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PHYTOCHEMICAL CONSTITUENTS, ANTIOXIDANT, AND CYTOTOXIC POTENTIAL OF THE ETHANOL EXTRACTS FROM DIFFERENT PLANT PARTS OF *Saccharum edule* Hassk.

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ABSTRACT

Several species of *Saccharum* exhibit antioxidant activity, among which *Saccharum edule* Hassk. is commonly used as a vegetable. This study aims to determine the phytochemical content, total phenolic, total flavonoid, total alkaloid, antioxidant activity, and cytotoxicity of a 96% ethanol extract of *S. edule* stem, leaf, bract, and flower. Extracts were obtained via maceration, followed by qualitative phytochemical screening. Total phenolics, flavonoids, and alkaloids were quantified using UV-Vis spectrophotometry, antioxidant activity by the DPPH method, and cytotoxicity by the BSLT assay. Phytochemical screening of the 96% ethanol extracts from the stem, leaf, and bract of *S. edule* revealed the presence of alkaloids, flavonoids, saponins, and tannins, while the flower extract contained only alkaloids, flavonoids, and saponins. The highest antioxidant activity was found in the 96% ethanol extract of the leaf, with an AAI value of 1.092 (strong category), total phenolic content of 5.112 ± 0.624 mg GAE/100g extract, and total flavonoid content of 2.514 ± 0.053 mg QE/100g extract. The highest lethal concentration (LC_{50}) was observed in the leaf and bract extracts (LC_{50} < 500 µg/mL), with alkaloid concentrations of 1.873 ± 0.129 mg CE/g extract and 1.840 ± 0.074 mg CE/g extract, respectively. The results suggest that the 96% ethanol extracts of the leaf and bract of *S. edule* have the potential for further testing as antioxidant and anticancer agents.

Keywords: *Saccharum Edule* Hassk., Total Phenolic, Total Flavonoid, Total Alkaloid, Antioxidant, Cytotoxicity.
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INTRODUCTION

Free radicals are highly reactive molecular species produced endogenously through mitochondrial respiration, enzymatic reactions, and immune responses, as well as exogenously from pollutants, UV radiation, gamma rays, X-rays, and cigarette smoke.[1] Excessive accumulation of free radicals can disrupt cellular homeostasis by inducing oxidative damage to nucleic acids, proteins, lipids, and DNA, thereby contributing to the pathogenesis of various chronic and degenerative diseases such as hypertension, atherosclerosis, neurological disorders, diabetes, asthma, aging, and cancer.[2] Antioxidants play a crucial role in neutralizing these harmful species and mitigating oxidative stress, and they are broadly classified as synthetic or natural.[3] However, the prolonged use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has been associated with toxic effects, allergic reactions, and carcinogenicity, which has increased interest in safer, plant-derived alternatives.[4] Phenolic compounds, particularly flavonoids, are abundant in plants and play a key role in antioxidant defense by neutralizing reactive oxygen species (ROS), thereby reducing the risk of cardiovascular disease, inflammation, cancer, and diabetes.[5,6] Flavonoids also exhibit additional pharmacological properties, including antimicrobial, anti-inflammatory, and hepatoprotective effects, which further highlight their relevance in the prevention of chronic diseases. In parallel, alkaloids, nitrogenous compounds derived from plants, are recognized for their wide spectrum of biological activities, such as antioxidant, cytotoxic, antimicrobial, and anticancer effects.[7] Numerous plant species produce alkaloids as part of their defense mechanisms, making them an important target for drug discovery and natural product research. The Brine Shrimp Lethality Test (BSLT) serves as a simple, rapid, and cost-effective preliminary bioassay to evaluate

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the general cytotoxic potential of plant extracts, with several studies confirming a correlation between alkaloid content and cytotoxic activity.[8] *Saccharum edule* Hassk. It is a species of grass from the Poaceae family, widely distributed in Sulawesi. In Central Sulawesi, local communities consume the flowers of *S. edule* as a vegetable. Previous studies have demonstrated that Saccharum species, such as *Saccharum officinarum* and *Saccharum munja*, exhibit significant antioxidant activity, which plays a crucial role in protecting against cellular damage, a key factor in developing various degenerative diseases.[9,10] The composition of secondary metabolites in Saccharum species, particularly those with antioxidant properties, is influenced by genetic and environmental factors. This makes Saccharum valuable as a source of sugar and a promising source of bioactive compounds with potential health benefits. This study aims to evaluate the antioxidant activity and toxicity of ethanol extracts from various parts of *S. edule* (stem, leaf, bract, and flower), to provide scientific insights to support the development of this plant in the health sector, particularly in pharmaceuticals.

EXPERIMENTAL

Plant Materials and Extraction Procedure

Saccharum edule Hassk. was collected from cultivated plants in Sigi, Central Sulawesi, Indonesia. The Biodiversity Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Tadulako University, recognised the plant and gave the voucher number 127/UN.28.UPA-SDHS/LK/2023. The *S. edule* simplicia was then crushed and macerated in 96% ethanol at 1:10 (w/v) for 72 hours. The filtrate was then concentrated with a rotary vacuum evaporator and a water bath heated to 50°C to produce a dry extract.

Phytochemical Screening of Secondary Metabolites

The phytochemical constituents of the ethanol extract of *S. edule* were determined using standard qualitative methods, including the Shinoda test for flavonoids, the foam test for saponins, the Ferric chloride test for tannins, and the Dragendorff test for alkaloids.[11-12]

Determination of Total Phenolic

The total phenolic content was determined using the Folin-Ciocalteu reagent with slight modifications.[13] 10 milligrams of the extract were dissolved in 10 mL of 96% ethanol to achieve a final concentration of 1000 µg/mL. Subsequently, 0.5 mL of the test sample was combined with 5 mL of a Folin-Ciocalteu reagent solution (diluted at a 1:10 ratio) and 4 mL of 1 M sodium carbonate. The resulting mixture was then incubated for 15 minutes, after which its absorbance was recorded using a UV-Vis spectrophotometer at a wavelength of 748.0 nm. The total phenolic content was quantified and expressed in milligrams of gallic acid equivalent per 100 grams of dry weight extract (mg GAE/100g DW).

Determination of Total Flavonoid

The total flavonoid content was determined using the Aluminum chloride test with slight modifications.[14] 10 milligrams of the extract were dissolved in 10 mL of 96% ethanol to obtain a 1000 µg/mL concentration. To 0.5 mL of the test sample, 1.5 mL of 96% ethanol, 0.1 mL of 10% Aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water were added. The prepared mixture was subjected to incubation for 30 minutes, after which its absorbance was determined utilizing a UV-Vis spectrophotometer set at a wavelength of 444.8 nm. The total flavonoid content was quantified and expressed as milligrams of quercetin equivalent per 100 grams of dry weight extract (mg QE/100g DW).

Determination of Total Alkaloid

The total alkaloid content was determined using the bromocresol green (BCG) test with slight modifications.[15] 100 milligrams of the extract was dissolved in 5 mL of 2N hydrochloric acid (HCl) and subjected to agitation. The resulting solution was subsequently washed three times with 10 mL of chloroform ($CHCl_3$) using a separating funnel, after which the chloroform phase was discarded. The remaining aqueous phase was neutralized with 0.1N sodium hydroxide (NaOH), adding 5 mL of bromocresol green (BCG) solution and 5 mL of phosphate buffer. The mixture was then extracted with 5 mL of $CHCl_3$ while being stirred at a speed of 1500 rpm for 15 minutes, which was repeated twice. The collected chloroform phases were pooled and evaporated under a nitrogen gas stream, and the resulting residue was subsequently dissolved in 10 mL of chloroform. Finally, the solution was subjected to a tenfold

dilution, and its absorbance was measured at a wavelength of 275 nm. The total alkaloid content was expressed as milligrams of caffeine equivalent per gram of dry weight extract (mg CE/g DW).

Antioxidant Activity

The antioxidant potential of the extract was evaluated based on its capacity to neutralize free radicals through the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay.[16] The extract was dissolved in methanol at varying concentrations. 1 mL aliquot of the prepared extract was then combined with 1 mL of a 0.1 mM DPPH solution, and the resulting mixture was incubated in the dark at room temperature for 30 minutes. Following incubation, the absorbance of the solution was recorded at 515.3 nm using a UV-Vis spectrophotometer. Methanol served as the blank, while the DPPH solution functioned as the control. The extent of DPPH radical inhibition was determined using the following formula:

$$\text{Inhibition (\%)} = [(\text{Abs Control} - \text{Abs Sample}) / \text{Abs Control}] \times 100$$

The 50% inhibitory concentration (IC50) was determined by plotting the percentage of inhibition against the sample concentration. Vitamin C was used as a reference standard.

The antioxidant activity was expressed as the Antioxidant Activity Index (AAI), which was calculated using the formula:

$$\text{AAI} = \text{Concentration of DPPH (µg/mL)} / \text{Concentration of Sample (µg/mL)}$$

The AAI provides a relative comparison of antioxidant activity based on the concentration of DPPH and the sample used. In this study, plant extracts were classified as follows: poor antioxidant activity when AAI < 0.5, moderate antioxidant activity when AAI ranges between 0.5 and 1.0, strong antioxidant activity when AAI ranges between 1.0 and 2.0, and very strong antioxidant activity when AAI > 2.0.

Cytotoxicity Test

The eggs of *Artemia salina* were placed in a container filled with filtered salt water and exposed to light for 48 hours until they hatched successfully. The ethanol extract was dissolved in seawater to prepare concentrations ranging from 10 to 1.000 µg/mL. The extract solution was then added to a vial containing ten shrimp larvae, which were incubated at room temperature for 24 hours. After incubation, the number of surviving larvae was counted.[17] Three independent replicates were conducted. The lethal concentration of 50% (LC50) was determined by constructing a correlation curve between the log10 extract concentration (x-axis) and the probit value (y-axis).

RESULTS AND DISCUSSION

Phytochemical screening of the 96% ethanol extracts from the stem, leaf, and bract of *S. edule* identified the presence of alkaloids, flavonoids, saponins, and tannins. In contrast, the flower extract contained alkaloids, flavonoids, and saponins (Table-1). These phytochemicals are well-documented for their broad range of pharmacological and physiological activities. Alkaloids have been reported to exhibit antispasmodic, antibacterial, and anticancer properties.[18,19] Flavonoids demonstrate various bioactivities, including antiviral, antioxidant, anti-atherosclerotic, anti-inflammatory, neuroprotective, and anticancer effects.[20-23] Saponins are primarily recognized for their anti-inflammatory properties.[24,25] Tannins are associated with antifungal and antibacterial activities.[26,27]

The total flavonoid content of the 96% ethanol extract of *S. edule* was determined to have the highest total phenolic and total flavonoid concentration in the leaf (Table-2). Phenolic and flavonoid compounds are plants' major classes of antioxidant compounds. They are crucial in stabilizing lipids against peroxidation, inhibiting oxidizing enzymes, and scavenging free radicals.[28,29]

The structural characteristics of phenolic and flavonoid compounds, such as the number and position of hydroxyl groups, influence their antioxidant properties.[30] Several studies have demonstrated the strong antioxidant activity of phenolic and flavonoid-rich plant extracts. The antioxidant capacity of these compounds is often assessed using in vitro assays, such as DPPH, ABTS, and FRAP. The antioxidant activity of phenolic and flavonoid compounds is directly correlated with their concentration in the plant extracts.[31-33]

Table-1: Phytochemical Screening and Yield of the 96% Ethanol Extract of *S. edule*

Phyto-constituents	Test performed	Parts			
		Stem	Leaf	Bract	Flower
Alkaloids	Dragendorff's	+ ve	+ ve	+ ve	+ ve
Flavonoids	Shinoda	+ ve	+ ve	+ ve	+ ve
Saponins	Froth formation	+ ve	+ ve	+ ve	+ ve
Tannins	FeCl3	+ ve	+ ve	+ ve	- ve
Yield (%)		11.06	3.52	3.35	14.60

Table-2: Total Phenolic, Total Flavonoid, and Total Alkaloid of the 96% Ethanol Extract of *S. edule*

Extract of *S. edule*	Total Phenolic (mg GAE/100g extract)	Total Flavonoid (mg QE/100g extract)	Total Alkaloid (mg CE/g extract)
Stem	1.282 ± 0.397	0.896 ± 0.184	0.796 ± 0.090
Leaf	5.112 ± 0.624	2.514 ± 0.053	1.873 ± 0.129
Bract	3.430 ± 0.096	0.179 ± 0.064	1.840 ± 0.074
Flower	2.647 ± 0.457	1.104 ± 0.282	0.822 ± 0.095

GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; CE: *Caffeine Equivalent*

The highest antioxidant activity (IC50) of the 96% ethanol extract of S. edule was observed in the leaf (Fig.-1). The antioxidant activity of *S. edule* exhibits variation across different plant parts, with the highest activity observed in the leaf, categorized as "strong" based on the Antioxidant Activity Index (AAI) (Table-3). This variation in antioxidant activity is likely attributed to differences in the content of phenolic and flavonoid compounds in the extracts. The elevated antioxidant activity in *S. edule* leaf correlates with a higher concentration of phenolic and flavonoid compounds than in other plant parts. These compounds are well-known for their free radical scavenging properties, central to their antioxidant activity.

Table-3: The antioxidant Activity Index (AAI) of the 96% Ethanol Extract of *S. edule*

Extract of *S. edule*	AAI	Category
Vitamin C	13.540	Very strong
Stem	0.125	Poor
Leaf	1.092	Strong
Bract	0.285	Poor
Flower	0.140	Poor

The lethal concentration of 50% (LC50) for the 96% ethanolic extract of *S. edule* was found to be < 700 mg/L (Fig.-2).

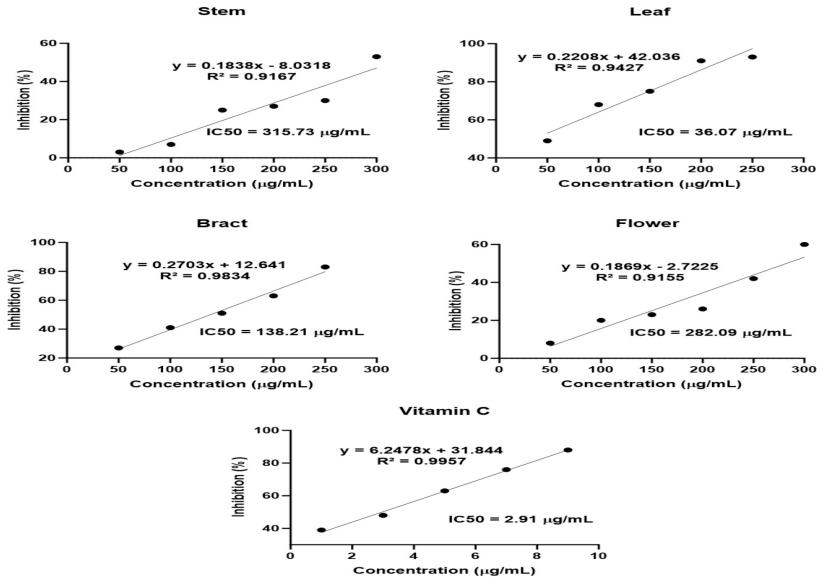

Fig.-1: Antioxidant Activity (IC50) of the 96% Ethanol Extract of *S. edule*

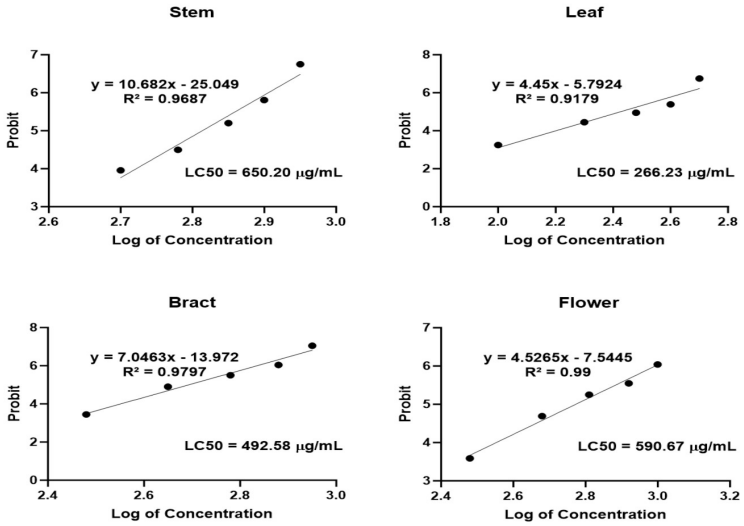

Fig.-2: LC50 value of the 96% Ethanol Extract *S. edule*

The LC50 value of the 96% ethanol extract of *S. edule* was < 1.000 mg/L, indicating it was toxic to *A. salina* larvae.[34] These results suggest that the 96% ethanol extract of *S. edule*, particularly from the leaf and bract (LC50 < 500 mg/L), may warrant further investigation for its anticancer potential.[35] Alkaloids have been extensively investigated for their cytotoxic and anticancer properties. Numerous studies have highlighted the potent anticancer effects of various alkaloids, including isoquinoline alkaloids, aporphine alkaloids, and chromone alkaloids, which have demonstrated significant cytotoxic activity against a range of cancer cell lines.[36-38] The determination of total alkaloid content (Table-2) revealed that the leaf and bract contained higher alkaloid levels compared to other parts, which correlates with their higher cytotoxicity.

CONCLUSION

The 96% ethanol extract from the stem, leaf, bract, and flower of *S. edule* contains varying levels of phenolics, flavonoids, and alkaloids. The leaf extract exhibited the highest antioxidant activity, while both the leaf and bract extracts showed potential anticancer properties. Further studies are needed to isolate the active compounds and elucidate their mechanisms, supporting the plant's potential in herbal medicine.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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