International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Comparative Phytochemical Screening and Antioxidant Potential of Nerium Cordifolia, Strobilanthes Veronicifolia, and Nyctanthes Arbor-Tristis

Mr. Arvind Kumar[1], Dr. Garima Gupta[2]

[1]Research Scholar, Department of Pharmacy, Monad University, Pilkhuwa, Hapur, Uttar Pradesh, India
[2]Department of Pharmacy, Monad University, Pilkhuwa, Hapur, Uttar Pradesh, India

Abstract:
Medicinal plants contain structurally diverse secondary metabolites that may protect biological systems against oxidative injury. The present study was designed to comparatively investigate the phytochemical composition and in-vitro antioxidant potential of the leaves of Nerium cordifolia and Strobilanthes veronicifolia and the stem bark of Nyctanthes arbor-tristis. Shade-dried plant materials were powdered and extracted using a hydroalcoholic solvent. The resulting extracts were subjected to qualitative screening for phenolics, flavonoids, alkaloids, tannins, terpenoids, saponins, glycosides, carbohydrates and proteins. Total phenolic content was determined using the Folin–Ciocalteu method and expressed as milligrams of gallic acid equivalents per gram of dried extract. Total flavonoid content was estimated by aluminium chloride colorimetry and expressed as milligrams of quercetin equivalents per gram of dried extract. Antioxidant potential was assessed through DPPH radical-scavenging, ABTS radical-cation decolourisation and ferric reducing-power assays. Ascorbic acid or Trolox was employed as a reference antioxidant. All experiments were designed to be conducted in triplicate, with the results expressed as mean ± standard deviation. One-way analysis of variance followed by an appropriate post-hoc test was proposed for comparative analysis. The integrated use of three antioxidant assays is expected to provide a more reliable evaluation than dependence on a single chemical reaction. The study may help identify the most promising extract for subsequent chromatographic characterisation, isolation of active constituents and pharmacological evaluation. However, any therapeutic application will require toxicological assessment, standardisation and confirmation in suitable biological models.

Keywords: Antioxidant activity; ABTS; DPPH; flavonoids; medicinal plants; phenolic compounds; phytochemical screening; reducing power.

1. Introduction

Oxidative stress develops when the production of reactive oxygen and nitrogen species exceeds the protective capacity of endogenous antioxidant systems. Reactive species may participate in normal cellular signalling; however, their excessive formation can damage lipids, proteins, carbohydrates and nucleic acids. Persistent oxidative injury is associated with inflammation, cellular dysfunction and the progression of several metabolic and degenerative disorders.

Plants synthesise numerous secondary metabolites as part of their defence against ultraviolet radiation, herbivores, pathogens and environmental stress. Phenolic acids, flavonoids, tannins, alkaloids and terpenoids are particularly important because many of these compounds can donate electrons or hydrogen atoms, chelate transition metals or interrupt radical-chain reactions. Their presence does not automatically establish clinical effectiveness, but it offers a rational basis for controlled phytochemical and pharmacological investigation.

No single in-vitro assay fully represents antioxidant action. DPPH measures the ability of a substance to neutralise a stable nitrogen-centred radical, whereas ABTS evaluates the decolourisation of a preformed radical cation. The reducing-power assay measures electron-donating capacity through the reduction of ferric ions. Results may vary with the solvent, reaction time, extract colour, pH and chemical composition. Using several complementary assays therefore produces a more balanced antioxidant profile.

The present investigation focuses on the leaves of Nerium cordifolia and Strobilanthes veronicifolia and the stem bark of Nyctanthes arbor-tristis. The latter is an accepted botanical species of the family Oleaceae and has a native distribution extending from the Himalayan region to parts of Southeast Asia according to Plants of the World Online. The selected plant parts may contain chemically different groups of metabolites because their biosynthetic and protective functions are not identical.

The comparative design of the study is important because variation in antioxidant activity may arise from differences in plant species, plant parts, geographical source, maturity, extraction solvent and storage conditions. Processing and evaluating all samples under the same experimental conditions can reduce procedural variation and permit a more meaningful comparison.

2. Taxonomic Verification:

Before the study is submitted or published, the identities of the plants must be authenticated by a qualified taxonomist. Voucher specimens should be deposited in a recognised herbarium, and the accession numbers should be reported.

The name **Nerium cordifolia** requires particular verification. Current authoritative botanical sources generally recognise **Nerium oleander L.** as the accepted species in the genus Nerium. Moreover, oleander is poisonous and may contain potent cardiac glycosides. Consequently, the name printed in the synopsis should not be changed informally, but it must be reconciled with the plant-authentication certificate before publication. Similar verification is advisable for **Strobilanthes veronicifolia**.

For the present draft, the names recorded in the approved Monad University letter have been retained.

3. Review of Related Literature:

Blois (1958) introduced the use of the stable DPPH radical for antioxidant determination. The method became widely used because it is relatively simple, rapid and suitable for screening plant extracts. A reduction in the violet colour of DPPH indicates radical neutralisation by an antioxidant substance.

Oyaizu (1986) described a reducing-power procedure based on the capacity of antioxidant compounds to reduce ferric ions. Greater absorbance of the reaction mixture generally indicates stronger electron-donating activity.

Re et al. (1999) developed an improved ABTS radical-cation decolourisation assay applicable to both water-soluble and lipid-soluble antioxidants. The method is valuable for comparing chemically diverse samples, although reaction conditions must remain standardised.

Singleton et al. (1999) explained the Folin–Ciocalteu method for measuring total phenolic constituents. The test is widely employed in plant research but is not completely specific to phenols because other reducing substances may also react with the reagent.

Chang et al. (2002) used an aluminium chloride colorimetric method for estimating total flavonoids. Complex formation between aluminium ions and flavonoid functional groups produces a measurable colour response.

Prior comparative studies have shown that DPPH, ABTS and ferric-reducing assays can produce different rankings because they involve different reaction mechanisms. Accordingly, several assays should be interpreted collectively rather than treating a single value as definitive evidence of antioxidant superiority.

4. Research Gap:

Although the selected plants have potential ethnomedicinal and phytochemical significance, a directly standardised comparison of their specified plant parts under identical extraction and analytical conditions is insufficiently documented. Differences between published studies may reflect dissimilar solvents, concentrations, geographical sources and assay procedures. The present work addresses this limitation by applying the same extraction procedure, concentration range, reference standards and statistical framework to all three samples.

5. Aim

To comparatively evaluate the phytochemical constituents and in-vitro antioxidant potential of the leaves of Nerium cordifolia and Strobilanthes veronicifolia and the stem bark of Nyctanthes arbor-tristis.

6. Objectives

1. To collect, authenticate and standardise the selected plant materials.
2. To prepare hydroalcoholic extracts under uniform experimental conditions.
3. To qualitatively screen the extracts for major groups of secondary metabolites.
4. To quantify the total phenolic and total flavonoid contents.
5. To evaluate antioxidant activity using DPPH, ABTS and reducing-power assays.
6. To compare the phytochemical contents and antioxidant activities statistically.
7. To investigate the relationship between phenolic or flavonoid content and antioxidant potential.

7. Research Hypotheses:

H_{01}: There is no significant difference in the total phenolic content of the three extracts.
H_{11}: At least one extract differs significantly in total phenolic content.
H_{02}: There is no significant difference in the total flavonoid content of the extracts.
H_{12}: At least one extract differs significantly in total flavonoid content.
H_{03}: The three extracts do not differ significantly in DPPH, ABTS or reducing-power activity.
H_{13}: At least one extract demonstrates significantly different antioxidant activity.
H_{04}: Phenolic and flavonoid contents are not significantly associated with antioxidant activity.
H_{14}: Phenolic or flavonoid content is significantly associated with antioxidant activity.

8. Materials and Methods:

Research Design: A laboratory-based, comparative and experimental research design was adopted. All three extracts were prepared and analysed under the same conditions to improve comparability.

Plant Materials: The following plant parts were selected:

Sample code	Plant	Plant part
NCLE	Nerium cordifolia	Leaves
SVLE	Strobilanthes veronicifolia	Leaves
NATBE	Nyctanthes arbor-tristis	Stem bark

The plant materials should be collected from pesticide-free locations during the same season, as far as practicable. Collection date, geographical coordinates and environmental conditions should be recorded.

Authentication: A qualified botanist should authenticate each specimen. A voucher sample of every plant should be preserved and deposited in an institutional or recognised herbarium.

8.4 Chemicals and Reagents:

DPPH, ABTS, potassium persulfate, Folin–Ciocalteu reagent, gallic acid, quercetin, aluminium chloride, sodium carbonate, potassium ferricyanide, ferric chloride, trichloroacetic acid, ascorbic acid or Trolox and analytical-grade solvents should be obtained from certified suppliers.

Preparation of Plant Materials: Plant materials should be washed with clean water to remove adhering matter and then shade-dried at room temperature with adequate ventilation. Direct sunlight should be avoided because it may degrade light-sensitive phytoconstituents. The dried materials should be pulverised separately, passed through a suitable sieve and stored in labelled airtight containers.

Extraction: Approximately 100 g of each powdered sample may be extracted separately with 70% ethanol using maceration or Soxhlet extraction. For maceration, a 1:10 plant-to-solvent ratio may be maintained for 72 hours with intermittent shaking. Each mixture should be filtered, and the solvent should be removed under reduced pressure below 45°C. The concentrated extracts should be dried to constant weight and stored at 4°C.

Qualitative Phytochemical Screening: Standard procedures should be used to identify the following constituent groups:

Constituent	Indicative test	Positive observation
Phenolics	Ferric chloride test	Blue, green or dark coloration
Flavonoids	Alkaline reagent test	Yellow colour disappearing with acid
Alkaloids	Dragendorff's/Mayer's test	Orange or cream precipitate
Tannins	Ferric chloride test	Blue-black or greenish coloration
Terpenoids	Salkowski test	Reddish-brown interface
Saponins	Foam test	Stable persistent froth
Glycosides	Appropriate specific test	Characteristic colour or ring
Carbohydrates	Molisch's test	Violet ring
Proteins	Biuret test	Violet coloration

Total Phenolic Content: Total phenolic content should be estimated using the Folin–Ciocalteu method. A measured volume of extract should be mixed with diluted Folin–Ciocalteu reagent, followed by

sodium carbonate solution. After incubation in darkness, absorbance should be measured at approximately 765 nm.

A gallic acid calibration curve should be prepared, and the result should be expressed as:

mg GAE/g of dried extract\text{mg GAE/g of dried extract}mg GAE/g of dried extract

The calibration equation, coefficient of determination and concentration range must be

Total Flavonoid Content: Total flavonoid content should be determined through aluminium chloride colorimetry. The extract should be mixed with aluminium chloride solution and incubated for the validated reaction period. Absorbance may be measured at approximately 415 nm, depending on the selected protocol.

A quercetin calibration curve should be prepared, and the result should be expressed as:

$$\frac{\text{mg QE}}{\text{g of dried extract}\backslash\text{text}\left\{\text{mg}\frac{\text{QE}}{\text{g}}\text{ of dried extract}\right\}\text{mg QE}}{\text{g of dried extract}}$$

DPPH Radical-Scavenging Assay:A freshly prepared methanolic DPPH solution may be mixed with different concentrations of each extract, for example 25, 50, 100, 200 and 400 µg/mL. The reaction mixtures should be incubated in darkness for approximately 30 minutes. Absorbance should be recorded at 517 nm.

$$\textbf{DPPH inhibition } (\%) = \textbf{AcAc} - \textbf{As} \times \textbf{100}$$

Where:

- A_c = absorbance of the control
- A_s = absorbance of the sample

The IC_{50} value represents the concentration required to inhibit 50% of the DPPH radicals. A lower IC_{50} indicates stronger radical-scavenging activity.

ABTS Radical-Cation Scavenging Assay: The ABTS radical cation should be generated by reacting ABTS solution with potassium persulfate and allowing the mixture to stand in darkness for 12–16 hours. Before use, the solution should be diluted to the validated initial absorbance at 734 nm.

The test extract should be added to the working ABTS solution, and absorbance should be

$$\textbf{ABTS inhibition } (\%) = \textbf{AcAc} - \textbf{As} \times \textbf{100}$$

Results may be presented as percentage inhibition, IC_{50} or Trolox-equivalent antioxidant capacity.

Reducing-Power Assay: Different extract concentrations should be mixed with phosphate buffer and potassium ferricyanide and incubated at 50°C. The reaction should be stopped using trichloroacetic acid. After centrifugation, the supernatant should be mixed with distilled water and ferric chloride. Absorbance should be measured at 700 nm.Unlike the DPPH and ABTS IC_{50} values, a higher absorbance in the reducing-power assay indicates greater electron-donating ability.

Control of Interference: Plant extracts can possess natural colour or turbidity that interferes with spectrophotometric readings. A separate sample blank containing the extract and all reagents except the radical or chromogenic reagent should therefore be included. Blank-corrected absorbance should be used for calculations.

Statistical Analysis: All experiments should be conducted independently in triplicate, and the results should be expressed as mean ± standard deviation. Statistical analysis may be conducted using SPSS, GraphPad Prism or R.

- One-way ANOVA should compare TPC, TFC and extract yields.

- Two-way ANOVA may compare concentration-dependent antioxidant responses.
- Tukey's post-hoc test should identify pairwise differences.
- Pearson's correlation may assess relationships among TPC, TFC and antioxidant measures.
- Statistical significance should be fixed at $p<0.05p<0.05p<0.05$.
- IC_{50} values should preferably be calculated by nonlinear regression.

9. Result:

Extraction Yield

Extract	Powder used (g)	Dried extract (g)	Yield (%)	Sourcing Context
NCLE (Nervilia cordifolia)	100	**11.45**	**11.45%**	Standard hydro-alcoholic maceration yields for Nervilia species run between 10% and 13%.
SVLE (Strobilanthes veronicifolia)	100	**14.20**	**14.20%**	Corresponds to traditional polar solvent extraction yields for Strobilanthes leaves.
NATBE (Nyctanthes arbor-tristis)	100	**6.48**	**6.48%**	Extracted from established ethnobotanical maceration values for Nyctanthes.

Qualitative Phytochemical Screening

Phytochemical	NCLE	SVLE	NATBE	Descriptive Interpretation
Phenolics	+++	+++	++	Strong presence was observed in NCLE and SVLE, whereas NATBE showed a moderate reaction, suggesting appreciable phenolic constituents in all three extracts.
Flavonoids	++	+++	++	Flavonoids were strongly detected in SVLE and moderately detected in NCLE and NATBE.
Alkaloids	++	++	+	NCLE and SVLE exhibited moderate alkaloid reactions, while NATBE demonstrated comparatively weak presence.
Tannins	++	+++	+++	Tannins were strongly present in SVLE and NATBE, whereas NCLE showed a moderate reaction.
Terpenoids	++	++	+++	All extracts contained terpenoids, with NATBE displaying the strongest characteristic reaction.
Saponins	+	++	++	Saponins were weakly detected in NCLE but occurred at moderate levels in SVLE and NATBE.
Glycosides	+++	++	++	NCLE showed a strong positive reaction for glycosides, while SVLE and NATBE demonstrated moderate positivity.
Carbohydrates	++	++	+++	Carbohydrates were present in all extracts, with comparatively stronger detection in NATBE.
Proteins	+	+	++	Protein-related constituents were weakly detected in

				NCLE and SVLE and moderately detected in NATBE.

Key: − = absent; + = weak; ++ = moderate; +++ = strongly present.

Total Phenolic and Flavonoid Contents

Extract	TPC (mg GAE/g)	TFC (mg QE/g)
NCLE	**68.42 ± 2.15**	**41.36 ± 1.48**
SVLE	**84.75 ± 2.62**	**56.28 ± 1.73**
NATBE	**59.63 ± 1.94**	**35.47 ± 1.29**

Among the three extracts, **SVLE showed the highest total phenolic content (84.75 ± 2.62 mg GAE/g)** and total flavonoid content (**56.28 ± 1.73 mg QE/g**). NCLE exhibited intermediate values, whereas NATBE showed comparatively lower phenolic and flavonoid contents.
The order of total phenolic content was:

SVLE > NCLE > NATBE
Similarly, total flavonoid content followed the order:

SVLE > NCLE > NATBE
The comparatively higher phenolic and flavonoid contents of SVLE are consistent with the illustrative antioxidant pattern previously presented, where SVLE showed stronger DPPH, ABTS and reducing-power activity. However, the manuscript correctly notes that higher TPC or TFC alone should not be treated as definitive proof of superior antioxidant activity; the values should be interpreted along with IC_{50}, reducing power and statistical significance.

DPPH Radical-Scavenging Activity

Concentration (µg/mL)	NCLE (%)	SVLE (%)	NATBE (%)	Standard (%)
25	18.42 ± 0.74	24.65 ± 0.81	15.38 ± 0.62	39.72 ± 0.86
50	29.86 ± 0.89	37.48 ± 0.95	25.64 ± 0.77	55.63 ± 0.91
100	43.75 ± 1.02	54.92 ± 1.11	38.27 ± 0.94	71.84 ± 1.03
200	61.48 ± 1.18	72.36 ± 1.26	55.69 ± 1.08	86.57 ± 0.88
400	78.92 ± 1.31	87.46 ± 1.18	73.84 ± 1.21	94.26 ± 0.65
IC_{50} (µg/mL)	**≈135.7**	**≈82.1**	**≈159.0**	**≈41.2**

Interpretation: DPPH radical-scavenging activity increased progressively with concentration in all extracts. Among the plant extracts, **SVLE exhibited the strongest DPPH activity**, followed by NCLE and NATBE. The comparatively lower IC_{50} of SVLE indicates a greater free-radical-scavenging capacity. The reference standard remained more active than all three extracts.

ABTS Radical-Scavenging Activity

Concentration (µg/mL)	NCLE (%)	SVLE (%)	NATBE (%)	Standard (%)
25	22.36 ± 0.68	28.74 ± 0.76	18.82 ± 0.71	43.28 ± 0.82
50	34.75 ± 0.84	42.68 ± 0.91	30.15 ± 0.83	59.46 ± 0.94
100	48.92 ± 1.01	59.37 ± 1.08	43.62 ± 0.96	75.82 ± 0.89

200	66.47 ± 1.16	77.82 ± 1.19	60.48 ± 1.12	89.34 ± 0.75
400	82.65 ± 1.23	90.54 ± 1.05	78.36 ± 1.18	96.18 ± 0.58
IC_{50} (µg/mL)	**≈106.2**	**≈72.7**	**≈126.9**	**≈35.4**

Interpretation: A concentration-dependent increase in ABTS radical-cation scavenging was observed. **SVLE again produced the highest activity among the extracts**, reaching approximately 90.54% inhibition at 400 µg/mL. Its lower estimated IC_{50} suggests stronger ABTS-scavenging capacity than NCLE and NATBE.

Reducing Power

Concentration (µg/mL)	NCLE: A_{700}	SVLE: A_{700}	NATBE: A_{700}	Standard: A_{700}
25	0.184 ± 0.009	0.236 ± 0.011	0.157 ± 0.008	0.318 ± 0.012
50	0.296 ± 0.012	0.374 ± 0.014	0.254 ± 0.010	0.487 ± 0.015
100	0.438 ± 0.015	0.552 ± 0.017	0.381 ± 0.014	0.692 ± 0.018
200	0.627 ± 0.018	0.758 ± 0.020	0.543 ± 0.017	0.914 ± 0.021
400	0.846 ± 0.022	0.982 ± 0.024	0.751 ± 0.020	1.168 ± 0.026

The reducing-power assay demonstrated a clear concentration-dependent increase in absorbance at 700 nm for all tested extracts. At 400 µg/mL, the reducing power followed the order **Standard > SVLE > NCLE > NATBE**. SVLE exhibited the highest reducing capacity among the plant extracts, with an absorbance of **0.982 ± 0.024**, followed by NCLE (**0.846 ± 0.022**) and NATBE (**0.751 ± 0.020**).

The overall antioxidant activity across the three assays therefore followed the illustrative pattern:

Standard > SVLE > NCLE > NATBE

This pattern is also compatible with the manuscript's interpretation framework, which recommends evaluating concentration-dependent inhibition, IC_{50}, reducing power, and phytochemical content together rather than relying on one antioxidant test alone.

Important: These are suitable as a **sample/mock result dataset for drafting and statistical demonstration**, not as claimed experimental findings. For a thesis or journal article, the final values should come from actual triplicate absorbance readings and IC_{50} should preferably be calculated using nonlinear regression, as specified in the manuscript.

10. Framework for Interpretation

The extract showing the highest TPC or TFC should not automatically be declared the strongest antioxidant. Its DPPH and ABTS IC_{50} values, reducing power and statistical significance must also be examined.

A convincing pattern would consist of:

- increasing radical inhibition with increasing concentration;
- a significantly lower IC_{50} than the other plant extracts;
- stronger reducing power at comparable concentrations;
- comparatively high phenolic or flavonoid content; and
- a significant correlation between phytochemical content and antioxidant measurements.

If the assays produce different rankings, this should be reported openly. Such variation may reflect different solubilities, reaction kinetics and mechanisms of antioxidant compounds. It is not necessarily evidence of experimental failure.

11. Discussion

The proposed study combines qualitative phytochemical examination with quantitative measurement and three complementary antioxidant tests. This design is scientifically preferable to reporting only the presence or absence of metabolites. Qualitative tests offer preliminary evidence, whereas total phenolic and flavonoid measurements permit comparative estimation.

Phenolics and flavonoids can participate in antioxidant reactions through electron donation, hydrogen transfer and stabilisation of radical intermediates. Tannins may also contribute because of their multiple hydroxyl groups. Terpenoids and alkaloids may exhibit antioxidant behaviour through different chemical pathways. Nevertheless, a crude extract is a chemically complex mixture, and its total activity may result from synergistic or antagonistic interactions.

The Folin–Ciocalteu result should be interpreted as an estimate of total reducing phenolic equivalents, not as the concentration of a particular compound. Likewise, aluminium chloride colorimetry provides an overall flavonoid-equivalent value but does not identify individual flavonoids. HPTLC, HPLC or LC–MS analysis would therefore be necessary to characterise active constituents.

DPPH is simple and reproducible but may be influenced by solvent conditions, slow-reacting antioxidants and sample pigmentation. ABTS can assess a wider polarity range but is similarly dependent on reaction conditions. Reducing power measures electron donation rather than direct radical scavenging. Agreement among these methods would strengthen the evidence, whereas divergence would reveal mechanistic differences requiring further investigation.

Safety is especially important for material assigned to the genus Nerium. Oleander species are known for serious poisonous characteristics; therefore, antioxidant activity alone must never be interpreted as proof of therapeutic safety. Laboratory handling should include gloves, protective clothing, controlled disposal and prevention of accidental ingestion or inhalation. No pharmaceutical or nutraceutical recommendation should be made without comprehensive toxicity and cardiac-safety evaluation.

12. Conclusion

The proposed comparative study provides a systematic framework for investigating the phytochemical composition and in-vitro antioxidant potential of the leaves of Nerium cordifolia and Strobilanthes veronicifolia and the stem bark of Nyctanthes arbor-tristis. Qualitative screening, total phenolic and flavonoid estimation, DPPH, ABTS and reducing-power assays together can generate a multidimensional antioxidant profile.

A final conclusion concerning the most active extract can only be drawn after authenticated plant materials have been tested and the resulting data analysed statistically. The extract demonstrating consistent activity across the assays may be selected for chromatographic profiling and compound isolation. In-vitro antioxidant results should be regarded as preliminary chemical evidence rather than direct evidence of clinical efficacy.

13. Limitations

The present study has certain limitations that should be considered while interpreting the findings. In-vitro antioxidant assays provide useful preliminary information but do not completely represent biological conditions within living systems. The phytochemical composition and antioxidant activity of plant extracts may also vary according to extraction solvent, harvesting season, geographical origin, environmental conditions and storage practices. The colorimetric methods used for estimating total

phenolic and flavonoid contents provide only overall quantitative values and do not identify individual bioactive compounds. In addition, natural pigments and other reducing substances present in crude extracts may interfere with spectrophotometric measurements. Therefore, antioxidant activity alone cannot confirm pharmaceutical safety, therapeutic effectiveness or clinical usefulness. Finally, accurate botanical authentication and preservation of voucher specimens are essential to avoid plant-name uncertainty and to ensure reproducibility of the study.

14. Future Scope

Future investigations may focus on advanced chromatographic profiling using HPTLC, HPLC and LC–MS to identify and characterise the major bioactive constituents present in the selected plant extracts. Isolation and structural elucidation of promising compounds may further clarify their antioxidant potential. The observed activity should also be validated through suitable cell-based oxidative-stress models and followed by acute and repeated-dose toxicity studies. Particular attention may be given to cardiac glycoside profiling of the Nerium sample to address safety concerns. Further studies may also examine possible synergistic or antagonistic interactions among the extracts and evaluate their hepatoprotective potential using ethically approved experimental models.

References:

1. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181, 1199–1200. https://doi.org/10.1038/1811199a0
2. 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.
3. Everette, J. D., Bryant, Q. M., Green, A. M., Abbey, Y. A., Wangila, G. W., & Walker, R. B. (2010). A thorough study of reactivity of various compound classes toward the Folin–Ciocalteu reagent. Journal of Agricultural and Food Chemistry, 58(14), 8139–8144. https://doi.org/10.1021/jf1005935
4. Harborne, J. B. (1998). Phytochemical methods: A guide to modern techniques of plant analysis (3rd ed.). Chapman & Hall.
5. Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition and Dietetics, 44(6), 307–315.
6. Rajurkar, N. S., & Hande, S. M. (2011). Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. Indian Journal of Pharmaceutical Sciences, 73(2), 146–151. https://doi.org/10.4103/0250-474X.91574
7. 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
8. Royal Botanic Gardens, Kew. (2026). Nyctanthes arbor-tristis L. Plants of the World Online. https://powo.science.kew.org/
9. Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts of Torilis leptophylla L. BMC Complementary and Alternative Medicine, 12, 221. https://doi.org/10.1186/1472-6882-12-221

10. Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods in Enzymology, 299, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1
11. Trease, G. E., & Evans, W. C. (2009). Trease and Evans pharmacognosy (16th ed.). Saunders Elsevier.
12. World Health Organization. (2011). Quality control methods for herbal materials. World Health Organization.