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Original Research Article

Phytochemical Profile, Antioxidant Potential, and Hemolytic Safety of *Tecoma stans* (L.) Kunth Leaf and Flower Extracts Using Different Solvents

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ABSTRACT

Tecoma stans, a tropical shrub, is traditionally used to treat diabetes, inflammation, infections, and digestive issues. While its antioxidant and antimicrobial effects are recognized, limited data exist on the hemolytic safety of its extracts. This study examined the phytochemical composition, antioxidant potential, and hemolytic safety of *T. stans* leaf and flower extracts prepared with three different solvents: dichloromethane, ethyl acetate, and 70% ethanol. Extraction yields were highest with 70% ethanol, reaching 18.64% for flowers and 6.6% for leaves extracts. Qualitative screening identified nine of ten targeted classes, including alkaloids, phenolics, flavonoids, terpenoids, and glycosides, while anthraquinones were absent in all extracts. Ethanolic extracts demonstrated the most diverse phytochemical profiles. Quantitative analysis confirmed that the ethanol leaf extract contained the highest total phenolic content (261.788 ± 6.229 mg GAE/g) and total flavonoid content (794.641 ± 259.249 mg QE/100 g). Antioxidant activity, assessed via ABTS and DPPH radical scavenging assays, showed the strongest activity in ethanol extracts. Specifically, in the ABTS assay, the ethanol leaf extract exhibited an IC_{50} of 4.524 ± 0.122 µg/mL, surpassing the ascorbic acid standard. Similarly, the ethanol leaf extract exhibited the highest DPPH radical scavenging activity (IC_{50} = 12.693 ± 0.207 µg/mL). Hemolysis and anti-hemolysis tests indicated that all extracts, across concentrations from 0 to 512 µg/mL, were non-hemolytic and conferred protection against red blood cell lysis. These findings suggest that *T. stans*, particularly its ethanol extracts, are abundant in bioactive compounds, exhibit significant antioxidant effects, and excellent hemocompatibility, supporting their potential for therapeutic or nutraceutical applications.

Keywords: Antioxidant activity, Hemolysis assay, Phenolic and flavonoid content, Phytochemical screening, *Tecoma stans*

Introduction

Medicinal plants have long served as a vital source of therapeutic agents in both traditional and modern medicine. Over 50% of contemporary clinical drugs are derived from natural products or their derivatives, suggesting their significance in pharmaceutical development.[1] *Tecoma stans* (L.) Kunth, commonly known as yellow bell or yellow elder, is a perennial shrub extensively distributed across tropical and subtropical regions.[2] Traditionally, it has been employed to treat conditions such as diabetes, inflammation, microbial infections, gastrointestinal disorders, and as a general tonic.[3-5] In many regions worldwide, particularly South Asia and Latin America, *T. stans* holds a prominent place in folk medicine. It contains diverse phytochemicals, including alkaloids (e.g., tecomanine, tecostanine), flavonoids (quercetin, apigenin, kaempferol), phenolic acids (gallic acid, caffeic acid), and terpenoids.[6-8] These constituents are linked to potent antioxidant, antimicrobial, and anti-inflammatory activities, positioning *T. stans* as a promising candidate for natural product-based drug development.[9]

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Recent studies have highlighted the solvent-dependent variability in phytochemical yield and biological activity of plant extracts. Ethanol and methanol extractions are generally more effective in isolating phenolic and flavonoid compounds compared to non-polar solvents like dichloromethane.[3,10] Furthermore, advanced analytical techniques such as Ultraviolet-visible spectrophotometer (UV-Vis spectrophotometer), Fourier Transform Infrared Spectroscopy (FTIR), High-Performance Liquid Chromatography (HPLC), and Gas Chromatography-Mass Spectrometry (GC-MS) have facilitated detailed characterization of bioactive compounds in *T. stans*, reinforcing its pharmacological potential.[11]

Although the antioxidant and antimicrobial effects of *T. stans* are well documented, its safety, particularly regarding hemolytic activity, remains insufficiently explored. Evaluating hemolysis and anti-hemolytic effects is essential to determine the biocompatibility of plant extracts intended for therapeutic or nutraceutical applications.

Therefore, this study aims to investigate the phytochemical composition, antioxidant capacity, and hemolytic safety of *T. stans* leaf and flower extracts obtained using three solvents: dichloromethane, ethyl acetate, and 70% ethanol. The research employs solvent extraction and standard antioxidant assays to elucidate solvent-specific differences in bioactive compound yield and activity. Hemolytic and anti-hemolytic assays assess the extracts' safety concerning red blood cell integrity. This investigation provides novel insights into the solvent-dependent bioactivity and safety profile of T. stans, supporting its potential for use in natural health formulations.

Materials and Methods

Plant Material Collection and Preparation

Tecoma stans specimens were collected in May 2021 from a shrubland

area within the grounds of Mahasarakham University, Mahasarakham Province, Thailand (geographic coordinates: 16°14′55.2″N, 103°15′05.7″E). Botanical identification was confirmed using the Flora of Thailand guidebook along with other standard taxonomic references.[12] A voucher specimen was archived at the Natural Medicinal Mushroom Museum, Mahasarakham University, under accession number MSUT-8760.

Fresh leaves and flowers *T. stans* specimens were harvested (Figure 1A and 1B). Each plant part was thoroughly rinsed with water to remove debris, then cut into small segments to facilitate drying. Samples were air-dried in a hot air oven at 40 °C for three consecutive days until a constant weight was reached, indicating complete dehydration. The dried materials were stored in airtight polyethylene bags with silica gel as a desiccant at room temperature (25 °C) to prevent moisture reabsorption before extraction.[13]

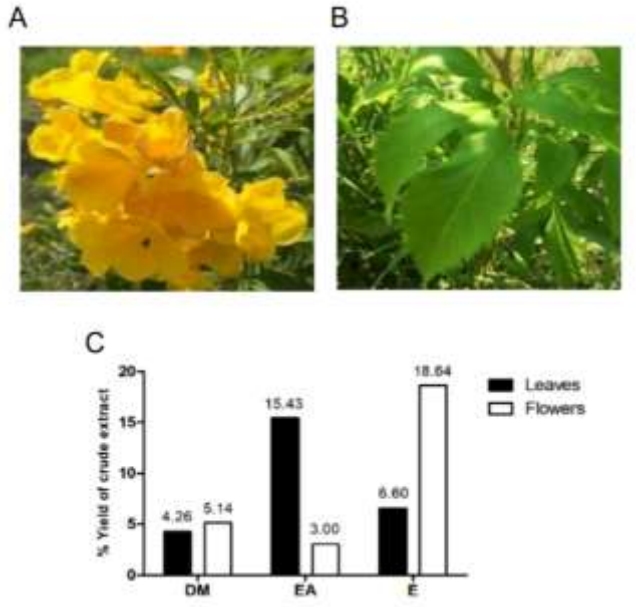

Figure 1: Morphological components and extraction yield of *Tecoma stans*. (A) Flower structure, (B) Leaf morphology, and (C) Percentage yield of crude extracts obtained using three different solvents: dichloromethane (DM), ethyl acetate (EA), and 70% ethanol (E).

Extraction Procedure

Dried leaves and flower samples were ground into a fine powder using a mechanical grinder. The powdered material was macerated in three different solvents, dichloromethane (Sigma-Aldrich, MO, USA), ethyl acetate (Sigma-Aldrich, MO, USA), and 70% ethanol (Sigma-Aldrich, MO, USA), at a plant-to-solvent ratio of 1:10 (w/v). Maceration was performed at ambient temperature for 72 hours. The mixtures were then filtered through Whatman No. 1 filter paper (Whatman, Maidstone, UK) to remove solids. Filtrates were evaporated to dryness on flat plates at 50 °C over three days.[14] Extraction yields were calculated using Equation 1. Dried extracts were stored at −20 °C until further use.

$$\% \text{ Yield} = \frac{\text{weight of crude extract (g)}}{\text{weight of dried sample (g)}} \times 100$$

Preparation of Extracts for Biological Assays

Each crude extract was reconstituted in 100% dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, USA) to a stock concentration of 100 mg/mL. The solutions were sequential filtered through a 100 µm nylon mesh, followed by 0.45 µm and 0.22 µm syringe filters (Whatman, Maidstone, UK) to ensure sterility and remove residual particulates. Sterile extracts were aliquoted into sterile microcentrifuge tubes and stored at −20 °C until phytochemical and biological evaluations.[14-15]

Phytochemical Screening

Qualitative screening of *T. stans* crude extracts was conducted to detect ten classes of secondary metabolites: alkaloids, phenolics, flavonoids, anthraquinones, coumarins, saponins, tannins, terpenoids, steroids, and glycosides. Each class was identified based on characteristic color changes or precipitate formation in specific chemical reactions.

Alkaloids were detected by the addition of Wagner's reagent, producing a yellow precipitate in positive samples. Phenolics were identified by reaction with ferric chloride ($FeCl_3$) (KemAus, Australia), yielding a dark green or bluish-black color. Flavonoids were confirmed by reaction with magnesium ribbon and hydrochloric acid, producing a strong yellow. Anthraquinones were revealed through treatment with sulfuric acid (KemAus, Australia) and ammonium hydroxide (KemAus, Australia), resulting in a reddish-pink coloration if present. Coumarins were detected by mixing extract with sodium hydroxide (NaOH) ((KemAus, Australia), which turned deep yellow for positive. The froth test confirmed saponins by persistent foam formation upon shaking. Like phenolics, tannins reacted with $FeCl_3$ to form a bluish or greenish-black solution. Terpenoids produced a brown ring at the interface between the dichloromethane layer and sulfuric acid. Steroids were identified by blue or green coloration after treatment with dichloromethane, glacial acetic acid, and concentrated sulfuric acid. Glycosides yielded a brown interface following addition of $FeCl_3$, glacial acetic acid, and H_2SO_4.[16-17]

Determination of Total Phenolic and Flavonoid Content

Total phenolic content (TPC) was measured using the Folin–Ciocalteu colorimetric assay. Extracts were prepared at concentrations of 10 and 20 µg/mL in deionized water. Gallic acid (Sigma-Aldrich, MO, USA) served as a calibration standard over 0–10 µg/mL. For each assay, 100 µL of sample or standard was mixed with 500 µL of 10% Folin–Ciocalteu reagent (Loba chemie, India). After 3 minutes, 400 µL of 7.5% sodium carbonate (Na_2CO_3) (KemAus, Australia) was added. The mixture was vortexed and incubated in the dark at room temperature for 30 minutes. Absorbance (Abs) was recorded at 731 nm using a UV–Vis spectrophotometer (GENESYS 180, Thermo Scientific, MA, USA). Results were expressed as milligrams of gallic acid equivalents per gram of crude extract (mg GAE/g).[18-20]

Total flavonoid content (TFC) was quantified using the aluminum chloride colorimetric method. Extracts were diluted to 10 and 20 µg/mL, while quercetin (Sigma-Aldrich, MO, USA) was the reference standard (0–10 µg/mL). Sample or standard aliquots (100 µL) were combined with 500 µL deionized water, followed by 30 µL of 5% sodium nitrite ($NaNO_2$) (Sigma-Aldrich, MO, USA) and incubated for 5 minutes. Subsequently, 60 µL of 10% aluminum chloride ($AlCl_3$) (KemAus, Australia) was added and incubated for 5 minutes. Then, 200 µL of 1 M sodium hydroxide (NaOH) (RCI Labscan, Bangkok) and 110 µL of water were added. After thorough mixing and a 5-minute incubation, absorbance was measured at 510 nm. TFC was calculated from the quercetin standard curve and reported as milligrams of quercetin equivalents per gram of crude extract (mg QE/g).[20, 21]

Antioxidant Activity Assay

Antioxidant potential was evaluated using two in vitro radical scavenging assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)). Ascorbic acid (RCI Labscan, Bangkok) served as the reference standard in both assays.

For the DPPH assay, extracts were serially diluted in deionized water to concentrations of 0, 25, 50, 100, 200, and 400 µg/mL. A volume of 100 µL of each dilution was mixed with 900 µL of 80 µM DPPH solution in methanol (Sigma-Aldrich, Germany). Mixtures were vortexed and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 515 nm using a UV–visible spectrophotometer.[22-23]

In the ABTS assay, $ABTS^{\bullet+}$ radicals were generated by reacting 7 mM ABTS solution (Sigma-Aldrich, MO, USA) with 2.45 mM potassium persulfate (PanReac AppliChem, Spain) and allowing the mixture to stand in the dark for 12–16 hours. The resulting radical solution was diluted with methanol (Sigma-Aldrich, MO, USA) until it reached an absorbance of 0.70 ± 0.01 at 734 nm was achieved. Extracts were

diluted to working concentrations ranging from 0 to 40 µg/mL, depending on the plant part. Equal volumes (1 mL) of extract and ABTS solution were mixed, vortexed, and incubated in the dark for 3–5 minutes. Absorbance was measured at 734 nm.[22]
Radical scavenging activity was calculated using equation 2:

$$\% \text{ Free radical scavenging activity} = \frac{Abs_{negative\ control} - Abs_{sample}}{Abs_{control}} \times 100$$

Hemolysis Assay Using Human Red Blood Cells
Hemolytic activity was assessed using human red blood cells (RBCs) from five healthy adult volunteers who provided written informed consent. The study protocol was approved by the Human Ethics Research Committee of Mahasarakham University (Approval No. 338-273/2564). Blood was collected from volunteers with normal health parameters.[12] Whole blood was centrifuged (Thermo Scientific, MA, USA) at 2,000 rpm for 10 minutes to isolate RBCs, which were washed and resuspended in phosphate-buffered saline (PBS) to prepare a 2% RBC suspension.[24]
Extracts were serially diluted two-fold to concentrations ranging from 0 to 400 µg/mL. For each test, 500 µL of extract was mixed with 500 µL of the RBC suspension and incubated at 37 °C for 30 minutes. After incubation, samples were centrifuged at 2,500 × g for 6 minutes. The supernatant (700 µL) absorbance was measured at 541 nm to quantify hemoglobin release as an indicator of cell lysis. Experiments were performed in triplicate. Controls included a negative control (PBS and 2% RBCs), a vehicle control (0.1% DMSO in PBS and RBCs), and a positive control (0.1% Triton X-100 inducing complete hemolysis). Hemolysis percentage was calculated using Equation 3, with the positive control defined as 100% lysis.[22]

$$\% \text{ Hemolysis} = \frac{Abs_{sample} - Abs_{negative\ control}}{Abs_{positive\ control} - Abs_{negative\ control}} \times 100$$

Anti-Hemolysis Assay Using Human Red Blood Cells
To assess inhibitory effects on RBC hemolysis, extracts were two-fold serially diluted to 0–400 µg/mL. For each sample, 400 µL extract was mixed with 400 µL of 2% RBC suspension and incubated at 37 °C for 5 minutes. Then, 200 µL of 3% hydrogen peroxide (H_2O_2) (Sigma-Aldrich, MO, USA) was added to induce oxidative hemolysis, and samples were incubated at 37 °C for 3 hours. After incubation, samples were centrifuged at 3,000 × g for 10 minutes. The supernatant (700 µL) absorbance was measured at 540 nm. Assays were performed in triplicate. Controls included a negative control (PBS and RBCs), a positive control treated with 0.1% Triton X-100 for full hemolysis, and a reference standard (ascorbic acid) for antioxidant protection. Anti-hemolysis percentage was calculated using Equation 4 relative to the positive control set at 100% hemolysis.[25-26]

$$\% \text{ Anti-hemolysis} = \frac{Abs_{negative\ control} - Abs_{sample}}{Abs_{positive\ control}} \times 100$$

Statistical analysis
All experiments were conducted in triplicate to ensure reproducibility. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons using GraphPad Prism version 10 (GraphPad Software Inc., CA, USA). Results are presented as mean ± standard deviation (SD). Differences with $p \leq 0.05$ were considered statistically significant.[22]

Results and Discussion

Extraction of Tecoma stans Leaves and Flowers
Leaves and flowers of *T. stans* were extracted using three solvents: dichloromethane, ethyl acetate, and 70% ethanol. The highest extraction yields were obtained with 70% ethanol, yielding 18.64% from flower and 6.6% from leaves. Ethyl acetate yield of 15.43% from leaves and 3.00% from flowers. Dichloromethane produced the lowest yields: 5.14% from flowers and 4.26% from leaves (Figure 1C). Extraction efficiency varied significantly among solvents, with 70% ethanol outperforming others. This align with prior studies showing showing ethanol's effectiveness as a polar protic solvent in solubilizing a broad range of phytoconstituents, especially phenolics and flavonoids.[6-7] Ethyl acetate afforded moderate yields, particularly from leaves, likely due to its intermediate polarity facilitating partial solubility of semi-polar compounds such as flavonoids and glycosides. Dichloromethane, a non-polar solvent, consistently showed lower yield, corroborating previous findings on its limited efficacy in extracting hydrophilic bioactives from *T. stans*.[8]

Preliminary phytochemical screening of Tecoma stans leaves and flowers extracts
Qualitative phytochemical screening using dichloromethane, ethyl acetate, and ethanol extracts revealed the presence of nine out of ten tested phytochemical groups. Alkaloids, phenolics, flavonoids, coumarins, saponins, tannins, terpenoids, steroids, and glycosides were detected. Anthraquinones were absent in all samples. Ethanolic extracts exhibited the most diverse phytochemical profile, notably strong for phenolics, tannins, terpenoids, and glycosides in both leaves and flowers. Ethyl acetate extracts, especially from the leaves, showed flavonoids and moderate levels of other compounds. Dichloromethane extracts contained fewer phytochemicals and notably lacked phenolics and tannins in the flower extracts. These results confirm ethanol as the most effective solvent for broad phytochemical extraction from *T. stans* (Table 1). These findings are consistent with previous reports regarding solvent-dependent phytochemical profiles and underscore ethanol's superior ability to extract compounds relevant to antioxidant and antimicrobial activities.[6,9-10,27-28]

Quantification of Total Phenolic and Flavonoid Contents in Tecoma stans Extracts
Total phenolic content was quantified using a gallic acid standard curve ($y = 0.0522x + 0.0014$, $R^2 = 0.9989$) (Figure 2A) and expressed as milligrams of gallic acid equivalent per gram of crude extract (mg GAE/g). Phenolic contents for flowers and leaves extracts using dichloromethane, ethyl acetate, and ethanol were 50.370 ± 1.595, 65.824 ± 1.991, 76.296 ± 1.738, 45.390 ± 1.171, 142.324 ± 12.675, and 261.788 ± 6.229 mg GAE/g, respectively. The 70% ethanol leaves extract had the highest phenolic content, significantly exceeding other extracts (Figure 2B).

Table 1: Phytochemical screening profile of *Tecoma stans* extracts

Compound groups	Solvents					
	Dichloromethane		Ethyl acetate		70% Ethanol	
	F	L	F	L	F	L
Alkaloids	+	+	+	+	+	+
Phenolic	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-
Coumarins	+	+	+	+	+	+
Saponins	-	-	-	-	-	-
Tannin	-	+	-	+	+	+
Terpenoids	+	+	+	+	+	+
Steroids	-	+	-	+	-	+
Glycosides	+	-	+	-	+	+

"+" indicates the presence of the respective phytochemical compound; "–" indicates its absence; "F" indicates flowers; "L" indicates leaves.

Total flavonoid content was determined using a quercetin standard curve ($y = 0.0051x + 0.0003$, $R^2 = 0.9869$) (Figure 2C) and reported as milligrams of quercetin equivalent per 100 grams of crude extract (mg QE/100 g). Flavonoid contents of flowers and leaves extracts in dichloromethane, ethyl acetate, and ethanol were 133.268 ± 12.606, 227.059 ± 3.922, 120.196 ± 1.961, 299.935 ± 34.262, 514.902 ± 106.172, and 794.641 ± 259.249 mg QE/100 g, respectively. Among all

samples, 70% ethanol leaves extract showed the highest flavonoid content (Figure 2D).

Quantitative analyses confirmed that 70% ethanolic leaf extracts contained the highest phenolic and flavonoid concentrations, surpassing previous reports for *T. stans* leaves.[7,11] These elevated values likely reflect effective extraction of antioxidant phytochemicals such as gallic acid, caffeic acid, quercetin, apigenin, and kaempferol, previously identified in related studies.[6,8] These compounds are well-known for their radical scavenging and metal-chelating properties, contributing to their potential in nutraceutical development.

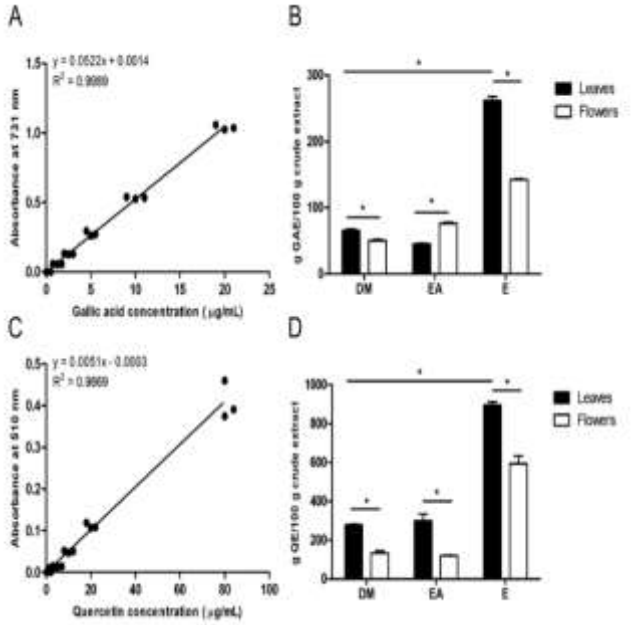

Figure 2: Total phenolic and flavonoid contents of *Tecoma stans* extracts. (A) Calibration curve of gallic acid used for phenolic quantification; (B) Total phenolic content expressed as milligrams of gallic acid equivalent (GAE) per gram of crude extract; (C) Calibration curve of quercetin used for flavonoid analysis; and (D) Total flavonoid content expressed as milligrams of quercetin equivalent (QE) per 100 grams of crude extract. Extracts were obtained using dichloromethane (DM), ethyl acetate (EA), and 70% ethanol (E). An asterisk (*) indicates statistically significant differences at $p \leq 0.05$.

Antioxidant Activity of Tecoma stans Extracts (ABTS and DPPH Assays)

The antioxidant capacity of *Tecoma stans* leaves and flowers extracts was evaluated using ABTS and DPPH radical scavenging assays, with ascorbic acid as a reference standard (Figure 3A and 3C). In the ABTS assay, the 70% ethanol leaves extract exhibited the strongest antioxidant activity (IC_{50} = 4.524 ± 0.122 µg/mL), followed by the ethanol flower extract (5.871 ± 0.144 µg/mL). Ethyl acetate and dichloromethane extracts showed considerably weaker activity, with IC_{50} values ranging from 34.549 to 65.078 µg/mL (Figure 3B).

Consistent trends were observed in the DPPH assay, with the ethanol leaf extract demonstrating the highest activity (IC_{50} = 12.693 ± 0.207 µg/mL), followed by flower ethanol extract (16.933 ± 5.376 µg/mL). Ethyl acetate extracts exhibited moderate activity, while dichloromethane extracts were the least active (flower: 112.004 ± 2.258 µg/mL; leaf: 99.679 ± 5.329 µg/mL). Ascorbic acid's IC_{50} was 56.045 ± 1.857 µg/mL (Figure 3D).

The strong antioxidant activities observed in ethanol extracts, particularly from leaves, is correlated with their high phenolic and flavonoid contents. Phenolic compounds such as gallic acid and caffeic acid, along with flavonoids like quercetin, kaempferol, and apigenin—previously identified in *T. stans* through HPLC and GC-MS analyses[6,8], are well known for their ability to scavenge free radicals through hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms.[30] These results concur with previous findings indicating superior antioxidant capacity in alcoholic solvent extracts versus non-polar solvents.[7,11,28] The stronger antioxidant activity in leaves likely reflects their higher polyphenolic content, while the lower polarity of dichloromethane limits solubility of hydrophilic antioxidants.

The potent free radical scavenging activity of ethanolic *T. stans* extracts suggests promising applications in pharmaceutical, cosmeceutical, and nutraceutical industries. These extracts could serve as natural antioxidants protecting skin from UV damage, premature aging, and inflammation.[30] Additionally, the abundance of polyphenols and flavonoids supports their potential in managing oxidative stress-related diseases such as diabetes, cardiovascular disorders, and neurodegenerative conditions.[6] Phytochemicals like caffeic acid and apigenin have been linked to hepatoprotective, neuroprotective, and wound healing effects through modulation of oxidative and inflammatory pathways.[10,31] Collectively, these bioactivities highlight *T. stans* extracts as valuable ingredients for diverse health-promoting products.

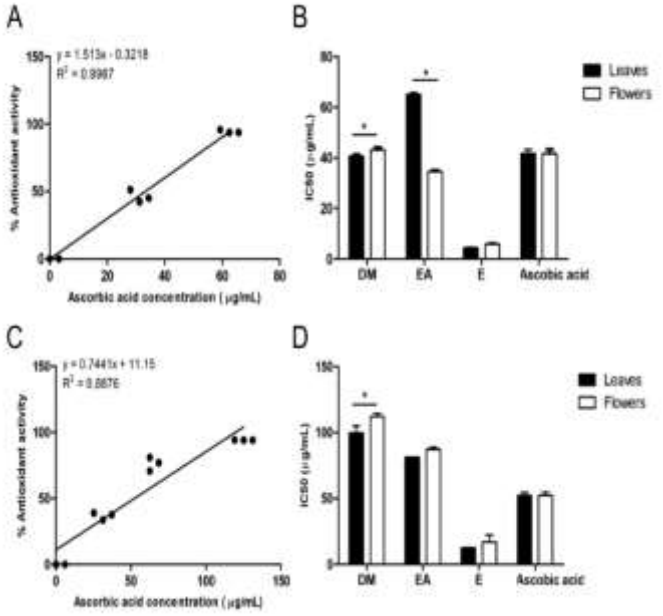

Figure 3: Antioxidant activity of *Tecoma stans* extracts evaluated by ABTS and DPPH radical scavenging assays. (A) Calibration curve of ascorbic acid for the ABTS assay; (B) ABTS radical scavenging activity of *T. stans* extracts from different plant parts; (C) Calibration curve of ascorbic acid for the DPPH assay; (D) DPPH radical scavenging activity of *T. stans* extracts. Extraction solvents include dichloromethane (DM), ethyl acetate (EA), and 70% ethanol (E). The asterisk (*) denotes statistically significant differences at $p \leq 0.05$.

Hemolysis and Anti-Hemolysis Activity of Tecoma stans Extracts

The effects of *T. stans* extracts on red blood cell (RBC) membrane stability were assessed via hemolysis and anti-hemolysis assays over a concentration range of 0 to 512 µg/mL, using extracts obtained via dichloromethane, ethyl acetate, and ethanol from leaves and flowers. No hemolytic activity was observed at any concentration tested (Figure 4A-F). Moreover, all extracts exhibited anti-hemolytic activity, effectively protecting RBCs from lysis. The absence of hemolytic activity effects confirms the membrane compatibility and safety profile of *T. stans*, supporting its use in pharmaceutical and cosmeceutical applications where cytocompatibility is critical. RBC membrane stability is a common indicator of cytotoxicity, and membrane-protecting agents are favored for systemic or topical formulations.[26]

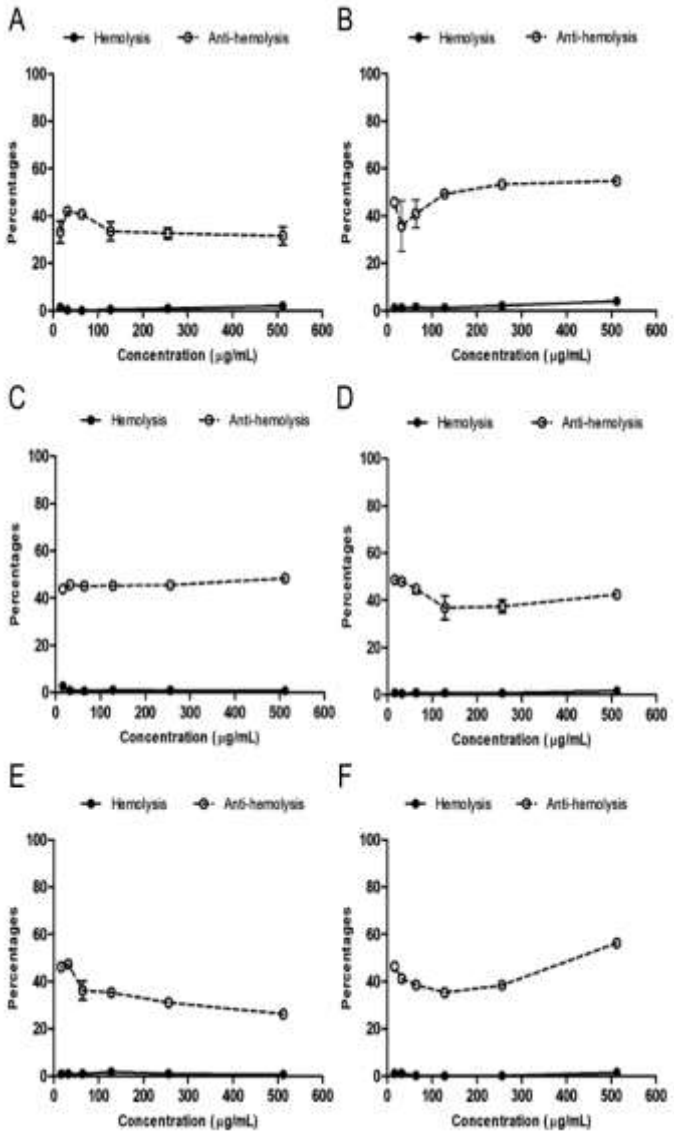

Figure 4: Hemolytic and anti-hemolytic effects of *Tecoma stans* extracts on human red blood cells. (A) Hemolysis and protection against hemolysis by flower extract obtained using dichloromethane; (B) Hemolysis and protection by leaf extract with dichloromethane; (C) Hemolytic and anti-hemolytic activity of flower extract using ethyl acetate; (D) Corresponding activity of leaf extract with ethyl acetate; (E) Hemolysis and inhibition by flower extract with 70% ethanol; (F) Hemolysis and inhibition by leaf extract with 70% ethanol.

The anti-hemolytic effect observed in this study can be attributed to the presence of membrane-stabilizing phytochemicals such as flavonoids, phenolics, and saponins. Compounds including quercetin, apigenin, and gallic acid, previously identified in *T. stans* extracts via HPLC and GC-MS[6,8], are known to protect erythrocyte membranes by preventing oxidative damage and lipid peroxidation. These bioactives interact with phospholipid bilayers, enhancing membrane stability under oxidative stress conditions.[7] Similar membrane-protective effects of *T. stans* have been reported, demonstrated enhanced wound healing activity attributed to antioxidant-mediated stabilization of cellular membranes.[10] Additionally, neuroprotective effects of *T. stans* extract in oxidative injury models have been described, supporting its general cytoprotective role.[6]

The results of this study expand upon earlier findings by confirming that *T. stans* extracts are not only rich in antioxidant compounds but also provide physical protection to cell membranes. This property is particularly relevant for the development of therapeutic agents aimed at preventing or alleviating hemolytic disorders, oxidative stress-related anemia, and inflammation-induced vascular injury. Moreover, the absence of hemolysis supports the use of these extracts in dermatological formulations and natural drug delivery systems where biocompatibility is essential.

In summary, *T. stans* extracts are non-hemolytic and exhibit significant anti-hemolytic activity, especially in ethanol-derived fractions. These findings validate the safety and therapeutic potential of *T. stans*, particularly for antioxidant-rich formulations intended for internal or external applications.

Conclusion

This study demonstrated that *T. stans* leaves and flowers extracts contain a diverse array of bioactive phytochemicals and possess significant antioxidant and membrane-stabilizing properties, with solvent selection critically influencing extraction efficiency. Among the solvents tested, 70% ethanol was most effective in extracting high yields of phenolics and flavonoids, particularly from leaves. These ethanolic extracts exhibited the strongest radical scavenging activity in both ABTS and DPPH assays, surpassing standard ascorbic acid and correlating with their high total phenolic and flavonoid contents. Furthermore, all extracts were non-hemolytic across tested concentrations and displayed dose-dependent anti-hemolytic effects, indicating safety and promise for therapeutic applications. The presence of antioxidant and membrane-protective compounds such as quercetin, gallic acid, apigenin, and saponins supports the traditional uses of *T. stans* and emphasizes its potential in developing natural health products. Particularly, ethanol-extracted *T. stans* leaves offer strong prospects for application in antioxidant-based nutraceuticals, cosmeceuticals, and safe herbal therapeutics. Future research should prioritize the isolation and characterization of specific active constituents, validation of their mechanisms of action through in vivo models, and the exploration of innovative formulation strategies to optimize clinical efficacy and delivery. Additionally, investigation into pharmacokinetics, toxicity profiling, and synergistic effects with conventional therapies will be critical to advancing *T. stans* toward pharmaceutical and nutraceutical applications.

Conflict of Interest

The author`s declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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