antibacterial activity at a concentration of 50% with a diameter of 10,53 mm. The minor concentration of the nanoparticle had a diameter of 3,00 mm The activity test results of the ethanol extract of Crassochepallum crepidioides with nanoparticles demonstrated that the inhibitory diameter of the ethanol extraction results remained more strong compared to the nanoparticles of simplisia powder.

Introduction

Crassocephalum crepidioides (Benth.) S. Moore leaves are distributed over several regions of Asia, Africa, and Australia. Crassocephalum crepidioides (Benth.) S. Moore leaves are conventionally used for the treatment of indigestion, stomach ache, and wounds. Crassocephalum crepidioides has been shown to possess many biological activities, including antibacterial, hypoglycemic, antioxidant, anti-inflammatory, anticancer, and antidiabetic properties. According to Galba Jean et al. (2022), the leaves of Crassocephalum crepidioides contain many beneficial substances such as phenolic compounds and flavonoids. Additionally, the essential oil derived from these leaves includes cubebene, farnesene, and caryophyllene (Asil, 2023). Nanoparticles are solid particles that are distributed and have diameters ranging from 10 to 100 nm. According to Muller et al. (2000), the use of small particle sizes is employed

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in the design and composition of materials, resulting in the development of novel features and functionalities.

Methods

This research included the deliberate collection of Crassochepallum crepidioides leaves, without doing any comparative analysis with leaves from other locations of the same plant species. The specimen was identified at the Herbarium Medanese Universitas Sumatera Utara (MEDA) under the reference number 924/MEDA 2023. The detected plant sample is Crassochepallum crepidioides, which belongs to the Crassocephallum family.

Crepidioides (Benth) is a species of Crassochepallum. The leaves of S. Moore are subjected to maceration using a 96% ethanol solution. A quantity of 500 grams of simplisia powder is placed in a container with a cover. It is then mixed with 5 liters of 96% ethanol solvent. The mixture is agitated for the first 6 hours and allowed to stand for 18 hours. During this time, the mixture is sometimes stirred, and the resulting filtrate (maserat I) is filtered and collected. Perform the extraction procedure on the pulp once more using 96% ethanol, with a volume of 2.5 L, in order to get Maserati II. In order to generate a concentrated extract, the two maserats were mixed and subjected to evaporation using a Rotavapor device at a temperature of 40 °C.

Aggregate ash content: A 3 g portion of the crushed sample is placed into a silicate crust that has been incandescent. The crust is incandescent slowly until the charcoal is completely consumed, then it is allowed to cool down and then weighed again. If the charcoal could not be eliminated, a hot water filter was introduced using filter paper that does not contain ash. The residual paper and filter paper are incinerated in the same crucible. The filtrate was transferred into a crucible, followed by evaporation and subsequent weighing. The determination of the ash content of the desiccated substance was performed. The acid insoluble ash content was determined by boiling ash obtained from the ash content determination process with 25 ml of dilute sulfuric acid P for a duration of 5 minutes. The resulting ash, which was insoluble in the acid, was then filtered to achieve a fixed weight and then weighed. The concentration of acid-insoluble ash in the dry material was determined.

Analysis of the concentration of water-soluble essence: A quantity of 5 grams of basic powder was measured and then subjected to maceration for a duration of 24 hours using 100 milliliters of water-chloroform (2.5 milliliters of chloroform in 1 liter of water). The mixture was shook once during the first 6 hours, allowed to stand for 18 hours, and then filtered. In a calibrated shallow, flat-bottomed dish, a volume of 20 ml of the filtrate was subjected to evaporation. The remaining substance was heated to a constant temperature of 105oC. The percentage of water-soluble moisture content was determined for the dried material. Analysis of the concentration of ethanol-soluble substances, 5 grams of simplisia powder were measured and then subjected to maceration for 24 hours with 100 milliliters of 96% ethanol. This process included shaking the flask for the first 6 hours, allowing it to settle for 18 hours, and then filtering it. A volume of 20 mL of the filtrate was removed using a cup and then heated at a temperature of 105 oC until it reached a constant weight. The concentration of ethanol-soluble essence in the dried material was determined.

Glycoside Identification: After filtration using a combination of 95% ethanol with water and 10 ml of 2 N hydrochloric acid, a total of 3 g of extract was obtained. The resulting mixture was refluxed for a duration of 2 hours. Subsequently, 20 ml of the filtrate was combined with 25 ml of distilled water. A solution of 25 ml of 0.4 M lead (II) acetate was agitated and left undisturbed for 5 minutes. It was then filtered using a mixture of 20 ml of isopropanol and chloroform at a ratio of 2:1. The resulting water was then evaporated. The remaining substance was reconstituted using 2 milliliters of methanol. The experimental solution was transferred into a test tube and subjected to evaporation, with a volume of 0.1 mL. A purple ring was seen at the interface of the two liquids, showing the existence of sugar bonds, when 2 mL of water and five drops of Molisch reagent were combined, followed by the addition of 2 mL of concentrated sulfuric acid. In 1995, the Directorate General of POM

The Steroids/Triterpenoids were identified by weighing 1 g of extract, macerating it with 20 ml of N-hexane for 2 hours, filtering it, and then evaporating the resulting filtrate. In the remaining portion, a little quantity of the Liebermann-Burchard reagent was introduced. The visualization of a blue-green or purple-red hue indicates the existence of steroids or triterpenoids. Identification of A 1 g sample of tannin was weighed, subjected to boiling for a duration of 3 minutes in 100 ml of distilled water, and then cooled and filtered. 1-2 drops of a 1% iron (III) chloride reagent were added to the filtrate. The presence of tannin was indicated by the appearance of a blue-black or green-black tint. The saponins were identified by weighing 0.5 g of extract, transferring it into a test tube, adding 10 ml of boiling water, allowing it to cool, and thereafter rapidly shaking it for a duration of 10 seconds. The foam reaches a height of 1-10 cm and remains stable for a minimum of 10 minutes, even after the addition of 1 drop of hydrochloric acid. The presence of saponins is indicated by the value 2N.

The process of identifying flavonoids A quantity of 10g of extract was combined with 100 ml of boiling water, which was then cooked for a duration of 5 minutes. The resulting mixture was then filtered into 5 ml of filtrate. From this filtrate, 0.1 g of magnesium powder, 1 ml of strong hydrochloric acid, and 2 ml of amyl alcohol were added. The mixture was then agitated and allowed to separate. According to Farnsworth & Norman (1966), the presence of a red, yellow, or orange tint in the amyl alcohol layer indicates the presence of flavonoids. The mashed siplisia will undergo grinding using a high-energy ball mill tool, which operates on a reciprocal mechanism (Osborne et al., 2023). This mechanism involves the friction between two surfaces due to the pressure generated surpassing the inherent strength of the particles. This leads to frustration (fractures or cracks), friction force (shear force), fragmentation of particles into multiple parts, and disaggregation resulting from the collision between aggregates (Pollastri et al., 2022).

The characterisation of nanoparticles involves the use of a Particle Size Analyzer and Scanning Electron Microscopy methodologies. The sample is solubilized in a 3 ml solution of ethanol. Subsequently, the solution was transferred into a tube with a maximum altitude of 15 mm. A VASCO nanoparticle analyzer was used to measure the diameter distribution of the specimens. The test was performed using the Dynamic Light Scattering (DLS) technique with the aid of a Zetasizer Nano ZS instrument (Farkas & Kramar, 2021). Electron microscopy at a high resolution A double-sided tape was used to attach the specimen to the tip. The electrical conductivity of the powder was enhanced by subjecting it to a 30-second conditioning process using a thin layer of platinum beam from the coating. This conditioning process was conducted at a pressure below 2 Pa and a current strength of 30 mA. The authors (Sabdoningrum et al., 2021) captured photographs using an electron voltage of 10 kV at the specified magnification.

The antibacterial activity of Crassochepallum crepidioides (Benth) was evaluated by using the ethanol extract and nanoparticles. S. Moore departs. The agar diffusion technique was used to test it on paper plates. A petri dish was used to transfer 0.1 ml of bacterial inoculum, followed by the addition of 15 ml of Mueller Hinton agar medium in a temperature range of 45-50ºC. There was homogenization of the Petri dish. Subsequently, a series of paper plates were immersed in a test solution containing a potent leaf ethanol extract and Crassochepallum crepidioides (Benth) on the solid medium. Nanoparticles of S.Moore leaf with different concentrations were deposited. Dimethyl sulfoxide serves as a control and is thereafter incubated at a temperature of 37oC for a duration of 24 hours. Following this, the diameter of the inhibitory region is measured.

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Result and Discussion

The calculated water content yields a value of 5.92%, which satisfies the specified threshold of less than 10%.The measurement of water content is a crucial characteristic used to assess the residual water present during the drying process, as well as to evaluate the stability of simplisia. The water-soluble essence content yields more significant findings compared to ethanol, suggesting a higher solubility of chemicals in water. The percentage of water-soluble essence was found to be 14.33%. The study aimed to assess the solubility of water-soluble essence in polar solvents, including both polar and nonpolar chemicals, by determining the athanol-soluble Simplicia content of 9.34% and the ethanol-soluble simplicia content.

The presence of ash content is associated with the mineral composition of the substance, including both organic and inorganic salts. The ash content serves as an indicator of the sample's appropriateness for further processing. The assessment of ash content offers a comprehensive understanding of the mineral composition, both internal and external, during the whole process leading up to the production of the simplicia. The total ash percentage in essential leaves was determined to be 12.86%. The outcome is seen in Table 1.

Table 1. Simplicia Characterization

Characterization	Results
a water content	5.92 %
Total ash content	12.86 %
an acid-insoluble ash content	1.55 %
water-soluble essence content	14.33%
an ethanol-soluble simplicia content	9.34%

Simplicia contain chemical substances from the alkaloids, flavonoids, tannins, and steroid/triterpenoid groups (Table 2) screening study of simplicia result.

Table 2. Phytochemical Screening

Compound Group	Simplisia
Alkaloid	+
Flavonoid	+
Glikosida	+
Saponin	+
Tanin	+

+ indicates that the sample contains compounds

Nanoparticle with *high-energy ball mill*, rocessed in the laboratory of the integrated research unit of the Universitas Sumatera Utara (Figure 1).

Figure 1. Crassochepallum crepidiodes (Benth). S.Moore nanoparticles

The experiment using the particle size analyzer was carried out at the Nanotechnology Laboratory affiliated with the University of North Sumatra. The results of the particle size analyzer test indicated that the nanoparticles produced had a size of 64.37 nm, as seen in Figure

2. Abdullah et al. (2008) provide a definition of nanoparticles as particulates or solid particles that are distributed and possess particle sizes within the range of 1-100 nm. Nanoparticles may be manufactured from leaf simplisia powder using the ball mill process, as shown by the findings.

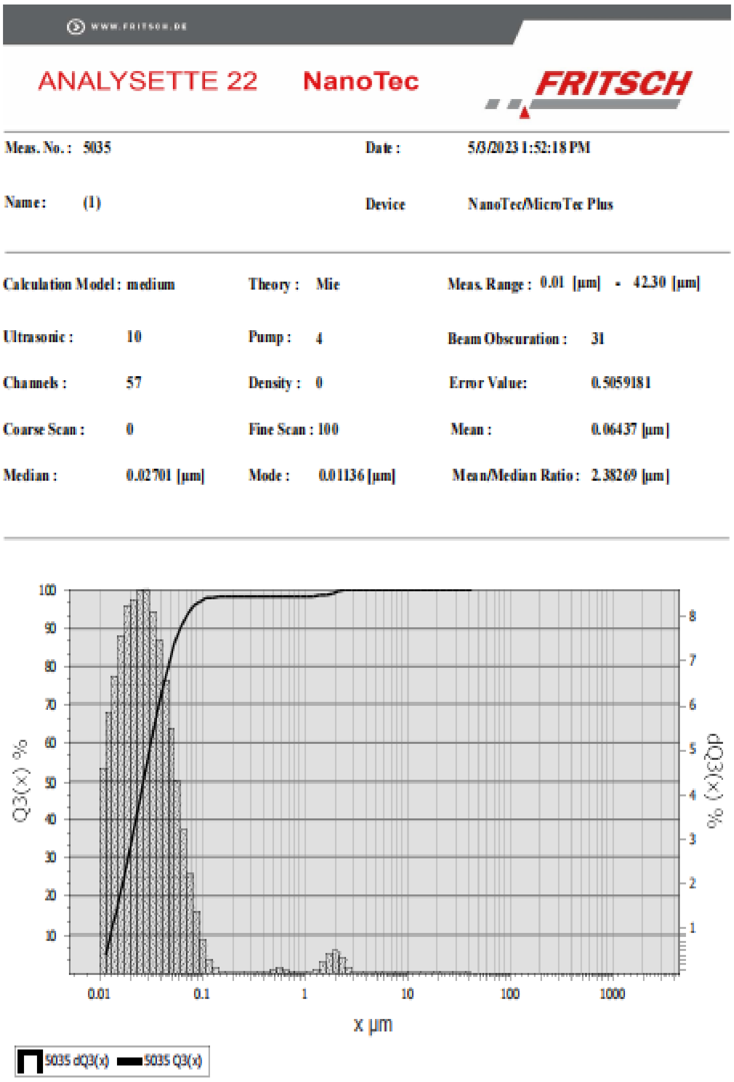
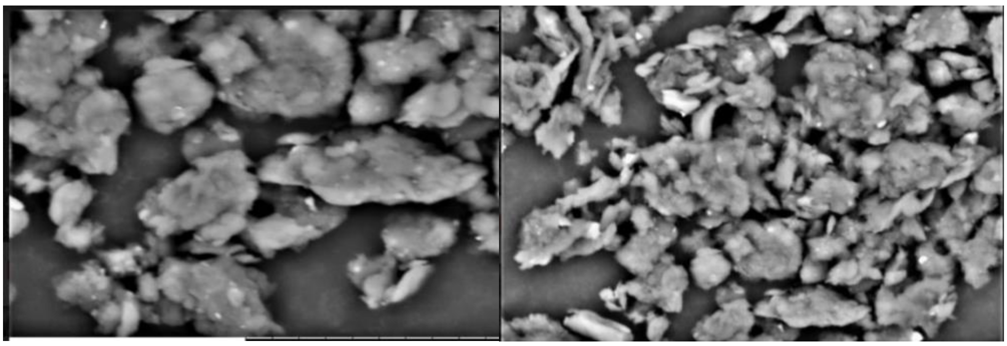


Figure 2. Results Particle Size Analyzer

The scanning electron microscopy assay was conducted at the Integrated Laboratory of the University Sumatera Utara.



2500x *5000x*

Figure 3. Morphology of Nanoparticles Crassochepallum crepidioides (Benth). S.Moore Magnification 1000 SEM

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The surface of Crassochepallum crepidioides (Benth) was seen using scanning electron microscopy. The nanoparticles of S. Moore leaf exhibited irregularity, nonuniformity, and amorphousness, as seen in Figure 3. This finding aligns with prior scholarly investigations (Sabdoningrum et al., 2021). During the milling procedure, the particles undergo agglomeration, resulting in the surface state of Crassochepalum crepidioides (Benth). The nanoparticles of S.Moore leaves exhibit irregularity, non-uniformity, and amorphousness.

The ethanol extracts of Crasochephallum Crepidioides (Benth) leaves exhibit antibacterial action. According to Alves et al. (2000), S. Moore demonstrated that the highest diameter required to impede the development of Staphylococcus aureus was seen at a concentration of 50%, resulting in a diameter of 12.00 mm (indicating partial activity). Conversely, a lower concentration of 1%, leading to a diameter of 3.16 mm (indicating inactivity), exhibited the same inhibitory effect.

Table 3. *Antibacterial Activity of Ethanol Extract* Crasochephallum Crepidioides

Consentrations (%)	**Antibacterial Activity (mm)**
1	3.16
2	5.33
3	6.33
4	7.20
5	7.76
6	8.03
7	8.53
8	8.73
9	9.03
10	9.16
20	10.36
30	10.86
40	11.76
50	12.00

Table 4. *Antibacterial Activity of Nanoparticle Crasochephallum Crepidioides*

Consentrasions (%)	**Antibacterial Activity (mm)**
1	0
2	3.00
3	4.00
4	6.10
5	7.00
6	7.60
7	7.80
8	8.10
9	8.40
10	8.50
20	9.00
30	9.30
40	10.20
50	10.53

The The study investigated the antibacterial activity of nanoparticles derived from the leaves of Crassochepallum Crepidioides. The results indicated that the nanoparticles had the greatest inhibitory effect on the growth of Staphylococcus aureus at a concentration of 50%, with a

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diameter of 10.53 mm (indicating partial activity) and the minor concentration of nanoparticle had a diameter 3.00 mm at a concentration 2%. However, the results indicated that the extraxt ethanol Crassochepallum crepidioides had the greates inhibitory effect on the growth of Staphylococcus aureus at the concentration of 50%, with a diameter 12,00 (partial activity) and the minor concentration of the extract had a diameter of 3,166 mm at a concentration 1%

Simplicia powder is composed of many substances, including alkaloids, tannins, flavonoids, steroids, and glycosides. With a water content ranging from 5% to 10%. The percentage of water-soluble essence was 14.33%, the content of athanol-soluble extract was 9.34%, and the content of acid-insoluble ash was 7.5%. A thick extract of 210 grams (21% yield) was produced by mixing 96% ethanol solvent with 1000 grams of sintrong leaf simplisia powder. The leaf simplicia powder of Crassochepallum crepidioides was transformed into leaf nanoparticles using the Ballmill technique, resulting in a particle size of 64.37 nm. The nanoparticles exhibited an uneven, non-uniform, and amorphous surface as seen using scanning electron microscopy. The ethanol extract derived from sintrong leaves exhibited significant antibacterial activity, as shown by its ability to inhibit the development of Staphylococcus aureus bacteria. Notably, at a concentration of 50% extract, the inhibition zone width was 12.00 mm, indicating a moderately active category. Diameter of 3.166 mm and a concentration of 1% were classified as inactiv. The antibacterial activity of nanoparticles at concentrations ranging from 1% did not exhibit inhibition. However, inhibition was seen at concentrations ranging from 2% to 50%.

Conclusion

According to Barku et al. (2013) literature, the antibacterial activity of sintrong leaf ethanol extract and sintrong leaf nanoparticles is believed to be caused by the ability of tannin and alkaloid compounds, specifically flavonoid compounds, to dissolve the peptidoglycan components in bacterial cells. This hinders the formation of the cell wall layer and leads to cellular demise. The ethanol extract of Crassochepallum crepidioides (Benth) was compared for its antibacterial activities. The use of S. Moore leaves in conjunction with nanoparticles demonstrated that the effectiveness of Crassochepallum crepidioides ethanol extraction was more pronounced in terms of inhibition diameter compared to the nanoparticles derived from Crassochepallum crepidioides (Benth). Powdered S. Moore leaf simplisia.

Suggestion

The suggestion of this study is for further researchers to conduct research by comparing the antibacterial activity of ethanol extracts with nanoparticles of Crassochepallum crepidioides leaves that have gone through the extraction process.

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