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Phytochemical Profile and Antioxidant Capacity of Aqueous Hibiscus tiliaceus L. Flower-Petal Extract: Relevance to Green Silver Nanoparticle Synthesis

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Abstract
The present study characterized the phytochemical composition and antioxidant potential of an aqueous flower-petal extract of Hibiscus tiliaceus L. as a biochemical basis for its subsequent use in plant-mediated silver nanoparticle synthesis. Fresh petals were shade-dried, powdered and extracted with water (1:20, w/v) at 60 °C for 30 min. Qualitative phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), and DPPH and ABTS radical-scavenging assays were performed. Phenolics, flavonoids, saponins, carbohydrates, reducing sugars, proteins, free amino acids, lipids/fixed oils, alkaloids, terpenoids and anthraquinones were detected, whereas tannins, steroids, cardiac glycosides, coumarins and ester-containing constituents were not detected under the applied screening conditions. TPC increased from 8.29 ± 0.91 to 19.14 ± 1.10 mg GAE/g extract as the petal quantity increased from 1 to 3 g, while TFC increased from 6.31 ± 0.48 to 14.71 ± 0.88 mg QE/g extract. The petal extract displayed marked concentration-dependent antioxidant activity. DPPH scavenging increased from 3.29 ± 0.04% at 10 µg/mL to 89.88 ± 3.04% at 400 µg/mL, whereas ABTS scavenging increased from 2.77 ± 0.06% to 88.10 ± 2.66% over the same range. The corresponding IC50 values were 166.40 and 177.35 µg/mL, respectively. Ascorbic acid showed lower IC50 values (19.36 and 21.08 µg/mL), as expected for a purified reference antioxidant. The combined phytochemical and antioxidant findings demonstrate that H. tiliaceus petals contain a redox-active aqueous phytochemical matrix with potential to support metal-ion reduction and surface stabilization in green nanoparticle synthesis.

Keywords: Hibiscus tiliaceus; flower petals; phenolics; flavonoids; DPPH; ABTS; antioxidant activity; green synthesis.

1. Introduction

Plant-derived antioxidants remain important sources of chemically diverse reducing molecules, particularly phenolic acids, flavonoids, terpenoids and related metabolites capable of donating electrons or hydrogen atoms to reactive species. Their biological relevance extends beyond conventional antioxidant screening because the same functional groups that participate in radical neutralization can also interact with metal ions and inorganic surfaces. This dual chemical behavior has made

phytochemical-rich extracts increasingly important in green nanotechnology, where plant metabolites may serve simultaneously as reducing, capping and stabilizing agents during nanoparticle formation.

Hibiscus tiliaceus L. (Malvaceae) is a coastal and mangrove-associated species with a long history of traditional use and a chemically diverse phytochemical profile. Earlier investigations of its flowers demonstrated measurable phenolic and flavonoid contents together with antioxidant activity (Kumar et al., 2008; Wong et al., 2009). Methanolic flower extracts have also shown antioxidant and protective effects against oxidative injury, indicating that the floral tissues contain biologically active redox-responsive constituents (Rosa et al., 2006, 2007). Studies using leaves and other tissues have identified or inferred flavonoids, phenolics, terpenoids and related secondary metabolites, while recent chromatographic work on aqueous H. tiliaceus extracts confirmed flavonoid constituents including rutin and kaempferol-3-O-rutinoside (Borive Amani et al., 2025).

The antioxidant profile of H. tiliaceus is relevant to the growing interest in this species as a green-synthesis matrix. Alawfi et al. (2020) demonstrated that aqueous H. tiliaceus flower extract can mediate silver nanoparticle formation, confirming the reducing capacity of the floral phytochemical environment. More recently, Konduri et al. (2024) reported green synthesis of AgNPs using H. tiliaceus leaves and linked plant-derived functional groups with nanoparticle formation and antioxidant behavior. Nevertheless, the chemical behavior of isolated flower petals deserves specific attention because phytochemical distribution varies among plant organs, extraction solvents and environmental conditions (Abdul-Awal et al., 2016; Surana et al., 2022).

DPPH and ABTS assays are widely used complementary approaches for evaluating radical-scavenging capacity. DPPH is commonly employed in a predominantly organic reaction environment, whereas ABTS can respond to a broader range of hydrophilic and lipophilic antioxidants. Agreement between the two assays therefore provides stronger evidence of generalized redox activity than a single radical system alone (Brand-Williams et al., 1995; Re et al., 1999). When interpreted together with TPC and TFC measurements, these assays can provide a practical biochemical description of an extract before its use in downstream functional applications.

Accordingly, the present study investigated the aqueous flower-petal extract of H. tiliaceus through qualitative phytochemical screening, quantitative determination of total phenolic and flavonoid contents, and DPPH and ABTS radical-scavenging assays. Ascorbic acid was included as a reference antioxidant. The study aimed to establish whether isolated H. tiliaceus petals provide a sufficiently rich redox-active matrix to justify their subsequent application in green silver nanoparticle synthesis.

2. Materials and Methods

2.1 Plant material and aqueous extraction

Fresh, healthy and fully opened Hibiscus tiliaceus L. flowers were collected from [collection locality, district, state, India] during [month and year] and authenticated by a qualified taxonomist at [Department/Herbarium/Institution]; a voucher specimen number should be inserted before submission. Petals were separated from other floral tissues, washed, shade-dried at approximately 25-30 °C to constant mass, pulverized and stored at 4 °C. For extraction, 10 g dried petal powder was mixed with 200 mL ultrapure water (1:20, w/v), maintained at 60 °C for 30 min with continuous stirring, cooled,

centrifuged at 6,000 × g for 10 min and filtered through Whatman No. 1 paper followed by a 0.45 µm membrane. A portion was used fresh and the remainder was lyophilized and stored at -20 °C until analysis.

2.2 Qualitative phytochemical screening

The aqueous petal extract was screened using standard color- and precipitation-based phytochemical tests with minor laboratory-scale modifications (Harborne, 1998; Evans, 2009). Phenolics and tannins were examined using ferric chloride reactions; flavonoids by alkaline reagent and/or Shinoda tests; saponins by the froth test; carbohydrates by Molisch's test; reducing sugars by Benedict's test; proteins by the Biuret test; free amino acids by ninhydrin; lipids/fixed oils by Sudan III and/or grease-spot testing; alkaloids by Dragendorff's/Mayer's reagents; terpenoids by the Salkowski reaction; steroids by the Liebermann-Burchard reaction; cardiac glycosides by Keller-Killiani testing; anthraquinones by a Borntrager reaction; coumarin-like compounds by alkaline/UV response; and ester-containing constituents by an optional hydroxamic-acid reaction. Results were recorded as present (+) or absent (-).

2.3 Total phenolic and flavonoid contents

TPC was determined by the Folin-Ciocalteu method (Singleton & Rossi, 1965). Briefly, 0.5 mL diluted extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent; after 5 min, 2.0 mL of 7.5% sodium carbonate was added and the mixture was incubated for 30 min in the dark. Absorbance was measured at 765 nm and TPC was calculated from a gallic acid calibration curve (20-200 µg/mL) and expressed as mg GAE/g extract. TFC was determined by the aluminium chloride method (Chang et al., 2002): 0.5 mL extract was combined with 1.5 mL methanol, 0.1 mL 10% AlCl3, 0.1 mL 1 M potassium acetate and 2.8 mL water, incubated for 30 min and read at 415 nm. Quercetin (10-100 µg/mL) was used for calibration, and results were expressed as mg QE/g extract. Determinations were performed in triplicate.

2.4 DPPH and ABTS radical-scavenging assays

DPPH radical-scavenging activity was measured using 0.1 mM DPPH in methanol according to Brand-Williams et al. (1995), with minor modification. Petal extract concentrations of 10, 25, 50, 100, 200 and 400 µg/mL were incubated with DPPH for 30 min in the dark and absorbance was measured at 517 nm. ABTS radical cation was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate for 12-16 h and diluting the solution to an absorbance of 0.70 ± 0.02 at 734 nm (Re et al., 1999). The same extract concentrations were reacted with ABTS•+ for 6 min before measurement at 734 nm. Ascorbic acid was tested as the reference antioxidant at 5, 10, 20, 30 and 50 µg/mL in both assays. Radical-scavenging activity was calculated as inhibition (%) = [(Acontrol - Asample)/Acontrol] × 100. IC50 values were estimated by linear interpolation between adjacent concentrations bracketing 50% inhibition.

2.5 Statistical analysis

Experiments were performed in triplicate and results are presented as mean ± standard deviation. Concentration groups were evaluated by one-way analysis of variance followed by Tukey's multiple-comparison test, with $p < 0.05$ considered statistically significant. Lower IC50 values were interpreted as greater radical-scavenging potency.

3. Results and Discussion

3.1 Qualitative phytochemical profile

The aqueous flower-petal extract showed a broad phytochemical profile (**Table 1**). Phenolics, flavonoids, saponins, carbohydrates, reducing sugars, proteins, free amino acids, lipids/fixed oils, alkaloids, terpenoids and anthraquinones were detected, whereas tannins, steroids, cardiac glycosides, coumarins and ester-containing constituents were not detected under the applied qualitative conditions. The co-occurrence of polyphenolic, nitrogen-containing, carbohydrate-derived and terpenoid constituents indicates that the petals provide a chemically heterogeneous aqueous matrix rather than a single dominant metabolite class.

Table 1. Qualitative phytochemical screening of aqueous H. tiliaceus flower-petal extract

Phytochemical/metabolite	Qualitative test	Result
Phenolics	Ferric chloride	Present (+)
Tannins	Ferric chloride/gelatin	Absent (-)
Flavonoids	Alkaline reagent/Shinoda	Present (+)
Saponins	Froth test	Present (+)
Carbohydrates	Molisch test	Present (+)
Reducing sugars	Benedict test	Present (+)
Proteins	Biuret test	Present (+)
Free amino acids	Ninhydrin test	Present (+)
Lipids/fixed oils	Sudan III/grease spot	Present (+)
Alkaloids	Dragendorff/Mayer	Present (+)
Terpenoids	Salkowski	Present (+)
Steroids	Liebermann-Burchard	Absent (-)
Cardiac glycosides	Keller-Killiani	Absent (-)
Anthraquinones	Borntrager	Present (+)
Coumarins	Alkaline/UV	Absent (-)
Ester-containing constituents	Hydroxamic-acid	Absent (-)

The positive phenolic and flavonoid reactions are consistent with flower-specific reports of appreciable phenolic-associated antioxidant activity in H. tiliaceus (Kumar et al., 2008; Wong et al., 2009). The negative reactions should not be interpreted as proof of absolute chemical absence because classical screening depends on solvent polarity, tissue chemistry and analytical sensitivity. Tissue-specific variation has been reported between H. tiliaceus leaves, bark and flowers (Abdul-Awal et al., 2016), while methanolic flower extracts have revealed sterol-related constituents not detected by the aqueous qualitative steroid test used here (Rosa et al., 2006). The present profile is therefore most appropriately viewed as the qualitative chemical fingerprint of the aqueous petal extract used in this study.

3.2 Total phenolic and flavonoid contents

Both TPC and TFC increased consistently with increasing petal quantity (**Figure 1**). TPC increased from 8.29 ± 0.91 mg GAE/g extract at 1 g to 13.49 ± 0.78 mg GAE/g extract at 2 g and 19.14 ± 1.10 mg GAE/g extract at 3 g. TFC increased from 6.31 ± 0.48 to 10.83 ± 0.89 and 14.71 ± 0.88 mg QE/g extract

across the same petal quantities. Thus, the 3 g preparation contained approximately 2.3-fold higher phenolic and flavonoid equivalents than the 1 g preparation under the extraction conditions used.

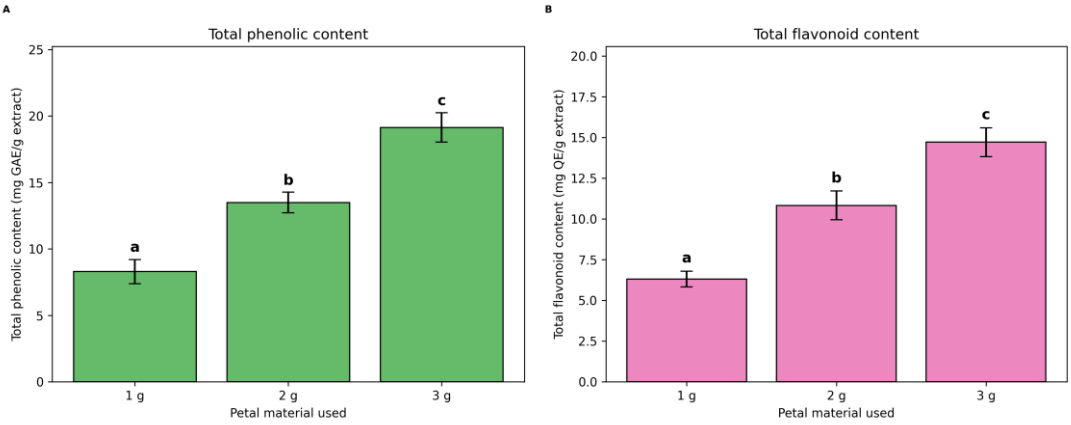

Figure 1. Total phenolic and flavonoid contents of H. tiliaceus flower-petal extract. (A) Total phenolic content (mg GAE/g extract) and (B) total flavonoid content (mg QE/g extract) at 1, 2 and 3 g petal quantities. Error bars represent standard deviation; different lowercase letters indicate Tukey groupings at $p < 0.05$.

The parallel TPC/TFC pattern supports the qualitative detection of phenolics and flavonoids and indicates progressive recovery of redox-active constituents with increasing petal loading. Earlier flower studies similarly linked phenolic and flavonoid abundance with antioxidant activity (Kumar et al., 2008; Wong et al., 2009). Recent phytochemical characterization of aqueous H. tiliaceus extracts identified rutin and kaempferol-3-O-rutinoside, confirming that this species can contain flavonoid glycosides with biologically relevant redox properties (Borive Amani et al., 2025). Vinh et al. (2021) also isolated antioxidant-active secondary metabolites from H. tiliaceus. These studies support the interpretation that the measured Folin- and aluminium-chloride-reactive fractions reflect a meaningful phytochemical reservoir rather than nonspecific colorimetric responses alone.

The quantitative phenolic/flavonoid content is also relevant to green nanotechnology. Phenolic hydroxyl groups can participate in electron transfer to metal ions, while oxygen-containing moieties can adsorb to growing particle surfaces. The ability of aqueous H. tiliaceus flower extract to generate AgNPs has been demonstrated directly by Alawfi et al. (2020), and a later H. tiliaceus leaf-based study similarly associated phytochemical functional groups with AgNP formation and antioxidant activity (Konduri et al., 2024). The present petal TPC/TFC values therefore provide a plausible chemical basis for subsequent use of this extract as a reducing and stabilizing matrix.

3.3 DPPH and ABTS antioxidant activity

The petal extract showed a clear concentration-dependent antioxidant response in both assays (**Figure 2**). DPPH scavenging increased from 3.29 ± 0.04% at 10 µg/mL to 8.14 ± 0.64, 14.19 ± 0.96, 37.41 ± 1.87, 56.37 ± 2.14 and 89.88 ± 3.04% at 25, 50, 100, 200 and 400 µg/mL, respectively. ABTS scavenging followed a closely comparable pattern, increasing from 2.77 ± 0.06% at 10 µg/mL to 7.81 ± 0.59, 16.14 ± 0.13, 35.69 ± 1.14, 54.19 ± 3.61 and 88.10 ± 2.66% at the corresponding concentrations. The highest extract concentration therefore produced approximately 90% radical scavenging in both systems.

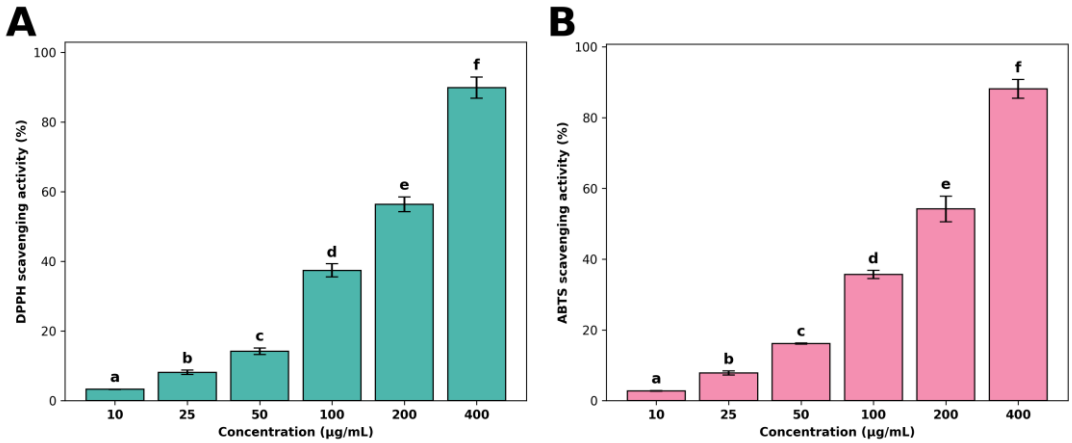

Figure 2. Concentration-dependent antioxidant activity of H. tiliaceus flower-petal extract in (A) DPPH and (B) ABTS assays. Bars represent mean values with standard-deviation error bars. Different lowercase letters indicate Tukey groupings at $p < 0.05$.

The close agreement between DPPH and ABTS responses is important because the two assays differ in radical chemistry and reaction environment. Concordant concentration-response behavior therefore indicates that the aqueous petals contain antioxidants capable of functioning across more than one assay system. The measured activity is consistent with the phenolic/flavonoid profile and with previous reports of concentration-dependent radical scavenging in H. tiliaceus extracts (Ramproshad et al., 2012; Surana et al., 2022). Borive Amani et al. (2025) further demonstrated DPPH and ABTS activity in aqueous H. tiliaceus extracts and identified rutin as a major antioxidant-active constituent, providing a mechanistic context for the strong response observed at higher petal-extract concentrations.

Ascorbic acid served as a benchmark and, as expected for a purified reducing molecule, produced high radical scavenging at lower concentrations. Its DPPH activity increased from 11.72 ± 0.94% at 5 µg/mL to 93.67 ± 4.14% at 50 µg/mL, whereas ABTS activity increased from 10.91 ± 0.53% to 86.14 ± 3.69% across the same range. At the directly comparable concentration of 50 µg/mL, the crude petal extract produced 14.19% DPPH and 16.14% ABTS scavenging compared with 93.67% and 86.14% for ascorbic acid. The contrast primarily reflects mass-based potency: ascorbic acid is a single active antioxidant, while the crude extract contains many compounds that contribute to total mass but not directly to radical scavenging.

Table 2. IC50 values of H. tiliaceus flower-petal extract and ascorbic acid

Sample	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)
H. tiliaceus flower-petal extract	166.40	177.35
Ascorbic acid	19.36	21.08

The IC50 values reinforce this interpretation. The petal extract required 166.40 µg/mL for 50% DPPH inhibition and 177.35 µg/mL for 50% ABTS inhibition, compared with 19.36 and 21.08 µg/mL for ascorbic acid (**Table 2**). The similar DPPH and ABTS IC50 values of the crude extract also mirror the close agreement of the full concentration-response curves. Importantly, the lower potency relative to ascorbic acid does not negate the functional significance of the botanical extract: at 200-400 µg/mL it

exhibited substantial to near-maximal scavenging, indicating a meaningful collective antioxidant effect from the aqueous phytochemical fraction.

Taken together, the qualitative screening, TPC/TFC data and two complementary radical assays form a coherent chemical-functional sequence. Phenolics and flavonoids were detected qualitatively, increased quantitatively with petal loading, and were accompanied by strong concentration-dependent radical scavenging. Because electron-donating phytochemicals can also reduce Ag+ and contribute to nanoparticle surface passivation, these findings provide a direct biochemical rationale for carrying the same aqueous petal extract forward into green AgNP synthesis. This rationale is supported by the independent demonstration of H. tiliaceus flower-mediated AgNP formation (Alawfi et al., 2020) and recent leaf-mediated nanomaterial work in the same species (Konduri et al., 2024).

4. Conclusion

The aqueous flower-petal extract of H. tiliaceus contained a broad range of primary and secondary metabolites, with phenolics and flavonoids representing particularly relevant redox-active classes. Both TPC and TFC increased with increasing petal quantity, reaching 19.14 ± 1.10 mg GAE/g extract and 14.71 ± 0.88 mg QE/g extract, respectively, in the 3 g preparation. The extract produced strong concentration-dependent DPPH and ABTS scavenging, approaching 90% inhibition at 400 µg/mL, with IC50 values of 166.40 and 177.35 µg/mL. Although ascorbic acid was more potent on a mass basis, the crude petal extract demonstrated substantial antioxidant capacity across two radical systems. These findings establish H. tiliaceus flower petals as a redox-active aqueous phytochemical source and provide a rational biochemical foundation for their subsequent use as a reducing and stabilizing matrix in green silver nanoparticle synthesis.

References

1. Abdul-Awal, S. M., Nazmir, S., Nasrin, S., Nurunnabi, T. R., & Uddin, S. J. (2016). Evaluation of pharmacological activity of Hibiscus tiliaceus. SpringerPlus, 5, 1209. https://doi.org/10.1186/s40064-016-2891-0
2. Alawfi, A. A., Henari, F. Z., Younis, A., & Manaa, H. (2020). Bio-inspired synthesis of silver nanoparticles using Hibiscus tiliaceus L. flower extracts for improved optical characteristics. Journal of Materials Science: Materials in Electronics, 31(23), 21073-21081. https://doi.org/10.1007/s10854-020-04619-6
3. Borive Amani, M., Frederich, M., Jansen, O., Bonnet, O., Ledoux, A., Memvanga, P. B., Batina Agasa, S., Mouithys-Mickalad, A., & Marini Djang'eing'a, R. (2025). Phytochemical characterization of Hibiscus tiliaceus L. leaves and evaluation of their antisickling, antioxidant, and anti-inflammatory activities. Molecules, 30(8), 1765. https://doi.org/10.3390/molecules30081765
4. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28(1), 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5
5. Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 10(3), 178-182.
6. Evans, W. C. (2009). Trease and Evans pharmacognosy (16th ed.). Saunders Elsevier.

7. Harborne, J. B. (1998). Phytochemical methods: A guide to modern techniques of plant analysis (3rd ed.). Chapman & Hall.
8. Konduri, V. V., Kalagatur, N. K., Gunti, L., Mangamuri, U. K., Kalagadda, V. R., Poda, S., & Krishna, S. B. N. (2024). Green synthesis of silver nanoparticles from Hibiscus tiliaceus L. leaves and their applications in dye degradation, antioxidant, antimicrobial, and anticancer activities. South African Journal of Botany, 168, 476-487. https://doi.org/10.1016/j.sajb.2024.03.035
9. Kumar, S., Kumar, D., & Prakash, O. (2008). Evaluation of antioxidant potential, phenolic and flavonoid contents of Hibiscus tiliaceus flowers. Electronic Journal of Environmental, Agricultural and Food Chemistry, 7(4), 2863-2871.
10. Ramproshad, S., Afroz, T., Mondal, B., Haque, A., Ara, S., Khan, R., & Ahmed, S. (2012). Antioxidant and antimicrobial activities of leaves of medicinal plant Hibiscus tiliaceus L. Pharmacologyonline, 3, 82-87.
11. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26(9-10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3
12. Rosa, R. M., Melecchi, M. I. S., da Costa Halmenschlager, R., Abad, F. C., Simoni, C. R., Caramao, E. B., Henriques, J. A. P., Saffi, J., & de Paula Ramos, A. L. L. (2006). Antioxidant and antimutagenic properties of Hibiscus tiliaceus L. methanolic extract. Journal of Agricultural and Food Chemistry, 54(19), 7324-7330. https://doi.org/10.1021/jf061407b
13. Rosa, R. M., Moura, D. J., Melecchi, M. I. S., dos Santos, R. S., Richter, M. F., Caramao, E. B., Henriques, J. A. P., de Paula Ramos, A. L. L., & Saffi, J. (2007). Protective effects of Hibiscus tiliaceus L. methanolic extract to V79 cells against cytotoxicity and genotoxicity induced by hydrogen peroxide and tert-butyl-hydroperoxide. Toxicology in Vitro, 21(8), 1442-1452. https://doi.org/10.1016/j.tiv.2007.06.004
14. Singleton, V. L., & Rossi, J. A., Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16(3), 144-158.
15. Surana, A. R., Kumbhare, M. R., Gunjal, A. R., Goswami, S. S., & Ghuge, D. M. (2022). Chemical characterization, thrombolytic and antioxidant activity of Hibiscus tiliaceus L. leaves. Natural Product Research, 36(23), 6106-6110. https://doi.org/10.1080/14786419.2022.2051705
16. Vinh, L. B., Nguyet, N. T. M., Thanh, C. D., Huong, T. T., Tram, L. H., Thong, N. V., Minh, N. H., Thao, N. P., Hwang, I., Yang, S. Y., & Kim, Y. H. (2021). Chemical constituents of Vietnamese mangrove Hibiscus tiliaceus with antioxidant and alpha-glucosidase inhibitory activity. Natural Product Research, 35(17), 2899-2904. https://doi.org/10.1080/14786419.2019.1672065
17. Wong, S. K., Lim, Y. Y., & Chan, E. W. C. (2009). Antioxidant properties of Hibiscus: Species variation, altitudinal change, coastal influence and floral colour change. Journal of Tropical Forest Science, 21(4), 307-315.