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EFFECT OF SOLVENT POLARITY ON ANTIOXIDANT ACTIVITY AND NMR-ASSISTED PHYTOCHEMICAL PROFILING OF YOUNG *Scaevola taccada* FRUITS

Azwin Apriandi[1,2], F. Feliatra[1]*, Rahman Karnila[3], Dian Iriani[3], Anggrei Viona Seulalae[2]
[1]Study Program of Marine Science, Faculty of Marine and Fisheries, Riau University Pekanbaru, Indonesia 28293
[2]Study Program of Fishery Product Technology, Faculty of Marine Science and Fisheries, Maritime Raja Ali Haji University, Tanjungpinang, Indonesia 29115
[3]Study Program of Fishery Product Technology, Faculty of Marine and Fisheries, Riau University, Pekanbaru, Indonesia 28293

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*Correspondence: feliatra@lecturer.unri.ac.id

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Abstract

The exploration of coastal plants as natural antioxidant sources has gained attention because of their adaptation to harsh environments and the presence of diverse bioactive metabolites in them. This study investigated the effect of solvent polarity on the extraction yield, antioxidant activity, and metabolite characteristics of young *S. taccada* fruits using an NMR-assisted phytochemical approach. Sequential extraction was performed using n-hexane, ethyl acetate, and ethanol. Antioxidant activity was evaluated using the DPPH radical scavenging assay, and metabolites were characterized using one-dimensional ^{1}H and ^{13}C NMR spectroscopy. The extraction yield increased with solvent polarity, with ethanol providing the highest yield (1.17±0.00%), followed by ethyl acetate (0.73±0.00%) and n-hexane (0.23±0.00%) extracts. The ethanol extract showed IC_{50} moderate activity (113.99±5.07 ppm), followed by ethyl acetate (181.01±15.60 ppm), whereas n-hexane showed very weak activity (2,965.25±1,966.56 ppm). DPPH inhibition increased with increasing concentrations, indicating a concentration-dependent response. NMR analysis of the ethanol extract revealed dominant carbohydrate-associated signals (δH 3.1-4.0 ppm; δC 64.17-73.68 ppm), along with aliphatic, unsaturated, and carbonyl signals, indicating a predominance of carbohydrate-associated and oxygenated metabolites; however, their contribution to antioxidant activity could not be directly established. Overall, solvent polarity significantly (p<0.05) influenced the extraction yield, antioxidant activity, and metabolite distribution of the extracts. . Further studies using LC-MS/MS and two-dimensional NMR are required to confirm metabolite identities and clarify their contributions to the observed antioxidant activity.

Keywords: bioprospecting, coastal bioresources, DPPH, glycosylated metabolites, secondary metabolite

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Pengaruh Polaritas Pelarut Terhadap Aktivitas Antioksidan dan Karakterisasi Fitokimia Berbasis NMR pada Buah Muda *Scaevola taccada*

Abstrak

Eksplorasi tanaman pesisir sebagai sumber antioksidan alami semakin diminati karena kemampuannya beradaptasi terhadap lingkungan ekstrem serta potensinya menghasilkan beragam metabolit bioaktif. Penelitian ini mengkaji pengaruh polaritas pelarut terhadap rendemen, aktivitas antioksidan, dan karakteristik metabolit buah muda *S. taccada* menggunakan pendekatan fitokimia berbantuan NMR. Ekstraksi bertingkat dilakukan menggunakan n-heksan, etil asetat, dan etanol. Aktivitas antioksidan dievaluasi dengan metode peredaman radikal DPPH, sedangkan metabolit dikarakterisasi menggunakan spektroskopi NMR satu dimensi ^{1}H dan ^{13}C. Rendemen meningkat seiring polaritas pelarut, dengan etanol tertinggi (1,17±0,00%), etil asetat (0,73±0,00%) dan n-heksana (0,23±0,00%). Ekstrak etanol menunjukkan aktivitas tertinggi IC_{50} (113,99±5,07 ppm), etil asetat (181,01±15,60 ppm), sedangkan n-heksana sangat lemah (2.965,25±1.966,56 ppm). Inhibisi DPPH meningkat seiring konsentrasi, menunjukkan respons yang bergantung konsentrasi. Analisis NMR ekstrak etanol menunjukkan dominasi sinyal berasosiasi karbohidrat (δH 3,1–4,0 ppm; δC 64,17–73,68 ppm), disertai sinyal alifatik, tak jenuh, dan karbonil, yang menunjukkan dominasi metabolit berasosiasi karbohidrat dan teroksigenasi; namun kontribusinya terhadap aktivitas antioksidan belum dapat dibuktikan secara langsung. Secara keseluruhan, polaritas pelarut berpengaruh nyata (p<0,05) terhadap rendemen, aktivitas antioksidan, dan distribusi metabolit. Diperlukan penelitian lanjutan menggunakan LC-MS/MS dan NMR dua dimensi untuk mengonfirmasi identitas metabolit dan menjelaskan kontribusinya terhadap aktivitas antioksidan yang diamati.

Kata kunci: bioprospeksi, DPPH, metabolit terglikosilasi, metabolit sekunder, sumber daya hayati pesisir

INTRODUCTION

The exploration of coastal plants as sources of natural bioactive compounds has attracted increasing scientific interest because these species have evolved unique physiological and biochemical adaptations to survive in extreme environments. Coastal ecosystems are characterized by high salinity, intense ultraviolet radiation, tidal fluctuations, nutrient limitation, and oxidative stress, all of which stimulate the overproduction of reactive oxygen species (ROS). To maintain cellular redox homeostasis, coastal plants activate complex defense mechanisms involving the enhanced biosynthesis of secondary metabolites, particularly phenolic compounds, flavonoids, and other oxygenated phytochemicals with antioxidant properties (Mohammed *et al.*, 2023; Ciupei *et al.*, 2024; Hasnat *et al.*, 2024; Hilal *et al.*, 2024). Consequently, these adaptive responses have positioned coastal flora as promising, yet still underexplored, sources of natural antioxidants and other bioactive compounds with potential applications in the food, pharmaceutical, and nutraceutical industries (Yeshi *et al.*, 2022; Swensen *et al.*, 2024).

Among tropical coastal halophytes, *Scaevola taccada* (Gaertn.) Roxb. a member of the Goodeniaceae family, is widely distributed throughout the Indo-Pacific coastlines, including Indonesia, where it has long been used in traditional medicine (Jacob *et al.*, 2019; Prihantono *et al.*, 2020; Apriandi *et al.*, 2021). Previous phytochemical investigations have demonstrated that *S. taccada* contains diverse classes of bioactive constituents, including phenolic acids, flavonoids, flavonoid glycosides, terpenoids, alkaloids, and other oxygenated metabolites associated with antioxidant and anti-inflammatory activities (El-Sayed *et al.*, 2020; Jasna *et al.*, 2025; Nasution *et al.*, 2026). Consistent with these phytochemical findings, antioxidant activity has been reported in extracts prepared from the leaves, stems, fruits, and fruit juice

of *S. taccada* (Apriandi *et al*., 2021; Fatmawati *et al*., 2021). However, most previous studies have focused primarily on crude extracts or different plant organs, whereas information regarding the phytochemical composition and solvent-dependent metabolite distribution in young fruits remains extremely limited. As metabolite biosynthesis is strongly influenced by the developmental stage, young fruits are expected to exhibit distinct metabolite compositions and antioxidant-associated compounds compared to mature fruits (Yadav *et al*., 2021; Singh *et al*., 2022; Hilal *et al*., 2024). Therefore, investigating young fruits provides an important opportunity to improve our understanding of metabolite accumulation during early fruit development and identify the bioactive constituents that may contribute to the antioxidant potential of this underexplored coastal species.

Solvent polarity-based extraction is widely recognized as an effective strategy for selectively recovering plant metabolites based on their physicochemical properties, thereby facilitating the characterization of metabolite classes associated with biological activity (Do *et al*., 2014; Dirar *et al*., 2019; Nawaz *et al*., 2020). Plant extracts comprise chemically diverse constituents, ranging from nonpolar lipids and terpenoids to highly polar phenolic compounds, flavonoids, and glycosides. Consequently, solvent polarity strongly influences both the extraction efficiency and the composition of the resulting extracts. Sequential extraction using solvents of increasing polarity enables the partitioning of metabolites into fractions enriched with compounds of similar polarity, providing a more comprehensive representation of phytochemical diversity than single-solvent extraction, while facilitating the interpretation of solvent-dependent biological activity (Bitwell *et al*., 2023). This approach is particularly relevant for young fruits, whose metabolite compositions are expected to change dynamically during early developmental stages. However, despite the recognized importance of solvent polarity in phytochemical extraction, no previous study has systematically evaluated how sequential solvent extraction influences extraction yield, metabolite distribution, and antioxidant activity, specifically in young *S. taccada* fruits. This limitation represents an important knowledge gap in understanding the chemical basis of the antioxidant properties of this underexplored coastal plant species.

Understanding the chemical composition of plant extracts is essential for elucidating the molecular basis of their biological activities. In recent years, Nuclear Magnetic Resonance (NMR)-based phytochemical profiling has emerged as a powerful analytical approach for characterizing complex plant metabolite mixtures through the simultaneous detection of multiple metabolite classes with minimal sample preparation and high analytical reproducibility (Kim *et al*., 2010; Emwas *et al*., 2019; Chen *et al*., 2024; Dutta *et al*., 2025). Unlike conventional phytochemical screening, NMR provides comprehensive metabolite fingerprints that facilitate the annotation of structurally diverse compounds in complex botanical extracts. However, its application to coastal medicinal plants, particularly *S. taccada*, remains limited, and information regarding the solvent-dependent metabolite distribution in young fruits is still scarce. Because the present study employed one-dimensional ^{1}H and ^{13}C NMR spectroscopy, the reported metabolite assignments should be interpreted as tentative chemical annotations rather than definitive structural identifications, which require complementary analyses such as two-dimensional NMR and LC-MS/MS (Emwas *et al*., 2019; Liu *et al*., 2025; Zhang *et al*., 2025).

Despite the increasing interest in the antioxidant potential of *S. taccada*, the chemical basis underlying the solvent-dependent antioxidant activity in young fruits remains poorly understood (El-Sayed *et al*., 2020; Apriandi *et al*., 2021; Fatmawati *et al*., 2021; Jasna *et al*., 2025). Previous investigations have primarily focused on the antioxidant evaluation of crude extracts, fruit juices, and different plant organs. In contrast, phytochemical characterization has largely relied on qualitative screening without systematically assessing how solvent polarity influences metabolite recovery or integrating

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metabolite profiling with antioxidant evaluations. Collectively, these studies demonstrate the antioxidant potential of *S. taccada* at the extract level; however, current knowledge remains insufficient to explain how metabolite composition varies among solvent fractions or which metabolite classes are preferentially recovered during early fruit development (El-Sayed *et al*., 2020; Apriandi *et al*., 2021; Fatmawati *et al*., 2021; Nasution *et al*., 2026). Consequently, the relationship between solvent polarity, metabolite distribution, and antioxidant activity remains largely unresolved (Do *et al*., 2014; Dirar *et al*., 2019; Nawaz *et al*., 2020; Tripathi *et al*., 2025; Azzahra *et al*., 2025). From an applied perspective, clarifying the solvent-dependent antioxidant potential in an underexplored coastal species such as *S. taccada* is directly relevant to fisheries product processing and functional food development, given the growing demand for natural, coastal-derived antioxidant ingredients as alternatives to synthetic preservatives. To the best of our knowledge, this study represents the first attempt to systematically combine sequential solvent extraction, DPPH radical scavenging assay, and one-dimensional NMR-assisted phytochemical profiling specifically in young fruits of *S. taccada*, thereby offering a novel developmental stage-specific contribution toward understanding the chemical basis of antioxidant activity in this coastal halophyte. To address this knowledge gap, the present study investigated the effect of solvent polarity on extraction yield, antioxidant activity, and metabolite distribution in young fruits of *S. taccada* through sequential solvent extraction combined with the DPPH radical scavenging assay and one-dimensional ^{1}H and ^{13}C NMR-assisted phytochemical profiling. Rather than attempting definitive metabolite identification, this study provides preliminary chemical evidence describing solvent-dependent metabolite distribution in young fruits and establishes a foundation for future investigations employing two-dimensional NMR, LC-MS/MS, quantitative phytochemical analysis and bioactivity-guided fractionation.

MATERIALS AND METHODS

Sample Preparation

Primary material used in this study consisted of young fruits of *S. taccada* collected from Trikora Beach, Bintan Island, Indonesia (1°07′50″ N, 104°37′35″ E). A total of 30 young fruit samples were collected for morphometric characterization. The collected samples were washed, cut into small pieces, air-dried, and subsequently oven-dried at 40 °C for 48 h using a Memmert oven to reduce the moisture content while preserving the thermolabile compounds. The dried samples were ground into a fine powder using a Miyako blender and stored in airtight containers at room temperature before extraction.

Extraction

Powdered samples of young *S. taccada* fruits were subjected to bioactive compound extraction using a sequential maceration method. Sequential extraction was selected to fractionate metabolites according to their relative polarity, thereby reducing the complexity of individual extracts and facilitating comparative evaluation of solvent dependent metabolite distribution. This approach enables the progressive recovery of nonpolar, semipolar, and polar constituents, providing a broader representation of the phytochemical composition than single-solvent extraction (Bitwell *et al*., 2023). Briefly, 50 g of each powdered sample was successively extracted with analytical grade n-hexane, ethyl acetate, and ethanol (200 mL; 1:4 w/v for each solvent), applied in order of increasing polarity, so that non-polar constituents were removed first, thereby minimizing their interference with the recovery of more polar metabolites in subsequent extraction steps. The 1:4 w/v ratio and 48 h maceration period were adopted based on established protocols for exhaustive metabolite recovery from plant matrices. Each extraction step was performed once for 48 h at room temperature with intermittent stirring to facilitate the solvent penetration and metabolite diffusion. After each extraction cycle, the mixture was filtered using Whatman No. 42 filter paper to separate the filtrate from the residue. The

residue was subsequently re-extracted using solvents of increasing polarity. All extractions were performed in triplicate (n = 3) to ensure reproducibility. The obtained filtrates were concentrated under reduced pressure using a vacuum evaporator (INTBUYING RE-201) at 40°C to remove the solvents and obtain the crude extracts. The resulting semisolid extracts were weighed to determine the extraction yield and stored at 4 °C until further analysis. However, sequential extraction does not achieve complete separation of metabolite classes because compounds with intermediate polarity may be distributed across more than one solvent fraction. Therefore, the resulting fractions should be interpreted as relative enrichments of metabolites rather than chemically pure fractions.

Antioxidant Activity (DPPH Radical Scavenging Activity)

Antioxidant activity (DPPH Assay) was determined following the method described by Gulcin & Alwasel (2023). The extracts were dissolved in methanol to obtain concentrations of 75-375 ppm, and ascorbic acid (positive control) was prepared at 5-25 ppm. A 1 mM DPPH solution was then prepared. Briefly, 4.5 mL of the sample or standard solution was mixed with 500 µL of DPPH and incubated at 37 °C for 30 min (Memmert IN 55). The absorbance was measured at 517 nm using a UV-Vis spectrophotometer (UV-1800; Shimadzu). Methanol DPPH solution was used as a blank. All assays were performed in triplicate (n=3). Radical scavenging activity was calculated as the percentage of inhibition of DPPH.

$$\%\text{Inhibition} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

IC_{50} values were determined from the linear regression of the percentage of inhibition versus concentration (y = a + bx), where IC_{50} represents the concentration required to inhibit 50% of the DPPH radicals. The coefficient of determination (R^2) was calculated for each regression model to evaluate the goodness of fit.

NMR Measurements

NMR Measurements and data analysis were performed following the procedure described by Kim *et al.* (2010). Because the ethanol extract exhibited the highest extraction yield and the strongest DPPH radical scavenging activity among the three solvent fractions, it was selected as the representative extract for NMR-assisted phytochemical characterization to obtain preliminary chemical information on metabolites potentially associated with antioxidant activity. NMR spectra were acquired on a JEOL JNM-ECZ500R/S1 spectrometer (^{1}H at 500 MHz, ^{13}C at 125 MHz) at 298 K. Each extract (ca. 20 mg) was dissolved in 600 µL methanol-d_4 (99.8% D, Sigma-Aldrich). ^{1}H NMR parameters: spectral width 15 ppm, 64 scans, relaxation delay 2.0 s, 32k data points. ^{13}C NMR parameters: spectral width 220 ppm, 10,000 scans, 1.0 s relaxation delay. Chemical shifts were referenced to residual solvent signals (δH 3.31, δC 49.0 ppm for methanol-d_4). Spectral processing was performed using the JEOL Delta v5.3 software with exponential line broadening (0.3 Hz for ^{1}H and 1.0 Hz for ^{13}C). Metabolite assignments were based on a comparison of the ^{1}H and ^{13}C NMR spectral characteristics with previously reported data. Therefore, compound identification should be considered tentative and requires further validation using complementary analytical techniques such as 2D NMR or LC-MS/MS. Because only one-dimensional ^{1}H and ^{13}C NMR spectra were acquired, metabolite assignments were based on characteristic chemical shifts and comparisons with published spectral databases. Consequently, all reported metabolite identifications should be regarded as tentative annotations rather than definitive structural identifications.

Data Analysis

The experiment was arranged in a Completely Randomized Design (CRD) consisting of three treatments with three replicates. Before analysis, the data were tested for normality using the Shapiro–Wilk test and homogeneity of variance using Levene's test. Differences among treatments were evaluated using one-way analysis of variance (ANOVA).

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When significant differences were detected ($p < 0.05$), mean separation was performed using Duncan's multiple range test (DMRT). All statistical analyses were performed using SPSS software (version 29; IBM Corp., Armonk, NY, USA). Statistical analyses were performed to compare the extraction yields and antioxidant activities of the solvent fractions. No formal correlation analysis was performed between metabolite composition and antioxidant activity because metabolite identification was qualitative and based on one-dimensional NMR profiling.

RESULTS AND DISCUSSION

Fruits Morphology and Extraction Yield

Young fruits of *S. taccada* were collected from Trikora Beach on Bintan Island in Indonesia. The morphological characteristics of the young fruits are shown in Figure 1. In this preliminary study, morphological observations focused on fruit diameter as the primary indicator of the developmental stage, whereas other morphometric parameters, such as fruit weight, length, peel thickness, and moisture content were not measured. These parameters are recommended for inclusion in future studies to provide a more comprehensive morphological characterization and improve reproducibility.

The young fruits of *S. taccada* were characterized by a green color, firm texture, and an average diameter of 0.77±0.12 cm, indicating an early developmental stage with high metabolic activity. At this stage, parenchymal tissues actively synthesize secondary metabolites, particularly phenolics, which commonly accumulate during early growth as part of the plant defense mechanism (Arunachalam, 2013; Yadav *et al.*, 2021; Singh *et al.*, 2022; Hilal *et al.*, 2024). The predominance of polar secondary metabolites is particularly important because their polar nature strongly influences the extraction efficiency, which depends on solvent polarity. This green coloration further reflects active photosynthetic metabolism and the activation of the phenylpropanoid pathway, which regulates phenolic biosynthesis (Dai & Mumper, 2010; Singh *et al.*, 2022; Herwibawa, 2025). In coastal environments, this pathway is often enhanced by salinity and UV stress, resulting in increased accumulation of antioxidant-related compounds (Ksouri *et al.*, 2007; Mohammed *et al.*, 2023). It should be noted that this interpretation of pigmentation, phenylpropanoid activation, and antioxidant accumulation is drawn from general plant physiological literature rather than from direct measurements of photosynthetic activity or phenylpropanoid pathway markers in the present study. These observations are consistent with the possibility that young *S. taccada* fruits contain relatively high proportions of polar metabolites, although their abundance was not quantitatively determined in the present study. The extraction yields of each fraction are shown in Figure 2.

Ethanol produced the highest extraction yield (1.173±0.003%), followed by ethyl acetate (0.727±0.003%), and n-hexane (0.227±0.0001%). This pattern indicates that the extractable metabolites in young *S. taccada* fruits are predominantly polar to semi-polar compounds, which exhibit greater solubility in ethanol because of their oxygenated functional groups (Dai

Figure 1 Young fruit of beach naupaka (*S. taccada)*

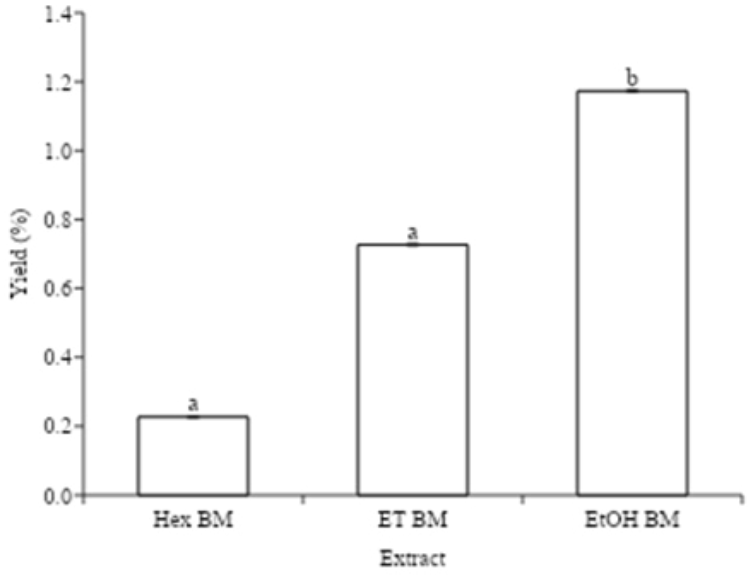

Figure 2 Yield of beach naupaka (*S. taccada*) young fruit extract; Different superscripts indicate differences (p<0.05);

& Mumper, 2010; Dirar *et al*., 2019; Nawaz *et al*., 2020; Ma *et al*., 2022; Mulyono *et al*., 2022; Emu *et al*., 2023). The predominance of ethanol-soluble metabolites observed in this study is consistent with previous findings for *S. taccada*. Nasution *et al*. (2026) reported that the methanolic extracts of *S. taccada* leaves and fruits contained higher levels of phenolic and flavonoid compounds than the less polar fractions, indicating that polar solvents are generally more effective for recovering antioxidant-associated metabolites from this species. Similar solvent-dependent extraction patterns have also been reported in coastal halophytes such as *Ipomoea pes-caprae* and *Cressa cretica*, where polar solvents preferentially recover phenolic-rich fractions with higher antioxidant activity (Qasim *et al*. 2017; Nazir *et al*. 2018).

Importantly, this polarity-dependent yield may reflect differences in the relative distribution of extractable metabolites among solvent fractions. The higher ethanol yield is consistent with the greater recovery of polar metabolites in the extractable fraction of young *S. taccada* fruits, potentially associated with antioxidant activity, in line with reports linking *S. taccada* activity to phenolic constituents (Apriandi *et al*., 2021; Fatmawati *et al*., 2021). In contrast, the low yield in n-hexane indicates a limited contribution of lipophilic compounds, whereas the intermediate ethyl acetate fraction indicates the presence of semi-polar metabolites, which may include phenolic constituents (Azmir *et al*., 2013; Do *et al*., 2014; Nawaz *et al*., 2020; Tripathi *et al*., 2025; Azzahra *et al*., 2025). This polarity-driven partitioning is further supported by the plant's coastal adaptation, in which environmental stress enhances phenolic accumulation (Ksouri *et al*., 2007; Jacob *et al*., 2019; Shekhawat *et al*., 2021; Emura *et al*., 2022). Consequently, the observed yield differences provide a meaningful basis for NMR assisted phytochemical characterization, as each fraction represents a distinct chemical profile that may provide a useful basis for subsequent comparison with antioxidant activity, although no formal statistical relationship was evaluated in the present study.

Antioxidant Activity

The antioxidant activity of extracts derived from the young fruits of *S. taccada* was evaluated using the DPPH radical-scavenging assay. Antioxidant performance was assessed using IC_{50} values as an indicator of radical

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Table 1 Antioxidant activity (IC_{50}) of the extract from young fruit *S. taccada*

Extract	IC_{50} (ppm)	Ascorbic acid (ppm)	Young fruit of *S. taccada**
N-hexane	2,965.25±1,966.56[a]		
Ethyl acetate	181.01±15.60[b]	7.15±0.15	130.71
Ethanol	113.99±5.07[b]		

Different superscripts indicate significant differences (p<0.05);
*Apriandi *et al.* (2021)

scavenging potency (Table 1), whereas concentration-dependent inhibition profiles were expressed as percentage inhibition (% inhibition) at different concentrations of the extracts (Figure 3).

The antioxidant activity of extracts derived from young fruits of *S. taccada* was assessed using the DPPH radical scavenging assay, with IC_{50} values used as an indicator of free radical inhibition efficiency (Table 1). The concentration-dependent inhibition profiles are presented in Figure 3. Based on Table 1 and Figure 3, the antioxidant activity of young *S. taccada* fruit extracts differed significantly among the solvent fractions (p<0.05). Linear regression analysis of DPPH inhibition against extract concentration produced the following equations: y=0.0215x+5.47 (R^2= 0.988) for the n-hexane extract, y = 0.1567x+21.43 (R^2=0.935) for the ethyl acetate extract, and y=0.1563x+32.20 (R^2=0.976) for the ethanol extract. These high coefficients of determination indicate a strong concentration-dependent increase in radical scavenging activity across all solvent fractions. The ethanol extract exhibited the moderate antioxidant activity (113.99 ± 5.07 ppm), followed by the ethyl acetate extract (181.01±15.60 ppm), whereas the n-hexane extract exhibited weak antioxidant activity (2,965.25±1,966.56 ppm). This polarity-dependent trend is consistent with the preferential recovery of metabolite classes that are commonly associated with antioxidant activity. Based on the DPPH IC_{50} classification (very strong <50 ppm, strong 50–100 ppm, moderate 100–150 ppm, weak

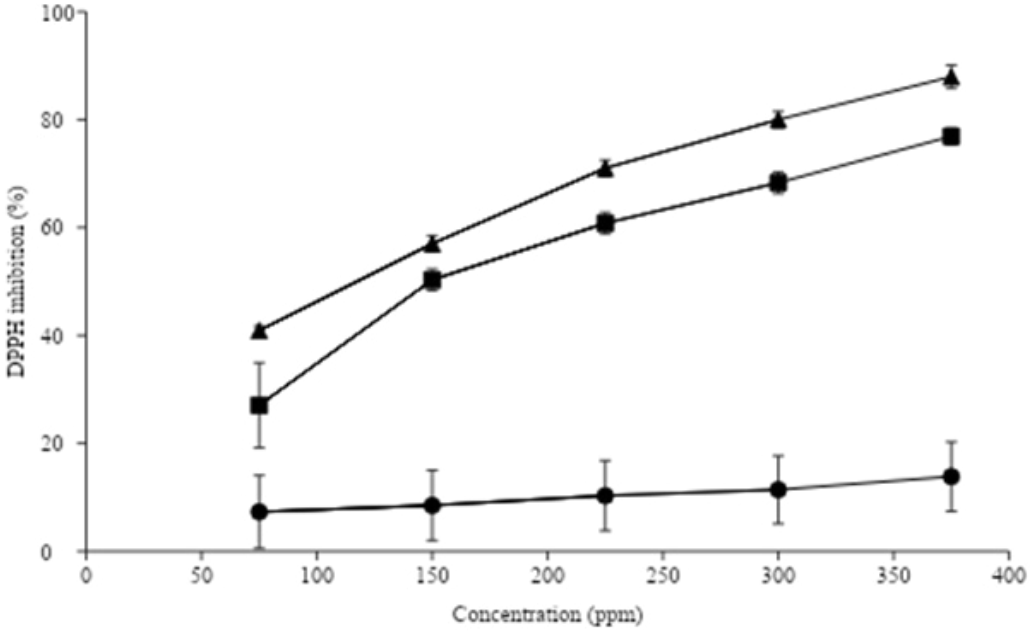

Figure 3 DPPH radical inhibition (%) of young *S. taccada* fruit extracts at different concentrations; values represent mean ± SD (n = 3);
• (n-hexane), ▪ (ethyl acetate), and ▲ (ethanol)

150–200 ppm, and very weak >200 ppm) (Molyneux, 2004), the ethanol, ethyl acetate, and n-hexane extracts fell within the moderate, weak, and very weak categories, respectively.

A simple comparison between the extraction yield and antioxidant activity revealed an inverse relationship between the extraction efficiency and IC_{50} values. Fractions with higher extraction yields exhibited stronger antioxidant activity, with the ethanol fraction showing the highest yield and lowest IC_{50} value. Although formal correlation analysis was not performed because only three solvent fractions were evaluated (n=3), this trend suggests that solvent polarity influences not only the extraction efficiency but also the recovery of antioxidant-associated metabolites. Nevertheless, this relationship should be interpreted cautiously because the total phenolic and flavonoid contents were not quantified in this study. Therefore, the observed relationship should be considered a qualitative trend rather than a statistically validated correlation.

The antioxidant activity observed in the present study is consistent with previous reports on *S. taccada*. Apriandi *et al.* (2021) reported an IC_{50} value of 130.71 ppm for young fruit extracts, whereas ethanol extracts of leaves and stems exhibited IC_{50} values of 109.81 and 87.15 µg/mL, respectively. These observations suggest that the antioxidant capacity of *S. taccada* varies among plant organs and may reflect differences in metabolite accumulation patterns. Similar organ-dependent variation has also been reported by (Budiana *et al.* (2019) and Nasution *et al.* (2026), who observed IC_{50} values of 109.81±0.71 and 87.15±0.71 µg/mL in leaf and stem extracts, respectively, accompanied by measurable phenolic and flavonoid contents. Comparable patterns have been reported in several medicinal halophytes, including *Atriplex stocksii, Cressa cretica,* and *Ipomoea pes-caprae*, where elevated phenolic and flavonoid contents are associated with stronger antioxidant activities (Qasim *et al.*, 2017; Nazir *et al.*, 2018).

The exceptionally large standard deviation observed for the n-hexane fraction likely reflects the very low inhibition values obtained across the tested concentration range. Because the inhibition percentages did not approach 50%, the IC_{50} estimate relied on substantial extrapolation from the regression model, reducing precision. Therefore, the reported IC_{50} should be interpreted mainly as an indicator of very weak radical scavenging capacity rather than a highly precise quantitative endpoint.

The concentration-dependent inhibition profiles shown in Figure 3 further support the influence of solvent polarity on the antioxidant performance. Increasing the extract concentration resulted in progressively higher DPPH inhibition percentages for all the solvent fractions. The ethanol extract consistently produced the highest inhibition values, increasing from 40.91±0.84% at 75 ppm to 87.94±2.15% at 375 ppm. Similarly, the ethyl acetate extract showed a marked increase from 27.05±7.88% to 76.90±1.52%, whereas the n-hexane extract exhibited limited inhibition, ranging from 7.29±6.77% to 13.84±6.43% across the tested concentrations. These findings demonstrate a clear dose-dependent response and indicate that polar extracts possess substantially greater free radical-scavenging capacity than non-polar extracts. The stronger antioxidant activity of the ethanol fraction is likely associated with the preferential extraction of polar oxygenated metabolites, which are commonly reported as radical-scavenging constituents in plant extracts (Dai & Mumper, 2010; Nawaz *et al.*, 2020; Ma *et al.*, 2022; Azzahra *et al.*, 2025; Tripathi *et al.*, 2025). However, this interpretation remains inferential because the total phenolic and flavonoid contents were not quantified in the present study.

Previous studies on *S. taccada* fruits have also demonstrated the effects of developmental stage on antioxidant capacity. Fatmawati *et al.* (2021) reported that juice prepared from young fruits exhibited stronger antioxidant activity (IC_{50} = 19.52 ppm) than that obtained from mature fruits (IC_{50} = 50.66 ppm), whereas mixed-stage fruits showed intermediate activity (35.52 ppm). The higher antioxidant activity in young fruits was accompanied by an elevated vitamin C content (20.24 mg), suggesting that antioxidant-related metabolites accumulate

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during early fruit development. Although these previously reported IC_{50} values. were substantially lower than those obtained in the present study, and several methodological differences may explain this discrepancy. Fruit juice contains naturally occurring water-soluble antioxidants, including ascorbic acid, which may not be recovered in equivalent proportions after sequential solvent extraction. Therefore, differences in sample preparation, extraction procedures, and analytical approaches may contribute to the observed variations in antioxidant activity.

Interestingly, preliminary qualitative phytochemical screening did not detect phenolics or flavonoids in these extracts. This apparent discrepancy may reflect the limited sensitivity of conventional qualitative screening methods when metabolites are present at low concentrations or in complex structural forms. The observed antioxidant activity, particularly in the ethanol fraction, suggests the presence of antioxidant-associated metabolites that require further chemical characterization to confirm their presence. Moreover, qualitative phytochemical screening generally exhibits lower sensitivity than instrumental approaches and may fail to detect compounds present at low concentrations or in their glycosylated forms.

NMR-Assisted Characterization of Metabolites in The Ethanol Extract

To obtain preliminary information regarding metabolites potentially associated with the antioxidant activity observed in the ethanol fraction, NMR-assisted phytochemical characterization was performed on the ethanol extract, which exhibited the strongest DPPH radical scavenging activity and the lowest IC_{50} value among all solvent fractions. The ethanol extract exhibited a concentration-dependent increase in DPPH inhibition, reaching 87.94 ± 2.15% at 375 ppm, with an IC_{50} value of 113.99 ± 5.07 ppm. These observations are consistent with the presence of polar metabolites in the ethanol fraction; however, the present data do not establish a causal relationship between these metabolites and their associated antioxidant activity. Therefore, ^{1}H and ^{13}C NMR analyses were conducted to obtain preliminary information regarding the chemical characteristics of the metabolites present in this bioactive fraction (Figures 4 and 5, respectively).

The ^{1}H NMR spectrum of the ethanol extract was dominated by intense resonances within the δH 3.1-4.0 ppm region, while the corresponding ^{13}C signals were observed at δC 64.17-73.68 ppm. These chemical shift regions are commonly associated with oxygenated protons and carbon environments in carbohydrates and glycosylated metabolites. These metabolites frequently accumulate in developing fruits as carbon reserves and metabolic intermediates required for growth and cellular metabolism (Emwas *et al*., 2019; Chen *et al*., 2024; Dutta *et al*., 2025; Joo *et al*., 2025; Liu *et al*., 2025). In addition, a prominent signal observed at approximately δH 4.93 ppm is consistent with the proton environments commonly reported for carbohydrate-derived structures and glycosylated metabolites (Emwas *et al*., 2019; Kim *et al.,* 2023).

The tentative assignments corresponding to the major ^{1}H and ^{13}C resonances in Figures 4 and 5 are listed in Table 2. An important observation from the NMR spectra is that the signals associated with carbohydrates were considerably more abundant than those associated with secondary metabolites. This finding suggests that primary metabolites constitute a substantial proportion of ethanol extracts. Therefore, although antioxidant-associated secondary metabolites may be present, their occurrence cannot be inferred solely from signal intensity because one-dimensional NMR does not provide sufficient resolution to distinguish all overlapping constituents in complex plant extract. In addition to the carbohydrate-associated signals, several resonances were detected in the aliphatic region. Signals observed at δH 0.8-2.0 ppm and δC 14.57-39.04 ppm are characteristic of aliphatic proton and carbon environments commonly reported in sterol-, lipid-, and terpenoid-related metabolites (Dutta *et al*., 2025; Zhang *et al*., 2025). Similar resonances have been observed in plant extracts containing phytosterols and other hydrophobic secondary metabolites. Although these signals

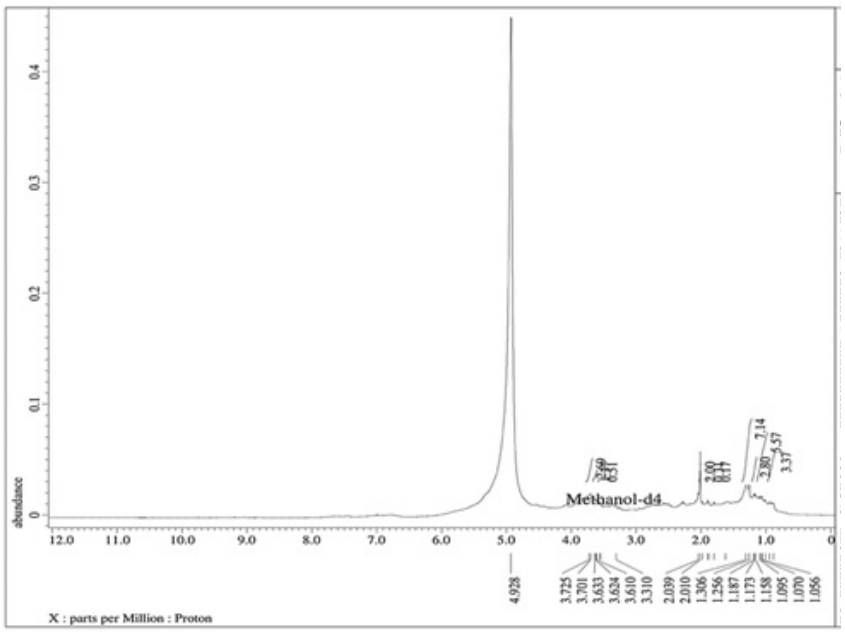

Figure 4 ^{1}H full NMR spectra of ethanol extract from young fruits

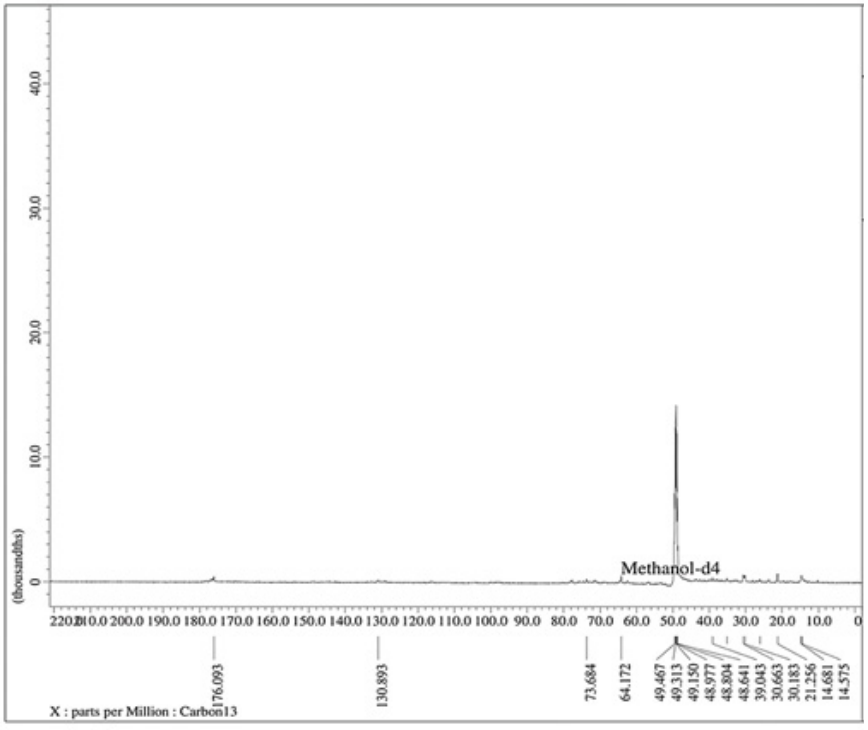

Figure 5 ^{13}C full NMR spectra of ethanol extract from young fruits

were less intense than those observed in the carbohydrate region, their presence indicates that the ethanol extract contains structurally diverse metabolite classes beyond primary carbohydrates.

The ^{13}C NMR spectrum also revealed signals at δC = 130.89 and 176.09 ppm. Resonances within the 120-140 ppm region are commonly associated with unsaturated carbon environments, including olefinic and aromatic carbons, whereas signals near 170-180 ppm are typically attributed to carbonyl-containing functionalities, such as esters, carboxylic acids, or related oxygenated

http://dx.doi.org/10.17844/av946v17

Table 2 Tentative assignment of major NMR signals detected in the ethanol extract of young *S. taccada* fruits

Tentative assignment	^{1}H NMR signal (ppm)	^{13}C NMR signal (ppm)	Interpretation	References
Carbohydrate-derived metabolites	3.10-4.00	64.17-73.68	Oxygenated proton and carbon environments commonly associated with sugars and carbohydrate derived metabolites	Chen *et al.* (2024); Emwas *et al.* (2019); Joo *et al.* (2025)
Carbohydrate-rich and glycosylated structures	~4.93	64.17-73.68	Signals consistent with glycosylated or carbohydrate-associated structures	Emwas *et al.* (2019); Kim *et al.* (2010)
Sterol-, lipid-, and terpenoid-related metabolites	0.80-2.00	14.57-39.04	Aliphatic proton and carbon environments characteristic of hydrophobic metabolites	Dutta *et al.* (2025); Zhang *et al.* (2025)
Unsaturated metabolites	-	130.89	Olefinic or aromatic carbon environment	Emwas *et al.* (2019); Kim *et al.* (2010)
Carbonyl-containing metabolites	-	176.09	Carbonyl carbon associated with esters, carboxylic acids, or related oxygenated metabolites	Emwas *et al.* (2019); Kim *et al.* (2010)

The assignments are tentative and based on one-dimensional ^{1}H and ^{13}C NMR spectral interpretation and comparison with the published literature;
Definitive structural identification requires complementary analyses such as 2D NMR and LC-MS/MS.

metabolites (Kim *et al.*, 2010; Emwas *et al.*, 2019; Dutta *et al.*, 2025). The occurrence of these signals suggests the presence of metabolites possessing unsaturated and oxygenated structural features, although their exact identities cannot be determined from one-dimensional NMR data alone.

Unlike previous studies that primarily reported phytochemical classes through conventional phytochemical screening or crude extract analysis, the present study provides a solvent-specific NMR-based metabolite distribution in young fruits, highlighting the developmental stage-specific chemical characteristics. Previous phytochemical studies on *S. taccada* have reported the presence of flavonoids, flavonoid glycosides, terpenoids, alkaloids, and phenolic constituents in different plant organs, including leaves, stems, and fruits (El-Sayed *et al.*, 2020; Jasna *et al.*, 2025; Nasution *et al.*, 2026). However, information on the chemical composition of young fruits is limited. The predominance of oxygenated signals observed in the present study is consistent with the tendency of developing fruits to accumulate carbohydrate-derived and glycosylated secondary metabolites during active growth. Therefore, the current findings contribute specifically to the understanding of metabolite distribution in young fruits, rather than extrapolating chemical characteristics from other plant organs. Overall, the NMR results provide a plausible chemical context for interpreting the antioxidant activity of ethanol extracts. However, because metabolite assignments were tentative and no quantitative phytochemical analysis or metabolite

bioactivity correlation was performed, the contribution of individual metabolite classes should be regarded as a qualitative hypothesis requiring further confirmation (Dai & Mumper, 2010; Gulcin & Alwasel, 2023).

Nevertheless, the current data do not allow the direct attribution of antioxidant activity to specific metabolites because the spectra are dominated by carbohydrate-associated resonances and only one-dimensional NMR data are available. The predominance of carbohydrate-associated resonances is consistent with the developmental stage of young fruits, during which primary metabolism remains highly active to support cell division, tissue differentiation, and subsequent maturation. Consequently, dominant carbohydrate resonances are expected and should be regarded as one of the principal biochemical characteristics of young *S. taccada* fruits rather than a limitation of the extraction process (Yadav *et al*., 2021; Singh *et al*., 2022). Importantly, the predominance of carbohydrate signals does not preclude the presence of secondary metabolites. Additional resonances assigned to aliphatic, unsaturated, carbonyl-containing, and oxygenated compounds indicate that structurally diverse secondary metabolites were also present in the ethanol extract, although at a relatively lower abundance or partially overlapping with intense carbohydrate signals (Kim *et al*., 2010; Emwas *et al*., 2019). Within this biochemical context, these less abundant metabolites provide a plausible chemical basis for the observed radical-scavenging activity (El-Sayed *et al*., 2020; Nasution *et al*., 2026), reinforcing the qualitative interpretation noted above, rather than establishing a new conclusion.

Similar observations have been reported in *Ipomoea pes-caprae* and other coastal halophytes, where elevated phenolic and flavonoid contents are associated with stronger antioxidant activity (Qasim *et al*., 2017; Nazir *et al*., 2018; Budiana *et al*., 2019; Nasution *et al*., 2026). Although the total phenolic and flavonoid contents were not quantified in the present study, the stronger antioxidant activity of the ethanol fraction is consistent with the possibility that polar oxygenated metabolites contributed to the observed radical scavenging activity. However, this requires confirmation through quantitative phytochemical analyses and compound identification. Although metabolite assignments remain tentative, these findings extend our current knowledge of the chemical characteristics of this underexplored developmental stage and provide a basis for future metabolomic studies.

In addition, the DPPH assay represents a simplified chemical radical-scavenging model and does not directly reflect the biological antioxidant efficacy under physiological conditions. Therefore, the antioxidant activity reported in this study should be interpreted as an indication of in vitro radical scavenging potential rather than direct evidence of the in vivo antioxidant or pharmacological effects of the extracts. This study had several limitations. Because the 1D NMR spectrum is dominated by carbohydrate-associated resonances, metabolite assignments should be regarded as tentative chemical class annotations rather than definitive compound identifications. Furthermore, the total phenolic and flavonoid contents were not quantified, preventing a direct correlation analysis between the antioxidant activity and metabolite abundance. Consequently, the proposed relationship between solvent polarity, metabolite distribution, and antioxidant activity should be interpreted qualitatively. Future studies incorporating LC-MS/MS, 2D NMR, TPC/TFC assays, and multivariate analyses are required to validate these observations.

CONCLUSION

Solvent polarity significantly affected the extraction yield, antioxidant activity, and metabolite distribution in young *S. taccada* fruit. The ethanol fraction exhibited the highest extraction yield and moderate antioxidant activity, indicating enrichment of polar metabolites. NMR-assisted phytochemical characterization revealed the predominance of carbohydrate-associated and oxygenated metabolite signals in the ethanol extract of

M. alba leaves. However, metabolite identification and metabolite activity relationships remain preliminary because the analysis relied solely on 1D ^{1}H and ^{13}C NMR spectroscopy data. Further validation using LC-MS/MS and 2D NMR is required.

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