Phytochemical Screening of Leaves from Three Sugarcane *(Saccharum officinarum L.)* Varieties: A Qualitative Analysis of Bioactive Compound Profiles

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Abstract. Sugarcane *(Saccharum officinarum L.)* is known as a primary sucrose crop and, especially the leaf, as a potential source of pharmacologically active secondary metabolites. However, comparative phytochemical data across various industrial types and cultivation areas remain limited. This study aimed to analyze and compare the qualitative phytochemical profiles of three sugarcane leaf varieties (PSKA 095, PSKA 942, and BL) collected from two plantation areas in East Java, Indonesia, namely Indonesian Sugar Research Institute (ISRI) and Kebon Agung Sugar Factory Malang. Five samples were analyzed: PSKA 095 and PSKA 942 from the cultivated area of Kebon Agung Sugar Factory Malang, along with PSKA 095, PSKA 942, and BL from the ISRI cultivar. All samples were subjected to qualitative phytochemical screening using colorimetric and precipitation methods. Compounds screened included flavonoids, alkaloids, tannins/phenols, terpenoids (steroid and triterpenoid fractions), and saponins. Flavonoids, tannins/phenols, triterpenoids, and saponins were detected in all five samples across both varieties and plantation origins. Steroids were uniformly absent in all samples. Alkaloids were detected in four samples but absent in PSKA 942 from ISRI, indicating inter-varietal variation in nitrogen-containing secondary metabolite biosynthesis. The presence of flavonoids and polyphenols across all varieties suggests a significant antioxidant capacity, while triterpenoids and saponins indicate potentially anti-inflammatory and surfactant bioactivities. This study confirms that all five sugarcane varieties possess diverse and pharmacologically relevant phytochemical profiles.These findings support the potential of sugarcane leaf as a source of bioactive compounds and provide a foundation for future quantitative analyses and bioactivity assays.

Keywords: *Saccharum officinarum*, PSKA 095, PSKA 942, BL, phytochemical screening.

1 Introduction

Sugarcane *(Saccharum officinarum L.)* has traditionally been cultivated as the world's principal source of sucrose, yet growing evidence over the past decade indicates that

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the plant, including its leaves, is also a reservoir of pharmacologically relevant secondary metabolites. Sugarcane straw, one of the major processing by-products, has been shown to be rich in phenolic compounds, with fifty distinct secondary metabolites, including hydroxybenzoic acids, hydroxycinnamic acids, and flavonoids have been identified through advanced mass spectrometry-based profiling. These extracts have demonstrated antioxidant, antimicrobial, antityrosinase, and antidiabetic activities in vitro [1]. Similarly, other sugarcane by-products such as molasses and vinasses have been characterized as containing a diverse range of phenolic compounds and heat-induced compounds with antioxidant potential, underscoring that bioactive phytochemicals are distributed across multiple tissues and processing fractions of the plant rather than being confined to the stalk juice alone [2]. Qualitative screening studies conducted directly on sugarcane cultivars have further confirmed the consistent presence of flavonoids, phenolics/tannins, triterpenoids, and saponins, alongside variable detection of alkaloids and steroids depending on plant part and solvent system used [3].

Despite this expanding body of evidence on the pharmacological potential of sugarcane-derived compounds, phytochemical profiling has rarely been extended to a systematic comparison across commercial sugarcane varieties and their cultivation origins. This gap is notable because secondary metabolite production in plants is known to be jointly shaped by genotype and growing environment: comparative studies in other species have shown that even within a single species, populations grown under different environmental conditions can display significant variation in the qualitative and quantitative profile of secondary metabolites such as flavonoids, terpenes, and steroids as demonstrated in congeneric willow herb populations, where the level of secondary metabolites was significantly correlated between species yet each compound still showed considerable variation among populations within species [4]. Comparable genotype-dependent metabolite variation has also been reported directly in sugarcane, where metabolomic comparison of three different sugarcane varieties detected nearly 1,200 metabolites spanning eleven chemical categories, with flavonoid, phenolic acid, and phenylpropanoid-related pathways showing marked differential abundance between varieties [5]. This genotype-dependent variability is consistent with the underlying biology of the crop. Sugarcane cultivars are derived from the highly heterozygous, cross-pollinated, and polyploid Saccharum complex, which comprises two wild species *(S. spontaneum and S. robustum)*, four cultivated species *(S. officinarum, S. sinense, S. barberi, and S. edule)*, and related genera such as Erianthus and Miscanthus, such that even accessions grouped under the same species can retain considerable genetic distance from one another [6]. These findings suggest that the qualitative phytochemical composition of sugarcane cannot be assumed to be uniform across cultivars or plantation sites, yet such comparative data remain scarce, particularly for Indonesian industrial varieties bred for sugar yield rather than phytochemical content.

In Indonesia, sugarcane breeding and germplasm development have historically been centered at the Indonesian Sugar Research Institute (ISRI), which maintains and evaluates numerous hybrid clones such as PSKA series and Bululawang (BL) variety for agronomic traits such as cane yield, sucrose content, and disease resistance [7].

Within this germplasm base, BL, that released in 2004, is still estimated to occupy 60–90% of the planted area in Java despite its growing susceptibility to smut disease. This variety continues to dominate commercial cultivation. Meanwhile, more recently released varieties such as PSKA 942, developed in 2020 from a BT 1323 polycross and noted for its early–middle maturity and tolerance to waterlogging, reflect ISRI's ongoing efforts, in collaboration with sugar mills such as PT Kebon Agung, to diversify the varietal base available to growers in Central and East Java [8]. PSKA 942 and related PS-series clones such as PSKA 095 from ISRI have previously been used as reference plant materials in molecular and tissue-culture studies, reflecting their established status as commercially relevant germplasm, yet their secondary metabolite composition remains largely uncharacterized.

Although numerous studies have characterized the phytochemical composition of sugarcane leaves, little is known about whether qualitative phytochemical profiles differ among Indonesian commercial sugarcane varieties grown under different environmental conditions. Consequently, the potential influence of varietal background and cultivation site on the occurrence of major bioactive compound classes has not been evaluated. Addressing this knowledge gap is important for identifying sugarcane varieties with potential pharmaceutical value beyond their conventional use as a sucrose crop. Therefore, the present study aimed to qualitatively analyze and compare the phytochemical profiles of three sugarcane varieties (PSKA 095, PSKA 942, and BL) cultivated at two plantation sites in East Java.

2 Methods

Five sugarcane leaf samples were analyzed in this study: PSKA 095 and PSKA 942 obtained from the cultivated area of Kebon Agung Sugar Factory, Malang, and PSKA 095, PSKA 942, and BL obtained from the ISRI cultivar collection, Pasuruan, East Java. All leaf samples were collected from mature, field-grown sugarcane plants at each respective plantation site. Leaves were harvested from the fourth internode of each stalk, cleaned to remove surface debris, and sorted to exclude damaged or discolored material. The sorted leaves were subsequently oven-dried and ground into a fine powder to obtain sugarcane leaf simplicia [9].

Qualitative phytochemical screening was performed using standard colorimetric and precipitation-based reactions adapted from established protocols for preliminary phytochemical analysis [10,11]. Tests for tannins were carried out by boiling the plant extract with distilled water and adding ferric chloride solution, with blue-black coloration indicating a positive result, while alkaloids were tested by combining the acidified filtrate with Mayer–Wagner reagent, with the appearance of a cream or brown-red precipitate indicating the presence of alkaloids. Saponins were assessed by vigorous agitation of the extract with distilled water, with stable foam formation confirming their presence, whereas flavonoids were tested by mixing the ethanolic filtrate with concentrated HCl and magnesium ribbon, with a pink or red coloration indicating a positive result. Steroids were identified by combining the extract with chloroform and sulfuric acid, with the formation of a bilayer (red upper layer, greenish

lower layer) indicating a positive result, while terpenoids were detected by the formation of a reddish-brown color following the addition of chloroform and sulfuric acid to the extract. Each phytochemical test was performed in a single qualitative run per sample, and results were recorded descriptively as positive or negative based on the presence or absence of the corresponding color/precipitate change, following the same class of colorimetric and precipitation methodology used in prior comparative screenings of sugarcane extracts [3].

3 Results and Discussion

3.1 Qualitative Phytochemical Profile of The Five Sugarcane Leaf Samples

Five sugarcane leaf samples have been measured qualitatively and profiled phytochemical using colorimetric and precipitation reactions, revealing a broadly conserved secondary metabolite profile across the two plantation origins, with a single point of inter-varietal divergence in alkaloid detection (table 1).

Flavonoids, tannins/phenolic compounds, triterpenoids, and saponins were detected as positive in all five samples, indicating that these four classes of secondary metabolites are consistently biosynthesized by *S. officinarum* regardless of variety or geographic origin. Steroids were uniformly negative across all five samples, suggesting either a genuine absence of free steroidal aglycones or a concentration below the detection threshold of the colorimetric/precipitation assay used. Alkaloids were detected as positive in four of the five samples (PSKA 095–Kebon Agung, PSKA 942–Kebon Agung, PSKA 095–ISRI, and BL–ISRI) but were negative in PSKA 942 obtained from ISRI. This is the only instance in the dataset where the same variety (PSKA 942) produced discordant results between the two cultivation sites, since PSKA 942 from Kebon Agung tested positive for alkaloids while PSKA 942 from ISRI did not**.**

Table 1. Qualitative phytochemical screening results of five *Saccharum officinarum L.* leaf samples from two plantation origins.

Compound Class	Reagent	Positive Indicator	PSKA 095 Kebon Agung	PSKA 942 Kebon Agung	PSKA 095 ISRI	PSKA 942 ISRI	BL ISRI
Flavonoids	Alkaline/Acid test	Yellow to intense coloration or persistent foam	+	+	+	+	+
Alkaloids	Meyer	White/cream precipitate	+	+	+	-	+
	Dragendorff	Orange/red precipitate	+	+	+	-	+

Compound Class	Reagent	Positive Indicator	PSKA 095 Kebon Agung	PSKA 942 Kebon Agung	PSKA 095 ISRI	PSKA 942 ISRI	BL ISRI
	Bouchardat	Brown/dark brown precipitate	+	+	+	-	+
Tannins	Ferric chloride	Dark green or blue-black coloration	+	+	+	+	+
Phenols	Ferric chloride	Intense green, blue, or black coloration	+	+	+	+	+
Steroids	Liebermann-Burchard	Blue-green or bluish-purple ring	-	-	-	-	-
Triterpenoids	Liebermann-Burchard	Red, purple, or brown ring	+	+	+	+	+
Saponins	Foam test	Persistent foam (>10 minutes)	+	+	+	+	+

+ = positive (compound detected); - = negative (compound not detected).

3.2 Flavonoids and Tannins/Phenolic Compounds as Conserved Antioxidant-Associated Metabolites

The consistent detection of flavonoids and tannins/phenolic compounds across all five leaf samples, irrespective of variety or plantation origin, is in agreement with previous phytochemical surveys of *Saccharum officinarum*. Ali et al. [12], in a comprehensive review of the phytochemistry and pharmacology of sugarcane, reported that phenolic and flavonoids compounds such as tricin, apigenin, naringenin, and luteolin derivatives are the most consistently identified secondary metabolites across sugarcane tissues and by-products. This compounds are strongly associated with antioxidant, anti-inflammatory, and antitumor activity. This is further supported at a much finer analytical resolution by Oliveira et al. [1], employing LC- MS to profile an ethanolic extract of sugarcane straw. This analysis identified fifty distinct phenolic compounds, spanning hydroxybenzoic acids, hydroxycinnamic acids, and flavonoids. The resulting phenolic-rich extract also exhibited strong free-radical scavenging activity in both the ABTS and DPPH assays. Similarly, Kerdchan et al. [3] screened four sugarcane cultivars from Maha Sarakham Province, Thailand, and found flavonoid and phenolic compounds present across all cultivars and plant parts examined. However, their absolute content varied considerably by cultivar and tissue type. This pattern was

further refined by Hewawansa et al. [13] in a synthesis of (poly)phenol data spanning the sugarcane plant body and its processing chain. Their analysis showed that only a small subset of compounds, namely apigenin, luteolin, and tricin C-glycosides, together with ferulic, caffeic, and chlorogenic acids, persist consistently across nearly all sugarcane tissues and processing stages. In contrast, many other phenolics fluctuate substantially depending on tissue type, cultivar, and processing conditions.

The present qualitative finding that flavonoids and tannins/phenols were positive in all five Indonesian samples is therefore consistent with the broader phytochemical pattern reported for this species, suggesting that the compounds responsible for the consistently positive reactions are likely derived from the conserved core flavonoid and phenolic acid pool rather than from more variable minor constituents, thereby supporting the inference that the analyzed sugarcane varieties possess a baseline antioxidant capacity attributable to phenolic-class metabolites. The present study only establishes the presence or absence of these phytochemical classes rather than their concentrations. Pant et al. [14] reported that although phenolic and flavonoid biosynthesis is broadly conserved across plant taxa, the quantitative accumulation of these metabolites is highly responsive to environmental factors, including temperature, soil water availability, and soil fertility. This phenomenon has also been demonstrated in sugarcane. Velázquez-Martínez et al. [15] investigated the influence of geographical origin on the phenolic yield and composition of sugarcane bagasse extracts and found that both extraction method and cultivation site significantly affected total phenolic yield and antimicrobial activity, despite the consistent detection of phenolic compounds in bagasse from all locations examined. In the present study, the two plantation sites, Kebon Agung and ISRI, differ in their soil characteristics and microclimatic conditions. Therefore, although flavonoids and phenolic compounds were detected in all samples, their concentrations may vary between the two locations. Such variation would be consistent with the site-dependent differences in phenolic yield reported for sugarcane bagasse extracts. However, this possibility cannot be evaluated using the qualitative colorimetric screening employed in the present study. Future investigations should therefore include quantitative determination of total flavonoid content and total phenolic content using spectrophotometric methods, together with antioxidant activity assays such as DPPH or ABTS, following the approaches described by Kerdchan et al. [3] and Oliveira et al. [1].

3.3 Alkaloid Variability

The most notable finding of this study is the absence of alkaloids specifically in PSKA 942 obtained from ISRI, while the same variety (PSKA 942) cultivated at Kebon Agung tested positive. This pattern is more parsimoniously explained by site-specific environmental modulation of alkaloid biosynthesis than by a fixed genotypic trait of the PSKA 942 variety. This interpretation is consistent with recent literature on the environmental and genetic control of nitrogen-containing secondary metabolites. Zhan et al. [16] reviewed how alkaloid biosynthetic pathways are jointly regulated by genetic factors (e.g., transcription factors controlling pathway-specific enzymes) and environmental stresses, noting that abiotic conditions can substantially up- or down-

regulate alkaloid accumulation even within a single genotype, while Pant et al. [14] reported that alkaloid content in several medicinal plant species fluctuates measurably with changes in temperature, soil water status, and soil fertility, independent of genetic background. This kind of within-genotype fluctuation has been documented directly in other species. Vishnyakova et al. [17], tracking 59 narrow-leafed lupine (*Lupinus angustifolius*) accessions grown at a single site over several years, found that total seed alkaloid content in the *same* accessions varied nearly two-fold between growing seasons, tracking closely with year-to-year differences in precipitation rather than any genetic change, indicating that a negative alkaloid result in one accession, as observed here in PSKA 942–ISRI, does not by itself imply a genetically distinct alkaloid profile.

Applied to the present dataset, the divergent alkaloid result in PSKA 942 may reflect differences in nitrogen availability, soil composition, or microclimate between the Kebon Agung industrial cultivation area and the ISRI research station, which could influence the amino-acid-derived biosynthetic pathways that give rise to alkaloids. A comparable geographic pattern has been reported in kratom (*Mitragyna speciosa*), where Sengnon et al. [18], analyzing 745 leaf samples collected across southern, central, and northern Thailand, found that mitragynine content varied several-fold between sites and seasons and linked this variability directly to differences in the soil at each collection location. This results supporting the plausibility that a site-level soil difference between Kebon Agung and ISRI underlies the discordant alkaloid result in PSKA 942. Because alkaloids are nitrogen-containing metabolites derived largely from amino acid precursors, their biosynthesis is often more sensitive to soil nitrogen status than that of carbon-based metabolites such as flavonoids or terpenoids [16]. The biosynthesis sensitivity has been demonstrated experimentally by Huang et al. [19], who showed in a controlled water-by-nitrogen factorial trial on the alkaloid-rich legume *Sophora alopecuroides* that soil nitrogen and water regime significantly altered total alkaloid accumulation, partly through changes in the rhizosphere soil environment. This provides a plausible physiological explanation for why alkaloid detection varied between cultivation sites for the same variety.

3.4 Absence of Steroids Alongside Positive Tripertenoids and Saponins

Steroids were negative in all five leaf samples, whereas triterpenoids and saponins were positive in all five. This pattern suggests that the terpenoid pool of the sugarcane leaf samples analyzed is dominated by the pentacyclic triterpenoid skeleton (e.g., oleanane-, ursane-, or lupane-type backbones) rather than by the tetracyclic steroidal skeleton, even though both classes share the same triterpenoid precursor pathway. Bildziukevich et al. [20], in a review of oleanane-, ursane-, and lupane-type triterpenoid saponins, distinguished this class from steroidal saponins on the basis of their aglycone skeleton and noted that many plant species preferentially accumulate one skeletal type over the other depending on the relative activity of downstream cyclization enzymes. The consistent triterpenoid/saponin-positive but steroid-negative profile observed across all five sugarcane samples in this study is compatible with a biosynthetic bias toward the triterpenoid branch of this shared pathway, although the possibility that steroidal compounds are present only in trace amounts undetectable by the qualitative

precipitation test used here cannot be excluded and would require confirmation using more sensitive methods such as thin-layer chromatography or GC-MS. This latter possibility is far from merely theoretical in sugarcane specifically. Kamchonemenukool et al. [21], extracting policosanols and phytosterols from sugarcane leaves, bagasse, and filter mud using subcritical liquefied dimethyl ether, recovered substantial phytosterol content (up to nearly 21 g/100 g in filter mud) from these same plant fractions, but only when using a non-polar, wax-targeting extraction approach rather than the aqueous colorimetric methods typically used in qualitative screening. This indicates that steroidal compounds in sugarcane are real but are concentrated in the epicuticular wax/lipid fraction, which is largely inaccessible to standard aqueous precipitation tests such as the one applied in the present study. This results offering a plausible distinct from a true biosynthetic absence. More broadly, this kind of divergence between structurally related compound classes within the same qualitative screening panel is not unusual. Dubale et al. [11], screening twelve traditionally used medicinal plants in Ethiopia for the same panel of phytochemical classes used in the present study (flavonoids, alkaloids, tannins, phenols, saponins, steroids, and terpenoids), likewise found that individual compound classes did not co-occur uniformly across species, underscoring that steroid- and terpenoid-class detection commonly diverge within a single screening panel even under identical qualitative test conditions.

The universal presence of triterpenoids and saponins across all varieties and both plantation origins is pharmacologically noteworthy, since both compound classes have been repeatedly associated with anti-inflammatory bioactivity in the recent literature. Miranda et al. [22] demonstrated that pentacyclic triterpenes exert anti-inflammatory effects primarily through suppression of pro-inflammatory mediators such as nitric oxide, prostaglandin E2, and NF-κB-dependent signaling. This mechanistic picture is reinforced by the more recent systematic review of Mantiniotou et al. [23] about leaf-extract studies, found that extracts containing a combination of triterpenoids and saponins consistently inhibited protein denaturation and nitric oxide production in vitro, in several cases at potencies approaching that of standard anti-inflammatory drugs such as diclofenac. The same protein-denaturation-based assay principle used by Boualam et al. [24] demonstrate anti-inflammatory activity in phenolic/flavonoid-rich extracts. Complementarily, Bildziukevich et al. [20] reported that triterpenoid saponins possess additional surfactant-related bioactivities, including antimicrobial, immunomodulatory, and membrane-disrupting properties conferred by their glycosylated structure. Boualam et al. [24] demonstrated that plant extracts containing a comparable combination of phenolics, flavonoids, and related secondary metabolites exhibited measurable in vitro anti-inflammatory activity via inhibition of heat-induced protein denaturation, the consistent triterpenoid and saponin positivity observed in the present sugarcane samples.

3.5 Implications for the Pharmacological Potential of Sugarcane

The qualitative phytochemical profile obtained in this study supports the growing evidence that *Saccharum officinarum* leaves are a source of pharmacologically relevant secondary metabolites [12]. Teixeira et al. [25], reviewing the recovery of bioactive

lipids such as phytosterols and triterpenoids from sugarcane tops, straw, filter cake, and bagasse, framed these residues as a starting point for developing health- and well-being-oriented products within a circular economy model, positioning compound classes structurally and functionally similar to those detected here (triterpenoids in particular) as commercially exploitable rather than incidental constituents. The combination of antioxidant-associated phenolics and flavonoids with anti-inflammatory-associated triterpenoids and saponins suggests that the sugarcane leaf samples from all varieties, regardless of cultivation site, may serve as potential raw material for nutraceutical or pharmacological applications. This finding is consistent with the broader valorization trend for sugarcane by-products described by Ali et al. [12] and Kerdchan et al. [3]. Carvalho et al. [26] demonstrated sugarcane straw extract that rich in phenolic and flavonoid, into a natural cosmetic preservative that met USP51 efficacy standards against common cosmetic-spoilage microorganisms. This provides a practical proof of concept that phytochemically comparable sugarcane material can be translated directly into a marketable cosmeceutical ingredient. In a review of sustainable valorization pathways for sugarcane processing waste, identified phytochemical-rich streams such as straw and bagasse as increasingly central to circular bioeconomy strategies, situating the nutraceutical/cosmeceutical potential proposed here within a wider industrial push to recover value from sugarcane residues rather than treating them solely as waste [27]. The single case of alkaloid variability further illustrates that phytochemical screening outcomes in *S. officinarum* cannot be assumed to be fixed by variety alone, and that cultivation environment should be treated as a relevant covariate in future comparative phytochemical studies of sugarcane.

4 Conclusion

This study provides a qualitative phytochemical baseline for five sugarcane (*Saccharum officinarum* L.) leaf samples, including PSKA 095 and PSKA 942 from Kebon Agung, and PSKA 095, PSKA 942, and BL from ISRI. Flavonoids, tannins/phenols, triterpenoids, and saponins were consistently detected across all five samples regardless of variety or plantation origin, while steroids were uniformly absent. Alkaloids were undetected specifically in PSKA 942 from ISRI, while present in the same variety grown at Kebon Agung, indicating a site-dependent variation within an otherwise consistent phytochemical profile. These findings demonstrate that all five sugarcane leaf varieties possess diverse and pharmacologically relevant phytochemical profiles, with minor inter-varietal differences notably in alkaloid content. The results support the potential of sugarcane leaves as a source of bioactive compounds, reinforcing its role beyond the conventional role of sugarcane as a sucrose crop. Sugarcane leaves represent a promising natural resource for the development of pharmacological product. Further quantitative phytochemical analysis and pharmacological studies are required to validate its bioactivity, elucidate the underlying mechanisms of action, and establish its safety and efficacy for therapeutic applications.

Disclosure of Interests. The authors have no competing interests to declare that are relevant to the content of this article.

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