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Total Phenolic Content and Antibacterial Activity of Nut Grass (*Cyperus rotundus* L.) Extract

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Abstract. The objectives of this research was to study the macroscopic, the microscopic and phytochemicals characterization of raw materials, total polyphenol content and the antibacterial activity of nut grass extracts (*Cyperus rotundus* L.) against *Shigella dysenteriae*, *Salmonella typhimurium*, *Staphylococcus epidermidis*, and *Pseudomonas aeroginosa*. The macroscopic and the microscopic characterization of raw materials were done with the determination of the water contents, the water soluble contents, the ethanol soluble contents, total ash and acid soluble ash. The extract was prepared by percolation using n-hexane, ethanol, and ethyl acetate as solvents. Phytochemical screening was done on the raw materials as well as n-hexane, ethyl acetate, and ethanol extracts. Total phenol contents were tested with the Folin-Ciocalteu reagent method. The antibacterial activity test was done by measuring the diameter of the inhibition zones using the diffusion agar method. The results showed that raw material retrieved 7.33% water content, 21.85% water soluble contents, 7.9% ethanol soluble contents, 1.53% total ash, and 0.67% total insoluble-acid ash. The phytochemical study showed that the nut grass contains various secondary metabolites including flavonoids, glycosides, saponins, anthraquinone glycosides, and steroid/triterpenoids. The total polyphenol contents of ethanol extracts were expressed as catechin equivalents 1.1616 mg/g extract. All of the nut grass extract showed antibacterial activity. Ethyl acetate extract of nut grass indicated the highest antibacterial activity against *Shigella dysenteriae, Salmonella typhimurium, and Pseudomonas aeroginosa.* Ethanol extract was only effective against *Staphylococcus epidermidis*.

Keywords: Antibacterial Activity, Bacteria, *Cyperus Rotundus* L Extracts, Phenolic

Abstrak. *Tujuan penelitian adalah untuk mengetahui skrining fitokimia, karakterisasi simplisia, kandungan total fenol dan uji aktivitas antibakteri dari beberapa ekstrak umbi rumput teki (Cyperus rotundus L.) terhadap bakteri Shigella dysenteriae, Salmonellatyphimurium, Staphylococcus epidermidis, Pseudomonas aeroginosa. Karakterisasi serbuk simplisia meliputi pemeriksaan makroskopik, mikroskopik, penetapan kadar air, penetapan kadar sari larut dalam air, penetapan kadar sari larut dalam etanol, penetapan kadar abu total dan penetapan kadar abu yang tidak larut dalam asam. Ekstraksi dilakukan dengan cara perkolasi dengan menggunakan pelarut yang berkesinambungan (n-heksana, etilasetat, dan etanol). Skrining fitokimia dilakukan terhadap serbuk simplisia, ekstrak n-heksana, ekstrak etilasetat dan ekstrak etanol. Kandungan total fenol diuji dengan metode Folin-Ciocalteau. Uji aktivitas antibakteri dilakukan secara in vitro dengan mengukur diameter zona hambat sekitar punch hole menggunakan metoda difusi agar. Hasil skrining fitokimia diperoleh adanya senyawa golongan alkaloida, flavonoida, glikosida, glikosida antrakuinon, saponin dan steroida/triterpenoida. Hasil karakterisasi serbuk simplisia diperoleh kadar air 7,33%, kadar sari larut dalam air 21,85%, kadar sari larut dalam etanol 7,9%, kadar abu total 1,53%, dan kadar abu tidak larut dalam asam 0,67%. Kadar total fenol ekstrak etanol rumput teki 1,1616 mg Katekin/g ekstrak. Semua ekstrak yang diujikan memiliki kemampuan sebagai antibakteri. Aktivitas antibakteri yang paling efektif ditunjukkan oleh*

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ekstrak etil asetat terhadap bakteri Shigella dysenteriae, Salmonella typhimurium, dan Pseudomonas aeroginosa, sedangkan ektrak etanol lebih efektif terhadap bakteri Staphylococcus epidermidis.

***Kata kunci**: Aktivitas Antibakteri, Bakteri, Cyperus Rotundus L, Ekstrak, Total Fenol*

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1. Introduction

Cyperus rotundus L., known as nut grass, is a herbaceous monocotyledonous plant that belongs to Cyperaceae. The extensive distribution of the nut grass is due to its ability to adopt to a wide range of environments. The spread of the plant is through its rhizomes. They may extend upward, downward or horizontally [1,2,3]. The plant is a herbal medicine widely used as analgesic, sedative, and antispasmodic drugs. Additionally, it is also used to treat stomach disorders and diarrhea [4]. Its rhizomes are used to treat dysentery, bronchitis, amenorrhea, and blood disorders [5]. The phytochemical analysis of nut grass rhizome revealed that alkaloids, tannins, flavonoids, glycosides, saponins, sesquiterpenoids, and essential oil were present in the plant [1,3].The prevalence of infections have increased from year to year and microbial resistant becomes an important global issue in the therapeutic problem. The uses drug-derived plants have been reported to be safe without side effects. Nut grass has been used by the people who live in few areas of Indonesia as a home remedy against stomach disorders especially dysentery. In addition, nut grass has possible antimicrobial properties due to the presence of polyphenols in the plant.

Therefore, there is a need to study the macroscopic, microscopic and phytochemical properties of the raw materials, total polyphenol contents, and the antibacterial activity of nut grass extracts against *Shigella dysenteriae*, *Salmonella typhimurium*, *Staphylococcus epidermidis*, and *Pseudomonas aeroginosa*.

2. Materials and Method

2.1. Materials

The materials used in this research were nut grass tubers (*Cyperus rotundus* L.), nutrient agar, nutrient broth, *Staphlococcus epidermidis* (ATCC No. 35668), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella typhimurium* (ATCC 29213) and *Shigella dysenteriae* (ATCC 25931), sodium chloride 0.9% solution, alpha-naphthol, amylalcohol, hydrochloric acid, acetic acid anhydrate, glacial acetic acid, nitric acid, sulphuric acid, iron (III) chloride, bismuth (III) nitrate, dimethylsulfoxide, n-hexane, ethanol, ethyl acetate, potassium iodide, chloral hydrate, anhydrous sodium sulphate, mercury (II) chloride, magnesium powder, zinc powder, lead (II) acetate, potassium bromide and isopropanol. Those chemicals and solvents used were analytical grade and are commercially available from Merck.

2.2. Equipments

The equipments used in this study were oven, autoclave, blender, incubator, *freeze dryer*, *laminar air flow cabinet*, refrigerator, analytical scale, analytical balance, rotary evaporator, micropipette, and glass wares.

2.3. Phytochemical Analysis

Phytochemical analysis of the dried raw material of nut grass was done on the extracts of n-hexane, ethyl acetate and ethanol that comprised the examination of alkaloids, glycosides, anthraquinone glycosides, saponins, flavonoids, tannins, and triterpenoid/steroid.

2.4. Preparation of Nut Grass Tuber Extracts

The nut grass tuber extracts were prepared according to the procedure as shown in Figure 1.

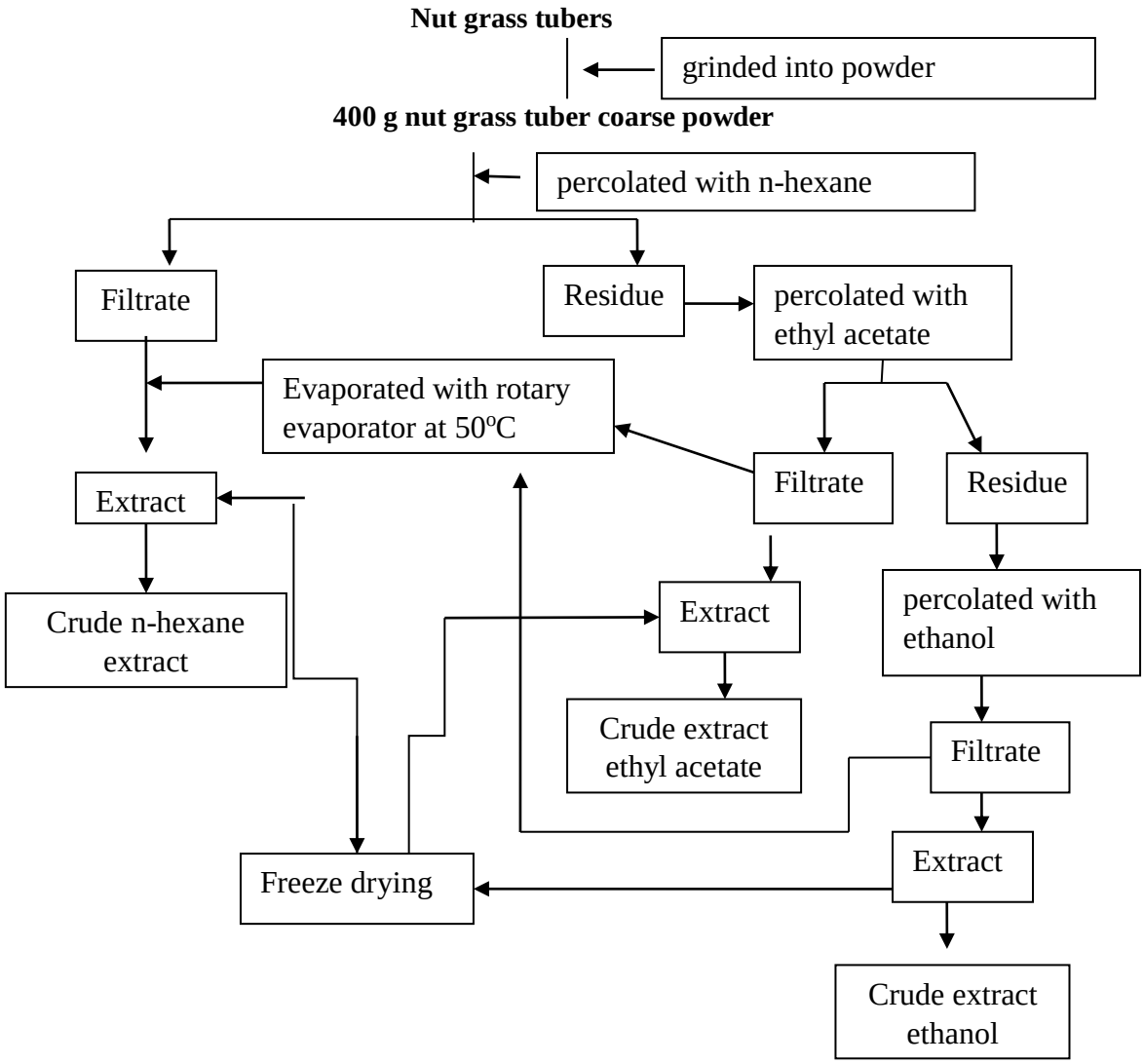

Figure 1. Flow Chart of the Extracts Preparation

2.5. Preparation and Dilution of Nut Grass Tuber Extracts

Five grams of the extract were dissolved in 10 ml of dimethylsulfoxide in a volumetric flask to obtain extract solution with concentration of 500 mg/ml. The solution was then diluted with dimethylsulfoxide to prepare the extract solutions with concentrations of 400 mg/ml, 300 mg/ml, 200 mg/ml, 100 mg/ml.

2.6. Preparation of Inoculums

The cultured bacteria were taken with OSE needle, then suspended with 10 ml of 0.9% of NaCl in a tube, subsequently shaked and matched with McFarland standards with concentration of 10^8 colony-forming unit (cfu)/ml, and finally diluted to10^6 cfu/ml. Inoculums preparations of *Salmonella typhimurium*, *Staphylococcus epidermidis* and *Pseudomonas aeroginosa* were prepared in the same way as that of *Shigella dysenteriae*.

2.7. Estimation of Total Polyphenol Content

The total polyphenol content present in the ethanol extract was analyzed using the Folin-Ciocalteau reagent as described by Nagulendran *et al* (2007) [1]. One milliliter of an extract solution was transferred into a 10 ml flask, and then diluted to obtain the final volume of 8 ml by the addition of distilled water. Then, 0.5 ml of Folin-Ciocalteu reagent was added into this mixture and allowed for one minute. Next, 1.5 ml of 20% of Na_2CO_3 was added. Subsequently, the mixture was shaken on a shaker for 90 minutes at room temperature, and then the absorbance of the solution was measured at a wavelength of 760 nm. Cathecin was used as the standard of calculation.

2.8. Antibacterial Activity Test of Nut Grass Extracts

As much as 0.1 ml of inoculum of *Shigella dysenteriae* suspension with concentration of 10^6 cfu/ml was transferred into a sterile Petri dish, then 20 ml of *Nutrient agar* with a temperature of 45-50°C was poured. The Petri dish was shacked until the medium and bacteria suspension were homogen and solid. Holes were made using a hole punch to place 0.1 ml of each of the n-hexane, ethyl acetate, and ethanol nut grass extracts in every hole at various concentrations. Pre-incubation was done for 15 minutes, and then was incubated in an incubator at a temperature of 36 ± 1°C for 18-24 hours. The diameters of growth resistance area noted as transparent area were measured around the hole. The same procedure was carried out for *Salmonella typhimurium, Staphylococcus epidermidis* and *Pseudomonas aeroginosa*.

3. Results

3.1. Characterization of Raw Material

The characterization result of coarse powder of nut grass tubers is described in the Table 1. It is noticeable that all of the requirements of raw materials in Materia Medica Indonesia (MMI) are met as the coarse powder of nut grass tubers [6].

Table 1. Characteristics of Coarse Powder of Nut Grass Tubers

Parameters	Result (%)	Requirements in MMI, 4th Ed (%)
Water content	7.33	Not more than10
Water soluble content	21.48	Not less than 6
Ethanol soluble content	7.90	Not less than 3.5
Total ash	1.53	Not more than 4.5
Total acid-insoluble ash	0.67	-

3.2. Phytochemical Contents

Identification of dried powder for chemical compounds which were present in the n-hexane, ethyl acetate and ethanol of nut grass tubers extracts with reagents for secondary metabolites classification, the data can be seen in the Table 2.

Table 2. Phytochemical Analysis Result of Row Material and Extract of Nut Grass Tubers

Tested Phytochemical compounds	Nut grass tubers (*Cyperus rotundus* L)			
	Raw material	Extract		
		n-hexane	Ethyl acetate	Ethanol
Alkaloids	+ (color)	-	+	+(color)
Flavonoids	+ (Red)	-	+ (Red)	+ (Red)
Saponin	+ (foam)	-	-	+ (foam)
Tannin	-	-	-	-
Glycosides	+ (purple)	-	+ (purple)	+ (purple)
Anthraquinone Glycosides	+	-	-	+
Steroids/Triterpenoids	+ (purple)	+ (purple)	-	-

Note: (+) = compound present
(-) = compound not present (absence)

The addition of Mg powder and Zn powder with concentrated hydrochloric acid gave a red color, indicating the presence of flavonoids. Glycosides were identified by the addition of Molisch's reagent and concentrated sulfuric acid, and formed a purple ring. The addition of 10 ml of hot water, cooled and then shaken vigorously for 10 seconds with a solid foam for not less than 10 minutes, as high as 1-10 cm and not disappear with the addition of 2N hydrochloric acid showed the presence of saponins. Addition Liebermann-Burchard gives purple color indicates the presence of steroid/triterpenoids. Finally, the addition of1% $FeCl_3$ gives green to brown color which indicates the presence of tannin.

3.3. Antibacterial activity of nut grass tuber extracts

The following Figure 2 was the result of the inhibition zones around the punch hole using the diffusion agar method.

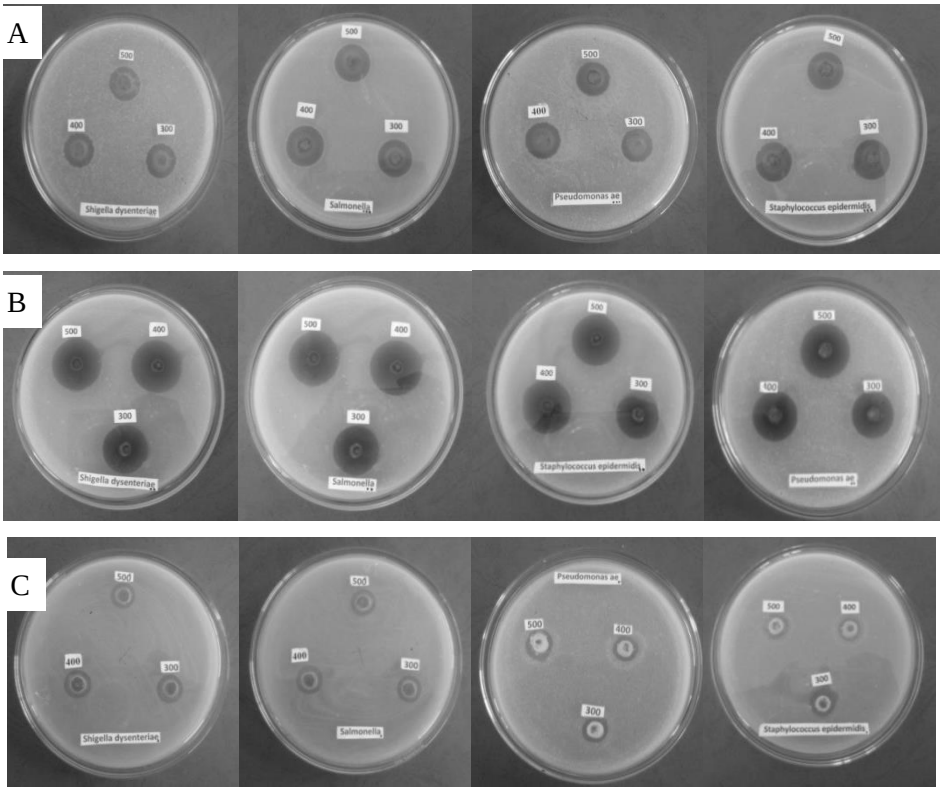

Figure 2. The Inhibition Zones Around the Punch Hole Using Diffusion Agar Method, (A) Ethanol extract; (B)Ethyl acetate extract (C) n-Hexane extract

The result of antibacterial activity test and the inhibition zone is presented in the Figure 3.

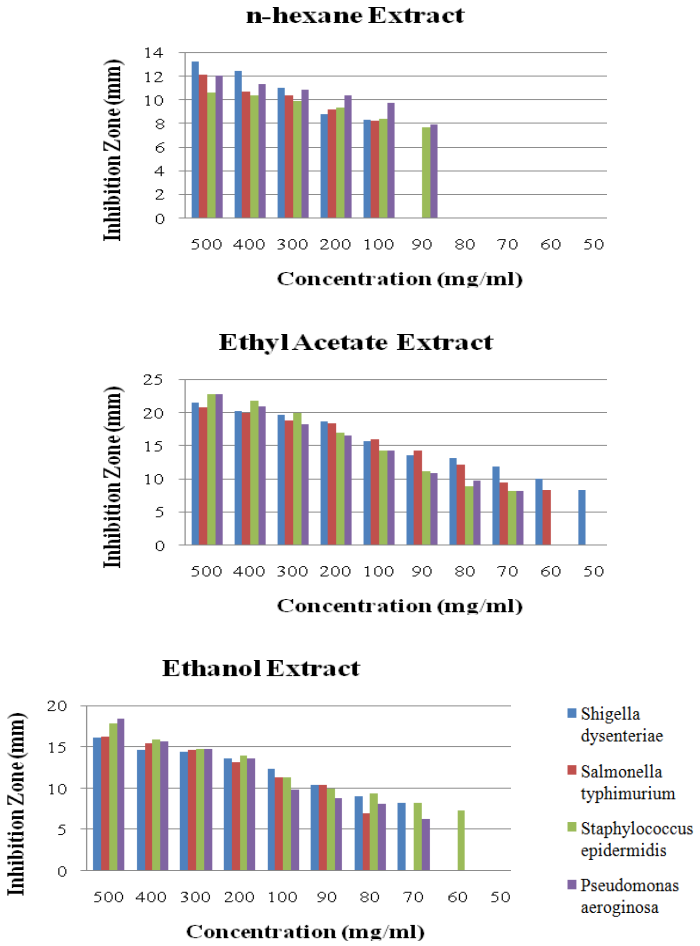

Figure 3. Inhibition Zone of Nut Grass Tuber Extracts Against Tested Bacteria

Based on the diagram, ethyl acetate extract of nut grass tubers shows the highest antibacterial activity by satisfactory inhibition the growth of *Shigella dysenteriae, Salmonella typhimurium, and Pseudomonas aeroginosa.* Ethanol extract was effective against *Staphylococcus epidermidis*.

4. Discussion

Based on the Table 1, the raw material contains more secondary metabolites than other extracts. The phytochemical groups of compounds present in raw material, including tannins, alkaloids, flavonoids, saponins, glycosides, and steroid/triterpenoids; while the n-hexane extract contains steroids/triterpenoids; ethyl acetate extract contains tannins, alkaloids, flavonoids, saponins, glycosides; and ethanol extract contain tannins, flavonoids, alkaloids, saponins, and glycosides. Previous phytochemical studies on *Cyperus Rotundus* revealed the presence of alkaloids, flavonoids, tannins, starchs, glycosides, furochromones, novel sesquiteroenoids, and two novel natural phenolic compounds (methoxy cyperotundol and cyperotundol) [7,3].

Phenolics are the most widespread secondary metabolite in the plant. These diverse groups of compounds have received much attention as a potential natural product. Flavonoid is the major phenolic compound in nature. A phenolic compound posses antimicrobial activity due to its protein denaturation ability in the bacteria that lead to cell death [1,9].

Based on the Figure 2, in vitro antimicrobial activity by agar well diffusion method was evaluated for ethanol, ethyl acetate and n-hexane extract. The ethyl acetate extract of nut grass tuber shows the highest antibacterial activity indicated by satisfactory inhibition on the growth of *Shigella dysenteriae* with the MIC 50 mg/ml, *Salmonella typhimurium* with the MIC 60 mg/ml, *and Pseudomonas aeroginosa* with the MIC 70 mg/ml. This antibacterial activity may be due to presence of polyphenolic (flavonoids), and the extraction of these compounds were more efficient with ethyl acetate than by n-hexane or ethanol.

Ethanol extract was effective against *Staphylococcus epidermidis* with the MIC 60 mg/ml and *Pseudomonas aeroginosa* with the MIC 70 mg/ml, while the n-hexane extract showed low antibacterial activity. Based on Kakaria, et al. (2014), the antimicrobial activity of ethanol extracts of *Cyperus rotundus* against *Staphylococcus epidermidis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Eschericia coli*, *Aspergillus niger* and *Candida albicans* were the most effective antimicrobial agent as compared to other extracts [7].

In this research, the ethanolic extract of nut grass tubers have mild antibacterial activity against four bacteria tested. According to Tambekar, *et al*. (2009), who compared the antibacterial activity of some herbs, one of them was *Cyperus rotundus* herb, showed that this herb had mild antibacterial activity at high doses [9]. Another part of nut grass has been reported to posses antibacterial activity was rhizome [7]. Uddin, *et al*. (2006) reported that the methanol extract of nut grass rhizome, given orally (205 and 500 mg/kg bw), showed significant antidiarrhea activity in castor oil induced diarrhea in mice [10]. Furthermore, the extract of other species, *Cyperus tegetum* Rox (MECT) also showed significant antidiarrhea activity using castor oil and magnesium sulfate-induced diarrhea in mice [11]. The oil of *Cyperus rotundus* showed a noticeable activity against gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis* [12,13].

5. Conclusions

The characterization of raw material shows that all of parameters meet the requirements of raw material specification in the *Materia Medika Indonesia*. The phytochemical components of nut grass shows that the extract contains alkaloids, flavonoids, glycosides, anthraquinone glycosides, saponins and steroid/triterpenoids. The present study suggests that ethyl acetate, ethanol and n-hexane possess antibacterial activity, and the former extract was more potent than other extracts.

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