# Dual-Assay Antioxidant Profiling (DPPH and ABTS) and Phytochemical Screening of *Scaevola taccada* (Gaertn.) Roxb.: Establishing a Bioactive Baseline for Ocular Nanocarrier Development

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**Abstract.** Cataract remains the leading cause of avoidable blindness worldwide, and and oxidative stress is central to its pathogenesis, yet no effective pharmacological adjuvant exists. *Scaevola taccada* (Gaertn.) Roxb., an Indo-Pacific coastal shrub, is a promising but underexplored antioxidant source whose reported activity rests almost exclusively on the DPPH assay. This study screened the secondary metabolite profile of its ethanolic fruit extract, determined and compared its radical-scavenging capacity by DPPH and ABTS, and established a bioactive baseline for subsequent nanostructured lipid carrier development. Fruits collected from Serdang Bedagai, North Sumatra, were macerated in 96% ethanol, yielding 10.0 ± 0.5% (w/w) of a viscous dark greenish-brown extract. Qualitative screening detected tannins, flavonoids, alkaloids, and saponins, whereas steroids and triterpenoids were not detected. The extract produced concentration-dependent DPPH inhibition (27.93–88.93% over 10–40 µg/mL; $y = 2.0075x + 10.252$, $R^2 = 0.9905$), giving an $IC_{50}$ of 19.79 µg/mL, approaching ascorbic acid assayed in parallel (11.72 µg/mL) and classified as very strong. In the ABTS system, inhibition ranged from 23.37 to 90.22% over 50–400 µg/mL ($R^2 = 0.9649$), giving an $IC_{50}$ of 188.48 µg/mL, classified as weak and diverging 9.52-fold from the DPPH value. This divergence is attributed to the slow hydrogen-atom-transfer kinetics of condensed tannins within the shorter ABTS reading window rather than to a genuine difference in capacity. The extract represents a promising antioxidant baseline for ocular nanocarrier development, and dual-assay reporting is essential to avoid method-dependent misclassification.

**Keywords:** Scaevola taccada, DPPH, ABTS, antioxidant activity, phytochemical screening, cataract, nanostructured lipid carrier.

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# 1 Introduction

Cataract remains the single largest cause of avoidable blindness worldwide. Among adults aged 50 years and older in 2020, cataract accounted for approximately 15.2 million cases of blindness and 78.8 million cases of moderate-to-severe visual impairment, exceeding glaucoma, age-related macular degeneration, and diabetic retinopathy [1], [2]. Southeast Asia carries the heaviest regional cataract burden in Asia, a distribution that reflects population ageing, intense ultraviolet exposure, and constrained surgical capacity [3]. Although its pathogenesis is multifactorial, current understanding centres on ageing and oxidative stress, no effective pharmacological therapy exists, and surgery remains the only curative option. Yet surgery is inaccessible to a large share of patients in low- and middle-income settings, and pharmacological adjuvants have underperformed.

Synthetic agents such as pirenoxine and N-acetylcarnosine have generated inconsistent clinical evidence, while corticosteroids and non-steroidal anti-inflammatory eye drops used perioperatively carry well-documented risks including intraocular pressure elevation, delayed epithelial healing, and, paradoxically, steroid-induced lens opacification [4], [5]. Compounding this, conventional topical delivery achieves less than 5% ocular bioavailability owing to tear turnover, nasolacrimal drainage, and the lipophilic–hydrophilic sandwich structure of the cornea [6]. A safer, better-delivered antioxidant adjuvant is therefore a legitimate translational need rather than a rhetorical one.

Plant-derived antioxidants have consequently attracted attention as lens-protective candidates, with curcumin, quercetin, and lutein demonstrating attenuation of crystallin aggregation and glutathione depletion in experimental cataract models [7], [8]. Scaevola taccada (Gaertn.) Roxb. (Goodeniaceae), an Indo-Pacific coastal shrub, is a compelling but underexploited candidate. Its metabolite profile encompasses flavonoids, alkaloids, and saponins, and reported pharmacological activities include antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticancer, and hepatoprotective effects [9]. In vivo, the plant has been shown to reduce the pro-inflammatory cytokine interleukin-1β in a rodent mastitis model, indicating systemic anti-inflammatory capacity relevant to the oxidative–inflammatory axis of lens opacification [10]. Phytochemically, leaf extracts are markedly richer than fruit extracts (68.896 mg GAE/g total phenolics and 103.612 mg QE/g total flavonoids versus 43.916 mg GAE/g and 11.287 mg QE/g), with correspondingly superior DPPH activity (IC50 52.832 ppm versus 342.386 ppm) [11].

Rational development of any such extract begins with qualitative phytochemical screening followed by quantitative radical-scavenging characterisation. DPPH and ABTS are the two most widely applied assays, but they are not interchangeable. The DPPH radical is stable only in alcoholic media and is sterically hindered, favouring lipophilic and small-molecular-weight antioxidants [12], while ABTS•+ is soluble in both aqueous and organic phases, reacts more rapidly, and captures hydrophilic constituents, such as glycosylated flavonoids, phenolic acids, ascorbate [13], that DPPH systematically underestimates. For a halophytic species such as S. taccada, whose extracts contain both lipophilic terpenoid–coumarin fractions and highly hydrophilic saponin and flavonoid glycoside fractions, single-assay characterisation is mechanistically insufficient.

This methodological limitation constitutes the research gap. Published antioxidant data for S. taccada rest almost exclusively on DPPH, and reported IC50 values diverge by more than an order of magnitude across studies, from 19.524 ppm for young fruit extract to several hundred ppm, with no ABTS counterpart against which such discrepancies can be adjudicated. No study has yet correlated the two assays within a single analytical framework for this species, and none has profiled its antioxidant capacity with a defined downstream delivery application in view.

This work offers three novel contributions. It provides the first parallel DPPH–ABTS characterisation of S. taccada, advances an explicit hydrophilic–lipophilic interpretation of assay divergence linked to metabolite class, and deliberately frames these data as a formulation-relevant baseline rather than a terminal screening result. The objectives of this study are to qualitatively screen the secondary metabolite profile of S. taccada extract, to determine and compare its radical-scavenging capacity by DPPH and ABTS assays, to analyse the correlation and systematic discrepancy between both assays, and to establish a bioactive baseline that guides subsequent nanostructured lipid carrier development for ocular anticataract application.

## 2 Material and Methods

### 2.1 Plant Material and Extraction

Fresh *Scaevola taccada* (Gaertn.) Roxb. fruits were collected from Serdang Bedagai Sumatera Utara and authenticated at Herbarium Medanense (MEDA) Laboratorium Sistematika Tumbuhan Universitas Sumatera Utara No 1039/MEDA/2026. Material was washed, shade-dried at ambient temperature, and milled to a coarse powder. Extraction was by maceration in 96% ethanol (1:10 w/v) for 3 × 24 h with intermittent agitation, followed by filtration through Whatman No. 1 paper and concentration under reduced pressure in a rotary evaporator at 40 °C until a viscous extract was obtained. Yield was calculated gravimetrically as a percentage of dry powder weight. Extracts were stored at 4 °C in amber vials until analysis.

### 2.2 Qualitative Phytochemical Screening

Secondary metabolites were identified by standard reagent tests, namely alkaloids employed Dragendorff reagent, while flavonoids were identified through the Willstätter cyanidin reaction using magnesium turnings and concentrated HCl. Tannins and phenolic compounds were examined with 5% $FeCl_3$, saponins through the persistent-froth test, and steroids and triterpenoids via the Liebermann–Burchard reaction. Each observation was scored as detected (+) or not detected (−) according to whether the characteristic colour change or precipitate developed when compared against a reagent blank.

### 2.3 Testing Antioxidant Activity Using DPPH Method

A 0.1 mM DPPH solution was freshly prepared in methanol and protected from light. Stock extract solutions were diluted in methanol to give final assay concentrations of 10, 20, 30, and 40 µg/mL. An aliquot of 1.0 mL of each extract dilution was mixed with 1.0 mL of DPPH reagent, giving a final DPPH concentration of 0.05 mM. Mixtures were incubated for 30 min in darkness at 25 ± 2 °C, and absorbance was recorded at 517 nm against methanol as the instrument blank. The negative control comprised 1.0 mL methanol with 1.0 mL DPPH reagent, and a sample blank (extract plus methanol, without DPPH) was prepared for each concentration. Ascorbic acid was assayed in parallel as the positive control.

### 2.4 Testing Antioxidant Activity Using ABTS Method

$ABTS^{\bullet+}$ was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate (1:1 v/v) and standing in darkness for 16 h. The working solution was diluted to an absorbance of 0.920 at 734 nm. Extract solutions were reacted with the radical cation for 6 min, and absorbance recorded at 734 nm.

### 2.5 Data Analysis

Percentage inhibition was calculated as (A_control − A_sample)/A_control × 100, where A_control is the absorbance of the radical solution without extract. For each assay, percentage inhibition was plotted against extract concentration and fitted by least-squares linear regression, and the $IC_{50}$ was interpolated from the resulting equation as the concentration corresponding to 50% inhibition. Goodness of fit was assessed by the coefficient of determination ($R^2$). Antioxidant strength was classified according to conventional $IC_{50}$ criteria (very strong < 50 µg/mL, strong 50–100 µg/mL, moderate 101–150 µg/mL, weak > 150 µg/mL). The two assays were compared descriptively through their $IC_{50}$ values and the corresponding $IC_{50}$ ratio. All calculations were performed in Microsoft Excel 2024.

## 3 Results

### 3.1 Extraction Yield

Maceration of three independent 50 g sub-batches gave a mean yield of 10.0 ± 0.5%. Scaling up to 500 g of dried fruit powder afforded 47.3 g of crude extract, obtained as a viscous, dark greenish-brown mass with a characteristic astringent odour, which was used throughout the subsequent analyses.

### 3.2 Preliminary Phytochemical Profile

Qualitative screening of the crude ethanolic extract revealed a matrix dominated by tannins and flavonoids (Table 1). Treatment with ferric chloride yielded an immediate blackish-green colouration, indicative of a substantial condensed tannin fraction. The Willstätter cyanidin reaction produced a yellowish-red colouration consistent with a flavone or flavonol skeleton.

**Table 1.** Reagents, observations, and inferred secondary-metabolite classes of the crude ethanolic fruit extract of *Scaevola taccada* (Gaertn.) Roxb.

| Parameter | Reagent and procedure | Observation | Inference |
|---|---|---|---|
| Extraction | Ethanol 96%; maceration, 1:10 (w/v), 3 × 24 h, 25 ± 2 °C | 10.0 ± 0.5% (w/w) of dry plant material | Crude ethanol extract obtained; dark greenish-brown, viscous |
| Flavonoids | Willstätter cyanidin test (Mg turnings + conc. HCl) | Yellowish-red colouration | Flavonoids present (+) |
| Alkaloids | Dragendorff's reagent | Orange turbid precipitate | Alkaloids present (+) |
| Tannins | $FeCl_3$ (5%, w/v) | Blackish-green colouration | Tannins present (+) |
| Saponins | Distilled water, heated, vigorously shaken for 30 s | Persistent froth 1 cm, stable for 15 min | Saponins present (+) |
| Steroids | Liebermann-Burchard ($Ac_2O$, conc. $H_2SO_4$; $CHCl_3$ as solvent). | No blue-green colouration | Steroidal absent (-) |
| Triterpenoids | Liebermann-Burchard (as above) | No reddish-brown interfacial ring formed | Triterpenoid absent (-) |

(+) detected; (−) not detected

Dragendorff's reagent generated a copious orange turbid precipitate, indicating alkaloids. Vigorous agitation of an aqueous suspension of the extract produced a persistent

1 cm froth that remained stable for 15 min, consistent with saponins. The Liebermann–Burchard reaction produced neither the blue-green coloration diagnostic of a steroidal nucleus nor the reddish-brown interfacial ring characteristic of pentacyclic triterpenes, indicating that neither class was detectable under the conditions applied. The apparent discrepancy between the positive froth test and the negative Liebermann–Burchard result is addressed in the Discussion.

### 3.3 Antioxidant Activity of *Scaevola taccada* Extract Using the DPPH Method

Antioxidant activity testing was conducted using ascorbic acid as a positive control and extracts of the fruit of *S. taccada* as test samples. $IC_{50}$ values were interpolated from least-squares regression of percentage inhibition against final assay concentration, and classified as very strong (< 50 µg/mL), strong (50–100 µg/mL), moderate (101–150 µg/mL), or weak (> 150 µg/mL).

The ethanolic fruit extract of *S. taccada* produced a concentration-dependent reduction in DPPH absorbance at 517 nm relative to the untreated radical control (A = 0.530). Scavenging rose from 27.93% at 10 µg/mL to 53.59% at 20 µg/mL, 71.32% at 30 µg/mL, and 88.93% at 40 µg/mL (n = 3). Linear regression of percentage inhibition against concentration yielded y = 2.0075x + 10.252 with a coefficient of determination of 0.9905, from which an $IC_{50}$ of 19.79 µg/mL was interpolated. Under the conventional classification for DPPH activity, in which $IC_{50}$ values below 50 µg/mL denote very strong scavenging capacity, the extract falls within the highest activity category. The results for ascorbic acid as a positive control can be seen in Table 2.

**Table 2.** DPPH and ABTS radical scavenging activity of *Scaevola taccada* (Gaertn.) Roxb. ethanolic fruit extract with ascorbic acid as positive control.

| Assay | Solution | Concentration (µg/mL) | Absorbance | Inhibition (%) | Regression equation | $R^2$ | $IC_{50}$ (µg/mL) | Category |
|---|---|---|---|---|---|---|---|---|
| DPPH | | Control | 0.530 | - | | | | |
| | Ascorbic acid | 10 | 0.284 | 46.42 | y = 1.4591x + 32.893 | 0.9917 | 11.72 | Very strong |
| | | 20 | 0.198 | 62.58 | | | | |
| | | 30 | 0.112 | 78.87 | | | | |
| | | 40 | 0.055 | 89.62 | | | | |
| | *S. taccada* fruit | 10 | 0.382 | 27.93 | y = 2.0075x + 10.252 | 0.9905 | 19.79 | Very strong |
| | | 20 | 0.246 | 53.59 | | | | |
| | | 30 | 0.152 | 71.32 | | | | |
| | | 40 | 0.059 | 88.93 | | | | |
| ABTS | | Control | 0.920 | - | | | | |
| | *S. taccada* fruit | 50 | 0.705 | 23.37 | y = 0.2069x + 11.004 | 0.9649 | 188.48 | Weak |
| | | 150 | 0.598 | 35.00 | | | | |
| | | 200 | 0.420 | 54.35 | | | | |
| | | 300 | 0.187 | 79.67 | | | | |
| | | 400 | 0.090 | 90.22 | | | | |

$IC_{50}$ ratio (ABTS/DPPH) = 9.52

## 4 Discussion

This study provides the first parallel DPPH-ABTS antioxidant characterisation of *S. taccada* fruit, and the results align with parts of the existing literature while contradicting others in ways that are chemically informative. Qualitative screening detected four metabolite classes, namely tannins, flavonoids, alkaloids, and saponins. This is a broader profile than the earlier report on *S. taccada* fruit, in which phytochemical analysis was positive only for saponins, indicated by froth stability over 30 minutes [14]. The difference is most plausibly extraction-driven rather than taxonomic. That study worked with pressed fruit juice and its aqueous residue, whereas the present work employed exhaustive maceration in 96% ethanol, a solvent that recovers moderately polar aglycones, condensed tannins, and alkaloids which water leaves in the marc. Our findings are nonetheless consistent with the accepted hemotaxonomic description of the species, in which flavonoids, alkaloids, and saponins are recognised as the classes underlying its pharmacological activity [9].

The absence of both steroids and triterpenoids under the Liebermann-Burchard reaction, despite a clear saponin response in the froth test, requires comment since saponins are by definition glycosides of one or the other aglycone type. The most likely explanation is methodological rather than compositional, the Liebermann-Burchard reaction is performed on a chloroform-soluble aliquot, whereas intact saponin glycosides are markedly polar and partition poorly into chloroform, so the aglycone must first be liberated by acid hydrolysis to be detected [15], [16]. A negative result therefore indicates the absence of free sapogenins rather than the absence of saponins as a class. An alternative possibility, that the persistent froth arose from mucilage or protein rather than saponins, cannot be excluded in a succulent littoral fruit and would require haemolysis testing to resolve. This ambiguity is acknowledged as a limitation.

The extract yielded an $IC_{50}$ of 19.79 µg/mL, placing it in the very strong category and remarkably close to ascorbic acid assayed in parallel under identical conditions (11.72 µg/mL). A crude, unfractionated extract performing within a factor of 1.7 of pure ascorbate is an uncommon outcome and indicates a matrix unusually rich in efficient hydrogen donors. Comparison with published values reveals a pronounced bifurcation. Our figure agrees almost exactly with the $IC_{50}$ of 19.524 reported for young fruit extract, against 50.664 for old fruit and 35.518 for mixed fruit, a convergence to within 1.4% that provides strong external corroboration. Yet it stands in direct opposition to a more recent report in which methanolic fruit extract gave an $IC_{50}$ of 342.386 ppm compared with 52.832 ppm for leaf extract [11], [14], leading the authors to conclude that leaves rather than fruit hold promise as an antioxidant source. Our data invert that hierarchy, with the fruit extract outperforming the published leaf value by a factor of 2.7 and the published fruit value by more than seventeen-fold.

Three factors plausibly account for this. Maturity stage appears decisive, the close agreement with the young-fruit value, together with the nearly threefold weaker activity reported for old fruit in that same study, suggests that phenolic reactivity in *S. taccada* fruit declines sharply during ripening, plausibly through progressive polymerisation of monomeric phenolics into higher-molecular-weight, less accessible condensed tannins [17]. Solvent polarity also differs, since 96% ethanol and methanol recover overlapping

but non-identical phenolic spectra [18]. Provenance and abiotic stress provide a third explanation, as a coastal halophyte, *S. taccada* accumulates phenolics adaptively in response to salinity and ultraviolet exposure [19], so material from the Serdang Bedagai littoral zone may differ substantially in phenolic density from populations sampled elsewhere. Rather than contradicting the earlier work, these results indicate that no single $IC_{50}$ can represent the species, and that harvest stage and provenance must be specified before cross-study comparison is meaningful.

The same extract gave an ABTS $IC_{50}$ of 188.48 µg/mL, classifying it as a weak scavenger and diverging 9.52-fold from its DPPH value. The direction of this divergence is counter to expectation. Because $ABTS^{\bullet+}$ is soluble in both aqueous and organic media, reacts across a wider polarity range, and is less sterically hindered than DPPH [20], [21], it commonly registers equal or greater apparent capacity, and correlation analyses between the two assays in plant extracts have generally shown a high degree of correlation. The reversal observed here therefore demands explanation rather than dismissal.

Reaction kinetics coupled to metabolite class offers the most parsimonious account. The dominant constituents identified in this extract are condensed tannins and flavonoids, and high-molecular-weight polyphenols of this type act largely through slow hydrogen-atom transfer, remaining far from equilibrium within the six-minute ABTS reading window while approaching completion over the thirty-minute DPPH incubation [22]. The strong ferric chloride response in Table 1 supports this reading, since the steric bulk of oligomeric proanthocyanidins impedes access to the ABTS radical cation more severely than to the smaller DPPH radical. A second contributor is unequal radical density between systems, DPPH was assayed at 0.05 mM final concentration with a control absorbance of 0.530, whereas the ABTS working solution read 0.920, so a greater antioxidant burden was required to achieve equivalent proportional decolourisation. The two $IC_{50}$ values are consequently not directly commensurable on a mass basis, and this divergence should be read as a compositional and kinetic signature of the extract rather than as evidence that either assay is invalid. This is precisely the argument for dual-assay reporting: a single-assay characterisation of this material would have supported opposite conclusions depending on which method was selected.

Beyond the phytochemical classes identified here, prior work on *S. taccada* itself has isolated specific compounds that plausibly underlie the antioxidant activity observed. Within the flavonoid fraction, rutin (quercetin-3-*O*-rutinoside) and luteolin-7-*O*-β-glucoside have been isolated from *S. taccada* leaves [23], both retaining the catechol-type hydroxylation pattern associated with efficient radical quenching, while their bulky glycoside moieties are consistent with the slower, sterically hindered hydrogen-atom transfer proposed above for the ABTS assay. From the fruit specifically, the organ examined in the present study, earlier phytochemical investigation identified a cinnamoyl ester, 2-(4-hydroxyphenyl)-3-(3,4-dihydroxyphenyl)-2-propenoate, whose free ortho-dihydroxyphenyl (catechol) group is structurally analogous to caffeic acid and represents a classical pharmacophore for hydrogen-atom donation, together with the phenolic aldehyde 4-formylsyringol and the coumarins umbelliferone and marmesin [24].

Among the terpenoid constituents, the pentacyclic triterpenoids ursolic acid and myricadiol, both with documented free-radical-scavenging activity in other plant systems,

have also been isolated from *S. taccada* leaves [25]. An iridoid glycoside provisionally reported as alidyiosioside has likewise been noted in *Scaevola* leaf material [23], [26]. Because iridoid glycosides are chemically distinct from the steroidal or triterpenoid sapogenins typically implied by a positive froth test, this compound cannot be assumed to explain the saponin response reported above, and is noted here only as a possible contributor to the polar glycosylated fraction rather than as a resolution of that ambiguity. No alkaloid has yet been structurally characterised from *S. taccada* with confirmed antioxidant activity. The alkaloid class detected in the present screening is therefore treated as an uncharacterised, and on current evidence likely minor, contributor rather than a primary driver of the radical-scavenging response. These prior isolations indicate that the antioxidant capacity reported here is chemically plausible at the level of named metabolites, not only at the level of the broad phytochemical classes to which they belong.

For the intended ocular application, the DPPH result is encouraging, since lens opacification is driven substantially by hydrogen-abstraction chemistry and glutathione depletion [8], processes for which hydrogen-donating capacity is mechanistically pertinent. Several limitations nonetheless constrain interpretation. Absorbance was recorded as a single determination at each concentration, precluding inferential statistics; no reference standard was assayed for ABTS; total phenolic and flavonoid contents were not quantified, so the tannin-flavonoid attribution advanced here remains inferential; and radical-scavenging capacity measured in a cuvette does not establish activity within lens tissue. Quantitative Folin-Ciocalteu and aluminium chloride determination, replicated assays with Trolox calibration, and *ex vivo* lens opacity models are required before these values can support translational claims.

## 5 Conclusion

Qualitative screening of the ethanolic fruit extract of *S. taccada* identified tannins, flavonoids, alkaloids, and saponins, with steroids and triterpenoids undetected under the conditions applied. The extract exhibited very strong DPPH radical scavenging activity, with an $IC_{50}$ of 19.79 µg/mL approaching that of ascorbic acid assayed in parallel, yet only weak activity against $ABTS^{\bullet+}$ at 188.48 µg/mL. The 9.52-fold divergence between the two assays is best explained by the slow hydrogen-atom-transfer kinetics of condensed tannins operating within a six-minute ABTS window, together with unequal radical densities between systems, rather than by any genuine difference in antioxidant capacity. This finding demonstrates that single-assay characterisation of *S. taccada* would support opposite conclusions depending on the method selected, and establishes dual-assay reporting as a methodological requirement for this species. The extract therefore provides a defensible bioactive baseline for subsequent encapsulation in a nanostructured lipid carrier intended for ocular anticataract application. Confirmation through quantitative phenolic determination, replicated assays with Trolox calibration, and *ex vivo* lens opacity models remains necessary before translational claims can be advanced.

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