

Effect of different solvent extracts on total phenol content, flavonoid content and antioxidant properties of *Strobilanthes cordifolia* (Vahl) J.R.I. Wood

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Abstract

The antioxidant quality of plant-derived products is widely acknowledged to be influenced by various extraction factors such as the method employed, temperature, duration and choice of solvent system. The objective of the current study was to assess how various solvents affect the extraction yields, phytochemical components and antioxidant properties of *S. cordifolia* leaves. In this study, hexane, chloroform, ethanol and aqueous were used as extraction solvents for *S. cordifolia*. Ethanol was found to be the most effective solvent, resulting in the maximum extraction yield ($24.46 \pm 1.03\%$) and the highest amount of phenolic (84.5 ± 0.5 mg GAE/g) and flavonoids (50.59 ± 0.65 mg QE/g) in the crude extract. The antioxidant activities of *S. cordifolia* were assessed using DPPH, ABTS and H_2O_2 assays. The ethanol extract showed substantial efficacy in scavenging DPPH, ABTS and H_2O_2 with IC_{50} values of $65.3 \mu\text{g/mL}$, $68.1 \mu\text{g/mL}$ and $75.8 \mu\text{g/mL}$ respectively.

Moreover, each solvent extract derived from *S. cordifolia* exhibited significant antioxidant potential, albeit with variations attributed to differences in their phytochemical composition. These findings highlight the potential of *S. cordifolia* leaves as a natural antioxidant source, promising in alleviating oxidative stress and holding significance for the nutraceutical and pharmaceutical industries. Future investigations should prioritize isolating and identifying the active secondary metabolites present in these extracts.

Keywords: *Strobilanthes cordifolia*, different solvent extraction, phenols, flavonoids, antioxidant activity.

Introduction

Free radicals are byproducts of physiological and metabolic processes in the human body that can lead to oxidative damage to biomolecules and a host of illnesses²². Plant-based antioxidant chemicals can prevent the synthesis of free radicals and can alleviate disorders induced by oxidative stress. Medicinal plants are renowned for their potent antioxidant properties, particularly due to the presence of phenolic and flavonoid compounds. These compounds are instrumental in the antioxidant activity of plants and serve as effective anti-inflammatory agents⁶. The plant-based antioxidants have recently gained significant interest since

plants are among the richest sources of naturally occurring antioxidants like flavonoids, phenolics and alkaloids^{32,33}.

Strobilanthes species are known for their medicinal uses such as antioxidant, antiarthritic, anti-inflammatory, gout, jaundice, antimicrobial, antidiabetic, anticancer, wound healing properties and rheumatism. Delving into the untapped potential of medicinal plants could reveal new drugs and treatments for various health conditions, potentially presenting more efficient and economical choices. *Strobilanthes cordifolia* (Vahl) J.R.I. Wood belongs to the family Acanthaceae. It is a shrub, branchlets viscid. [Leaves ovate-lanceolate, base attenuate, margin crenate, apex acute, petiole to 6 cm. Spikes terminal, interrupted, flower cluster 1 cm apart below, closer above, bracts oblong-ovate, 3-ribbed, bracteoles lanceolate. Calyx- lobes 5, subequal, parted above the middle of the tube. Corolla blue, narrowly tubular below, ventricose above, obscurely 2-lipped, lobes 2+3, subequal. Stamens 2+2, didynamous, attached above the middle of the tube, exserted. Ovules 8, style slender, stigma curved, seeds 8, with silky hairs around].

The flowering and fruiting season is yearly once. It is distributed in South India and Sri Lanka. Extraction stands as the primary method for acquiring bioactive compounds from biomass materials. The selection of solvents used in this process significantly influences the composition and yield of secondary metabolites derived from medicinal plants. More precisely, polar solvents extract phenolic compounds, glycosides and saponins whereas non-polar solvents are preferred for extracting fatty acids and steroids⁷. Therefore, selecting the appropriate solvent is essential to maximize the yield and quality of desired bioactive compounds during extraction from medicinal plants. The objective of this study was to evaluate how different extraction solvents affect the extraction yield, secondary metabolite levels and antioxidant properties in *S. cordifolia* leaves.

Material and Methods

Plant collection and identification: The fresh leaves of *Strobilanthes cordifolia* were collected from Kotagiri hills, Nilgiris District, Tamil Nadu, India. The plant specimen was identified and authenticated by the Botanical Survey of India, Southern Region, Coimbatore.

Preparation of plant material: The leaves were washed with running tap water followed by distilled water and

allowed to shade-dry for 5 weeks. The dried leaves were ground into a fine powder and stored for future use.

Extraction: In our prior research, the proportion of plant material to solvent was set at 1:10 according to the methodology of Venkatesan et al³⁰. The aqueous extract of leaves (25 grams in 250 mL of distilled water) was kept in a shaker for 24 hours, then it was filtered using Whatmann no. 1 filter paper and lyophilized. The powdered leaves (25 grams in 250 mL) were subjected to Soxhlet extraction with hexane, chloroform and ethanol. The extracts were then filtered using the Whatmann no. 1 filter paper, the filtrate was evaporated to dryness using rotary evaporator. The final extracts were weighed to ascertain yield percentage and the crude extracts were preserved at 4°C in a refrigerator for future analysis.

Extraction yield: The yield of the extraction was calculated:

Extraction yield (%) = Weight of crude extract (g) / Weight of powdered sample (g) × 100

Determination of total phenolic content (TPC): The total phenolic content (TPC) of leaf extracts was assessed using the Folin-Ciocalteu method as outlined by Truong et al²⁹, with slight adjustments. Initially, 1.5 mL of Folin-Ciocalteu reagent (10%) was combined with 1 mL of each plant extract solution (1 mg/mL). Following a 5-minute incubation period, 5 mL of Na₂CO₃ (7.5%) was introduced and the mixture was allowed to incubate at room temperature for 30 minutes. Standard solutions of gallic acid ranging from 10 to 100 mg/mL were prepared similarly to the extracts. A blank solution containing all reagents except the plant extract and standard solution was also prepared.

Subsequently, the absorbance of both the extracts and standard solutions was measured at 760 nm using a UV/Visible spectrophotometer against a reagent blank. The total phenolic content in the tested extracts was determined by the standard curve. This experimental procedure was repeated three times to ensure accuracy.

Determination of total flavonoid content (TFC): The determination of Total Flavonoid Content (TFC) in leaf extracts followed the aluminium chloride method as described by Rana et al²⁵ with minor adjustments. Extracts (0.5 mL of 1 mg/mL) were mixed with 1.5 mL of methanol, followed by the addition of 1 mL each of aluminium chloride (10%) and potassium acetate (1M) in the test tubes. The mixture was then thoroughly mixed and allowed to incubate at room temperature for 30-40 minutes. Standard solutions of quercetin (10 to 100 mg/mL) were prepared in a similar manner to the extracts. The experimental process was carried out three times.

Subsequently, the absorbance of the solutions was measured at 415 nm using a UV/Visible spectrophotometer against a blank containing all reagents except the sample/standard. A

calibration curve was used to determine the total flavonoid concentration in the tested extracts. The results were expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g extract).

DPPH free radical scavenging activity: The antioxidant capacity of the *S. cordifolia* extracts was assessed using the DPPH-free radical scavenging assay, as outlined by Mahdi-Pour et al¹⁸ with minor modifications. Briefly, 0.3 mL of extract at various concentrations (20 to 100 µg/mL) was mixed with 1 mL of DPPH solution (0.004% in methanol) and allowed to incubate in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured at 517 nm using a UV-visible spectrophotometer. Methanol and 1 mL of DPPH solution served as the negative control. The inhibition percentage of free radical scavenging was then determined using the following formula:

DPPH scavenging activity (%) = $\frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \times 100$

The concentration required for a 50% inhibition of DPPH (IC₅₀) was then calculated by plotting the percentage of residual DPPH against the sample concentration.

ABTS radical scavenging activity: The ABTS method measures the reduction of ABTS•+ radicals by antioxidants in plant extracts, following the protocol of Rafińska et al²⁴. Initially, the ABTS radical was generated by mixing 10 mL of a 7 mM ABTS stock solution with 10 mL of 2.45 mM potassium persulfate. This mixture was allowed to react for 16 hours until the reaction was complete and the absorbance stabilized. The ABTS•+ solution was then diluted with ethanol to reach an absorbance of 0.7 at 745 nm. Samples were prepared in methanol at various concentrations (µg/mL). For testing, 10 µL of each sample was added to a test tube and mixed thoroughly with 2.99 mL of the ABTS radical working solution. The absorbance of the resulting clear mixture was measured at 734 nm. The percentage of antioxidant activity of the sample was calculated using the following formula:

ABTS scavenging activity (%) = $\frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \times 100$

Ascorbic acid was used as a standard. The concentration required for a 50% inhibition of DPPH (IC₅₀) was then calculated by plotting the percentage of residual DPPH against the sample concentration.

H₂O₂ radical scavenging activity: The potential of different solvent extracts to scavenge hydrogen peroxide (H₂O₂) was evaluated following the method described by Mohanty et al²⁰. Leaf extracts at varying concentrations (20-100 µg/mL) were prepared and mixed with 0.1 mL of 0.2 M phosphate buffer (pH 7.4) and 0.6 mL of H₂O₂ solution. The mixture was incubated for 30 minutes at room temperature. The H₂O₂ scavenging activity was measured by recording the

absorbance at 230 nm, with phosphate buffer serving as the blank. A mixture of phosphate buffer and H₂O₂ was used as the control. The percentage of H₂O₂ scavenging activity was calculated using the following equation:

$$\text{H}_2\text{O}_2 \text{ scavenging activity (\%)} = \frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \times 10$$

Ascorbic acid served as a standard. The IC₅₀, representing the concentration at which 50% inhibition of H₂O₂ occurred, was determined by plotting the percentage of residual H₂O₂ against the concentration of the samples.

Results and Discussion

Effect of different solvents on extraction yield: The extraction procedure precedes the analysis of phytochemical compounds and antioxidant properties in samples. The quantity of crude extracts is significantly influenced by the choice of plant material and the solvent used for extraction. In our study, we aimed to understand how different solvents (hexane, chloroform, ethanol and water) affect the extraction of bioactive compounds from the leaves of *S. cordifolia*. By analyzing the extraction yields presented in table 1, we found that the yields of various solvent extracts ranged from 9.58±1.23% to 24.46±1.03%. Notably, ethanol consistently yielded the highest extraction rate, averaging 24.46%, while hexane showed the lowest yield, averaging 9.58%.

In a similar vein, in the leaves of *Caesalpinia bonduc*, ethanol proved to be the most efficient solvent, yielding the highest extraction rate and is favored for its efficacy in extracting phenolic compounds from plants¹⁴. These discrepancies in both the dry weight of the extracts and the yields among the organic solvents may be attributed to their differing polarities²⁷. Research indicates that the extraction efficiency of plant material frequently rises as the polarity of the solvent used increases^{8,17}. Moreover, the polarity of solvents significantly impacts both the extraction rate and the antioxidant activity of plant extracts⁹. Ethanol extraction is especially appropriate for acquiring deparaffinated extracts abundant in polyphenolic elements.

Conversely, water extraction is suitable for obtaining extracts containing water-soluble phenolic acids¹². Despite water's high polarity and tendency to wash away hydrophilic compounds, it was slightly less effective compared to ethanol in extracting these compounds as observed by Rafińska et al²⁴. Ethanol is widely considered safer for human consumption next to water³⁰. However, there are no available reports on the comparative yield analysis obtained

from different solvent extracts of *S. cordifolia* in existing literature.

Effect of different solvent extracts on TPC and TFC:

Phenolic compounds, regarded as secondary metabolites, are synthesized by plants in response to various environmental stresses such as ultraviolet radiation and pathogens³¹. These compounds are abundant in most medicinal plants and play a vital role in the human diet due to their antioxidant properties and numerous other health-promoting benefits³. The scavenging ability of all phenolic compounds tested exceeded 85%, primarily because of the strong reactivity of the hydroxyl radical¹⁹. Flavonoids are a group of secondary plant compounds with significant biological effects. The biological functions of flavonoids are determined by the arrangement and placement of hydroxyl groups on their structure¹⁶.

The assessment of total phenolic content and total flavonoid content of *S. cordifolia* leaves in different solvent extracts was conducted through the application of the Folin-Ciocalteu method and aluminium chloride colorimetric method. Results exhibited a range of 12.28 to 84.5 mg GAE/g crude extract for TPC and 15.15 ± 0.70 to 50.59 ± 0.65 mg QE/g of crude extract for TFC (Table 2). The results decisively showed statistical significance ($p < 0.05$) among the different solvent extracts. The ethanol exhibited the highest phenolic content (84.5 ± 0.5 mg GAE/g) and flavonoid content (50.59 ± 0.65 mg QE/g), while the hexane showed the lowest phenolic content recorded at 12.28 ± 0.87 mg GAE/g and flavonoid content (15.15 ± 0.70 mg QE/g).

These outcomes imply that the higher polarity of ethanol led to its superior efficiency in extracting polar phytochemicals (phenolics and flavonoids) from the leaves of *S. cordifolia*. Likewise, studies on *Strobilanthes crispus* and *Strobilanthes kunthiana* exhibited notable levels of phenols and flavonoids in the ethanol extract^{2,13}.

Furthermore, the ethanol extract demonstrated significant effects on the phenolic and flavonoid content in *Guiera senegalensis*⁷. In research on *Limnophila aromatica*, the highest concentration of phenolic compounds was obtained from extracts using 100% ethanol whereas lower levels were observed with 75% and 50% aqueous ethanol⁸. Conversely, in *Cuphea carthagenensis*, the highest total phenolic content (TPC) was achieved with a mixture of 50% ethanol and water⁵. This suggests that there is no easy formula to foresee how different compounds will behave during extraction from groups.

Table 1
Effect of different solvent extracts on total yield of *S. cordifolia*

S.N.	Extracts	Extract yield (%)
1.	Hexane	9.58±1.23
2.	Chloroform	13.75±1.48
3.	Ethanol	24.46±1.03
4.	Aqueous	17.28±1.08

Table 2

Quantification of Total Phenolic and Flavonoid Contents in Different Solvent Extracts of *S. cordifolia* Leaves

S.N.	Extracts	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)
1.	Hexane	12.28 ± 0.87 ^d	15.15 ± 0.70 ^d
2.	Chloroform	24.56 ± 0.87 ^c	18.5 ± 0.58 ^c
3.	Ethanol	84.5 ± 0.5 ^a	50.59 ± 0.65 ^a
4.	Aqueous	55.84 ± 1.01 ^b	27.60 ± 0.96 ^b

Values are mean of triplicate determination (n=3) ± standard deviation, GAE – Gallic Acid Equivalent, QE – Quercetin Equivalent, statistically significant at $p < 0.05$ where $a > b > c > d$

So, it is crucial to tailor the basic factors to the specific plant material and the desired compounds. Ultimately, ethanol and water proved to be superior solvents compared to others for extracting phenolic compounds from the extracts due to their excellent solubility and polarity.

Conversely, chloroform and hexane, which have lower polarity, exhibited reduced effectiveness in extracting phenolic compounds⁴. This highlights the essential importance of selecting the right solvent to optimize phytochemical extraction. Building upon the preceding discussion, it underscores the substantial influence of ethanol on extracting bioactive compounds from plants. These discoveries improve our comprehension of phytochemical extraction methods and stress the significance of solvent selection in maximizing the production of bioactive compounds from plant extracts.

Effect of different solvent extracts on Antioxidant activity: Excessive production of free radicals within the human body can lead to cellular damage, contributing to the onset of conditions like cancer, heart attacks, hardened arteries and neurodegenerative diseases. Nevertheless, natural antioxidants sourced from plants possess the ability to neutralize these harmful radicals, thereby playing a crucial role in the prevention of a wide array of disorders²⁸. The antioxidant potential of *S. cordifolia* leaves was assessed through analyses of DPPH free radical scavenging activity, ABTS radical scavenging activity and H_2O_2 scavenging activity. In this study, we evaluated the efficacy of different solvent extracts in scavenging free radicals.

Initially, the DPPH radical assay was conducted on all extracts. Essentially, the test relies on the ability of DPPH free radicals to interact with plant metabolites like phenolic and flavonoid compounds which act as donors of hydrogen ions (H^+). When this reaction occurs within the sample, the DPPH solution undergoes a colour change from purple to yellow, as it gains a proton from the donor species. The degree of colour alteration corresponds directly to the scavenging efficacy of the biological sample¹. Figure 1a depicts the DPPH radical scavenging potential of the different extracts compared to the positive control (Ascorbic acid) with significant difference $p < 0.05$. It was observed that the radical scavenging efficacy correlated with concentration in all solvent extracts. The antioxidant activity of the tested extracts is expressed in terms of IC_{50} values ($\mu g/mL$), where

a lower IC_{50} value signifies stronger antioxidant activity. The extracts obtained from ethanol (65.3 $\mu g/mL$) showed the highest potential in scavenging free radicals, nearing the effectiveness of the ascorbic acid (46.0 $\mu g/mL$). In contrast, the aqueous extract (91.6 $\mu g/mL$) exhibited the least activity among all the extracts tested. The free radical scavenging activity of *S. cordifolia* was also determined using ABTS radical. When an antioxidant capable of donating a hydrogen atom is added to a solution of ABTS radical, the blue-green colour of the solution transforms into colourless, resulting in a decrease in absorbance at 734 nm.

Therefore, the extent of colour intensity reduction is indicative of the antioxidants' ability to scavenge radicals²⁶. A statistically significant difference was observed between the ascorbic acid standard and all *S. cordifolia* extracts, with a significance of $p < 0.05$ (Fig. 1b). The ethanol leaf extracts exhibited the highest scavenging activity percentage (IC_{50}) at 68.1 $\mu g/mL$ followed by hexane at 78.1 $\mu g/mL$ and chloroform at 84.7 $\mu g/mL$. Conversely, the aqueous extract displayed the lowest scavenging activity among all extracts, recording 101 $\mu g/mL$. Nevertheless, the efficacy of all extracts was slightly inferior to that of ascorbic acid (45.9 $\mu g/mL$).

The assay was based on the principle of formation of a red-orange complex between ferrous iron (Fe^{2+}) and 1,10-phenanthroline that absorb light at 536 nm. While antioxidants fail to scavenge H_2O_2 , H_2O_2 can oxidize ferrous (Fe^{2+}) to ferric (Fe^{3+}) iron which is incapable of forming a complex. Hence, the amount of red-orange compound produced in the assay mixture is directly proportional to the H_2O_2 scavenging potential of antioxidants²¹. Figure 1c shows the radical scavenging activity of H_2O_2 of the different extracts in contrast to the standard, resulting with a significant difference of $p < 0.05$. The ethanol extract had the maximum potency, with an IC_{50} value of 75.8 $\mu g/mL$, similar to the effectiveness of ascorbic acid at 54.9 $\mu g/mL$. The hexane, chloroform and aqueous extracts had lesser radical scavenging activity (IC_{50} values of 83.3 $\mu g/mL$, 89.9 $\mu g/mL$ and 111 $\mu g/mL$ respectively) relative to the ethanol extract.

In brief, the assessment of antioxidant efficacy in *S. cordifolia* leaf extracts using DPPH, ABTS and H_2O_2 techniques revealed that the ethanol extract had the highest potency. Conversely, the aqueous extract had the lowest activity. Similarly, the ethanol extracts of *Lepidium sativum*

have higher antioxidant capacity than aqueous extracts²⁴. In contrary, previous research demonstrated that the aqueous extract of *Strobilanthes crispus* leaves exhibited greater levels of total phenolics, total flavonoid content and antioxidant activity compared to the ethanol extract^{11,23}.

However, the results of this present study contradicted these findings, as it was observed that the ethanol extract displayed higher levels of total phenolics, flavonoids and antioxidant activity compared to the aqueous extract. These disparities may be attributed to variations in plant age as well as differences in drying and extraction methods employed. Plant-derived natural phenols tend to exhibit higher solubility in solvents with intermediate polarity such as alcohol and acetone, as opposed to more polar solvents like water or less polar solvents like ethyl acetate¹⁰.

It is important to acknowledge that the choice of solvents for extraction significantly impacts the structural variety of the obtained antioxidant compounds¹⁵. In our investigation, the ethanol extract demonstrated highest radical scavenging activity compared to other solvent extracts, likely due to the

enhanced solubility of antioxidant compounds. Ethanol, being less polar than water according to Index Merck (2001), is possibly more efficient in extracting antioxidants. Consequently, the notable antioxidant activities observed in the ethanol extract may be attributed to the presence of polyphenolic compounds.²⁰

Conclusion

The study focused on extracting *S. cordifolia* leaves using different solvents, revealing varied impacts on extraction yield, phenol and flavonoid content and antioxidant activity. Significantly, the use of polar solvents in extraction greatly influenced total phenolic contents, total flavonoid content and antioxidant activity ($p < 0.05$). Among the solvents tested, ethanol emerged as the most effective for extracting bioactive compounds from *S. cordifolia*, yielding the highest extraction yield and content of phenolics and flavonoids. The ethanol extracts were notably rich in phenolic and flavonoid compounds, contributing to their observed high antioxidant activity.

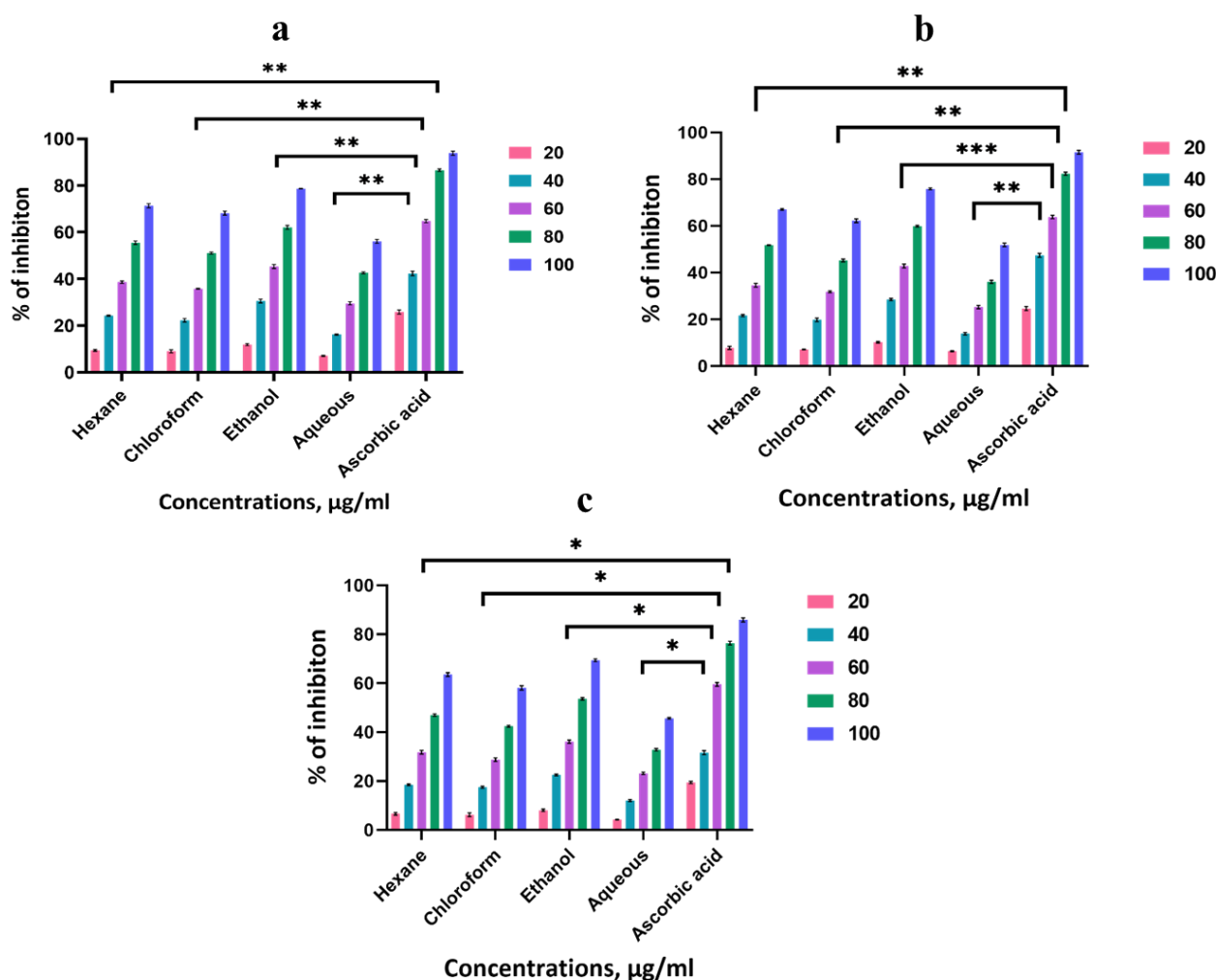


Figure 1: Antioxidant activity of *S. cordifolia* leaf extract (A) DPPH (B) ABTS (C) Hydrogen peroxide. Data are expressed as mean \pm SD (n=3); Significant variations on different leaf extracts were considered * denotes $p < 0.001$, ** denotes $p < 0.01$ and * denotes $p < 0.05$**

Moreover, each solvent extract derived from *S. cordifolia* exhibited significant antioxidant potential, albeit with variations attributed to differences in their phytochemical composition. These findings highlight the potential of *S. cordifolia* leaves as a natural antioxidant source, promising in alleviating oxidative stress and holding significance for the nutraceutical and pharmaceutical industries.

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