

# Phytochemical Screening, *in vitro* Anti-bacterial, and Antioxidant Efficacy of *Solanum virginianum* L. Aerial Vegetative Parts Extracted in Four Solvents

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## ABSTRACT

**Background:** *Solanum virginianum* is a key component of Ayurveda's "Dasmul Asava," used to treat inflammation, respiratory, and gastric problems. Being a repository of countless bioactive compounds, the plant is well known for its traditional medicinal value. Modern pharmacological properties like anti-cancer, anti-diabetic, antioxidant, anti-microbial, and anti-inflammatory have also claimed its traditional uses. **Objectives:** The current research was designed to perform a comparative assessment of *in vitro* anti-bacterial, and antioxidant potential as well as the phytoconstituents make up of plant extracts prepared in four solvents. **Materials and Methods:** Freshly prepared plant extracts were subjected to standard qualitative and quantitative phytochemical screening protocols to identify significant phytochemicals. Different functional groups and chemical entities in plant extracts, were identified using FTIR spectroscopy. Anti-bacterial potential was assessed using disc diffusion and micro broth dilution assay. DPPH decolorization assay was adopted to determine free radical scavenging potential of plant extracts. **Results:** Methanolic plant extract exhibited highest number of total phenols and flavonoids. Plant extracts were also found to be effective against some ampicillin-resistant bacterial strains. Methanolic extract showed highest DPPH scavenging ability comparable to ascorbic acid. **Conclusion:** Although all plant extracts were endowed with good antioxidant and anti-bacterial activity but methanolic extract was found to be more potent free radical scavenger and anti-bacterial agent as it possessed appreciable levels of phenols and flavonoids. The current research could facilitate researchers to identify and isolate new bioactive compounds of medicinal importance and their predicted mode of action against various biological activities.

**Keywords:** Anti-bacterial, Antioxidant, FTIR, Phytochemical, *Solanum virginianum*.

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## INTRODUCTION

Plants assets have a significant impact on mankind and remained an integral component of humanity since time immemorial. The therapeutic value of plants became evident at the very beginning of human existence. There are bountiful confirmations from various sources that the bond between human and their search for drugs in nature dates back to ancient times.<sup>[1,2]</sup> Plants continue to serve a pivotal part in the healthcare sector for a majority of people around the globe, especially in developing nations, where the use of herbal treatment has an extensive history. Due to prohibitive cost and limited approaches to allopathic medicines and their strong believe in traditional medicines; rural people in developing countries are still relying on traditional medicinal system.<sup>[3-5]</sup> As per WHO (World Health

Organization) records, globally 80% people depends on plant based medicines for their primary medical care entails.<sup>[6-8]</sup> The golden veracity in the use of herbal treatments is that it is suitable for people of any age and gender.

Being the repositories of vast array of chemical compounds, plants serve as a substantial source of drugs not only in conventional medical treatments but also have multifaceted applications in modern medicines. Thus, there is urgent need to explore more and more plants to validate their traditional uses. *Solanum virginianum* L. is a perennial herb of solanaceae family; commonly known as 'Kantakari'. It is a key component of Ayurveda's "Dasmul Asava," and is used to treat inflammation, respiratory, and gastric problems. It is widely distributed in several Indian states, including Rajasthan, Gujarat, Uttar Pradesh, West Bengal, and Haryana.<sup>[9]</sup> Various parts of the plant, including leaves, stem, flower, root, and seeds, have traditionally been used to treat human ailments such as asthma, arthritis, hernia, earache, toothache, finger abscess, cough, and fever.<sup>[10-14]</sup> The therapeutic properties of the plant is due to the presence of many



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phytochemicals especially steroidal alkaloids.<sup>[15-17]</sup> Modern pharmacological properties of the plant have also claimed its traditional uses.<sup>[18-21]</sup> In the context of vast medicinal potential of *S. virginianum*, the current research was designed to carry out a comparative assessment of *in vitro* anti-bacterial, and antioxidant potential as well as the phytoconstituents make up of extracts prepared in four solvents. This could facilitate pharmacologists, scientists and researchers for search of new drugs from therapeutic compounds identified from this plant.

## MATERIALS AND METHODS

### Chemicals

Basic chemicals and solvents were procured from HiMedia, CDH, and SRL India and were of analytical quality. DPPH (2, 2-diphenyl-1-picryl-hydrazyl) was obtained from Merck Sigma-Aldrich, India. Ascorbic acid, gallic acid, quercetin, nutrient agar, nutrient broth, sterile discs and antibiotic discs were obtained by HiMedia.

### Collection and extraction of plant material

Aerial vegetative parts of *S. virginianum* were collected from Rohtak, Haryana, India between coordinates 28.9028° N, 76.6016° E. Freshly collected plant was first washed using tap water and then twice with Distilled Water (DW) to remove foreign particles. After drying in the shade for 20-25 days, the plant material was ground into a coarse powder. Plant extracts were prepared in four solvents (1:5 w/v) viz. aqueous (10.2), methanol (5.1), chloroform (4.1), and benzene (0.1) using soxhlet apparatus. Extraction was carried out till the solvent became colorless. Plant extract was passed through Whatman filter paper no. 1 and excess solvent was evaporated with rotary vacuum evaporator (Buchi Type, Gallen). The concentrated crude extract was kept at 4°C for future usage.

### Percentage Yield (PY)

Percentage yield of plant extract was calculated with the MS equation (1) given below:

$$\% \text{ PY} = \frac{\text{MS equation: 1}}{\text{Wt. of powdered plant material (g)}} \times 100$$

### Preliminary phytochemical screening

#### Qualitative analysis

Freshly prepared plant extracts were analyzed using standard phytochemical detection protocols to check the presence of phytochemical constituents viz. alkaloids, saponins, flavonoids, phenols, glycosides, terpenoids, steroids and tannins.<sup>[22-27]</sup>

### Quantitative analysis

#### Total Phenolic Content (TPC)

TPC of the plant extracts was quantified using Folin-Ciocalteu (FC reagent) colorimetric assay.<sup>[28]</sup> 1 mL of plant extract (1 mg/mL) was added to 2.5 mL of freshly made FC reagent (10%) and 2 mL of sodium carbonate (2%). The resulting solution was kept under dark conditions for half an hour and absorbance was taken at 765 nm with UV-vis spectrophotometer. Gallic acid (1mg/mL) was taken as reference. The results obtained were presented as mg GAE/g (milligram of gallic acid equivalent per gram dry extract).

#### Total Flavonoid Content (TFC)

TFC of the plant extracts was quantified using aluminum chloride colorimetric assay.<sup>[28]</sup> 1 mL of plant extract was mixed with 300 µL of aluminum chloride (10%) and potassium acetate (1M) each along with 3 mL of methanol. After half an hour of incubation period, the absorbance of sample was recorded at 417 nm. Quercetin (1mg/mL) was used as reference and results were presented as mg QE/g (milligram of quercetin equivalent per gram dry extract).

#### FTIR Spectroscopy

To identify different functional groups and chemical entities present in plant extracts, FTIR spectrum was recorded using Thermo Scientific™ Nicolet™ iS50 FTIR Spectrometer. A drop of extract was directly put onto the diamond tip of ATR and absorption spectra were documented at the resolution of 4 cm<sup>-1</sup> with wave numbers ranging from 4000-500 cm<sup>-1</sup>.

### Anti-bacterial activity

#### Bacterial strains

Seven bacterial strains viz. *Escherichia coli* (MTCC-41), *Chromobacterium violaceum* (MTCC-2656), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-2453), *Bacillus subtilis* (MTCC- 2057), *Mycobacterium smegmatis* (MTCC-992), and *Staphylococcus aureus* (MTCC-96) acquired from CSIR-IMTECH Microbial Type Culture Collection (MTCC) Chandigarh, India were used to analyse the anti-bacterial potential of plant extracts. Anti-bacterial efficacy of plant extracts was checked by disc diffusion and microbroth dilution assay. Four conc. (concentrations) of plant extract i.e., 100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5 mg/mL were prepared by re-constituting the plant extracts in DMSO.

#### Inoculum preparation

To prepare the inoculum, nutrient broth was dissolved in DW and autoclaved at 121°C for 25 min. A colony of bacteria was picked up and added to the sterilized culture tube having 15 mL of nutrient broth. Culture tubes were kept in shaker cum B.O.D. incubator at 37°C for 16 hr. Bacterial cultures were calibrated

to 0.5 McFarland ( $1.5 \times 10^8$  CFU/mL) and used for further experiments.

### Disc diffusion assay

Anti-bacterial efficacy of plant extracts was assessed using disc diffusion assay.<sup>[29]</sup> In brief, nutrient agar plates were impregnated with sterile discs after being inoculated with 100  $\mu$ L of bacterial inoculum. Discs were loaded with varying conc. of plant extract (100, 50, 25 and 12.5 mg/mL). Ampicillin (0.1 mg/mL) and DMSO were used as positive and negative controls, respectively. Petri plates were kept in B.O.D. incubator for 24 hr at 37°C. ZOI (zone of inhibition) obtained was recorded with HiMedia Antibiotic ZoneScale-C. The experiment was carried out in triplicates and mean of diameter of ZOI (mm) was taken as final value.

### Microbroth dilution assay

Minimum Inhibitory Conc. (MIC) of plant extracts that inhibit the growth of bacterial strains was obtained by micro broth dilution assay through two-fold serial dilutions.<sup>[30]</sup> 100  $\mu$ L of nutrient broth was added to each well of 96-microtiter plate (12x8 size; Tarson) up to twelve wells. 100  $\mu$ L of plant extract was added in first well to each row and serially diluted up to twelve wells. 10  $\mu$ L each of bacterial inoculum ( $1.5 \times 10^8$  CFU/mL) and resazurin dye (0.04% w/v in DW) were added into each well. Petri plates were covered with parafilm to prevent media evaporation and bacterial dehydration and set aside in a B.O.D incubator at 37°C for 24 hr. The conc. of plant extract at which no change in color was observed (blue to pink) was noted as MIC value for a given bacteria.

### Antioxidant activity

#### DPPH assay

Antioxidant capacity of plant extracts was measured using DPPH experiment.<sup>[31]</sup> Briefly, 1 mL of DPPH (0.3 mM DPPH in methanol) was mixed with varying conc. of plant extracts and sample was left to incubate for 30 min. (minutes) in the dark and the absorbance was recorded at 517 nm. Ascorbic acid served as reference compound. DPPH solution refrain from plant extract acts as control. Percentage inhibition of plant extract was calculated with the MS equation (2) given below:

$$\text{MS equation: } 2\% \text{ Inhibition} = \left\{ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{Control}}} * 100 \right\}$$

Here,  $A_{\text{control}}$  is absorbance of control (DPPH + respective solvent);  $A_{\text{sample}}$  is absorbance of sample (DPPH + plant extract/ standard).

#### IC<sub>50</sub> value

IC<sub>50</sub> is the proportion of plant extract needed to scavenge half of the free radicals (DPPH). It was calculated using the slope intercept formula ( $y=mx+b$ ) of a graph plotted between conc. and % inhibition.

### Statistical analysis

Graphical analysis was done using Origin Pro-2021. All the experimentation was done in triplicates to ensure the reproducibility of results and data is presented as mean value  $\pm$  SE and  $p$  value  $<0.05$  was taken as significant statistically. Pearson's correlation coefficient was applied to estimate the interdependence of IC<sub>50</sub> and TPC/TFC.

## RESULTS

*S. virginianum* is traditionally important plant known for its medicinal value and maintaining human health. The current research was aimed to explore the phytochemicals makeup, anti-bacterial and antioxidant potential of its aerial vegetative parts. Extraction was carried out in polar (Methanol, Aqueous) and non-polar (Chloroform, Benzene) solvents and % yield of plant extract was calculated. ME (methanolic extract) showed the highest % yield (23.74%), followed by AqE (Aqueous Extract) (8.3%) and CE (Chloroform Extract) (5.86%). Least % yield was obtained in BE (Benzene Extract) (2.98%) (Supplementary Figure 1).

### Phytochemical screening

Preliminary screening of phytochemicals is crucial to identify the class of phytoconstituents accountable for medicinal properties of the plant. All extracts of *S. virginianum* were assessed for their phytochemical constituents and results obtained are depicted in Table 1. All of the plant extracts contained phenols, flavonoids, tannins and terpenoids. Glycosides were found to be present in all the extracts except AqE. Saponins were found solely in BE whereas steroids were detected in all the extracts except BE.

### Quantitative analysis

#### Total Phenolic and Flavonoid Content

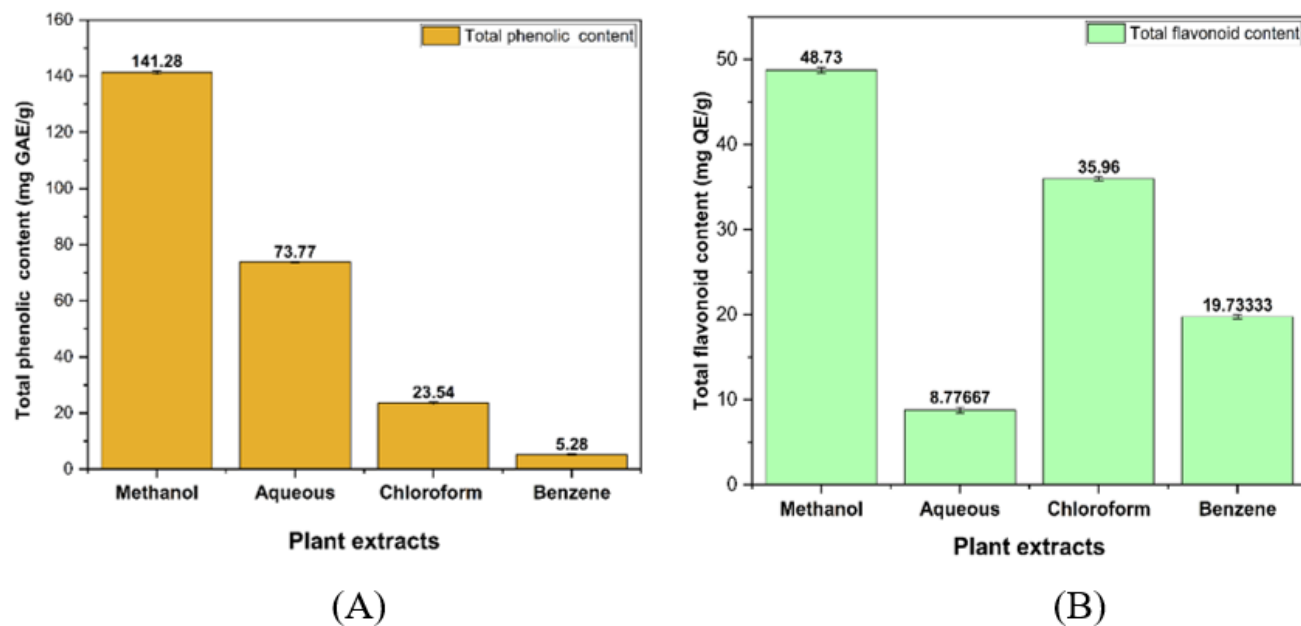
TPC and TFC of plant extracts are presented graphically in (Figure 1). Highest phenolic content was found in ME ( $141.28 \pm 0.66$ ), followed by AqE ( $73.77 \pm 0.24$ ). Least phenolic content was found in BE ( $5.28 \pm 0.20$ ). Similarly, highest TFC was found in ME ( $48.73 \pm 0.34$ ), followed by CE ( $35.96 \pm 0.21$ ). Least TFC was observed in AqE ( $8.77 \pm 0.32$ ).

#### FTIR

FTIR spectrum of all the extracts showed characteristic peaks of alcohols, amines, alkanes, alkynes and aromatic compounds etc. (Figure 2). Different bands and their corresponding functional groups present in plant extracts are presented in Table 2.

### Anti-bacterial activity

Disc diffusion assay: The anti-bacterial efficacy of plant extracts was assessed using disc diffusion assay, and the ZOI (in mm) obtained is shown in Figure 3. ME exhibited highest



**Figure 1:** TPC (A) and TFC (B) of *S. virginianum* extracts. Values are expressed as Mean  $\pm$  SE,  $n=3$ .

**Table 1:** Preliminary phytochemical screening of *S. virginianum* extracts.

Plant extracts	Phenols	Saponins	Flavonoids	Glycosides	Alkaloids	Tannins	Steroids	Terpenoids
AqE	+	ND	+	ND	+	+	+	+
ME	+	ND	+	+	ND	+	+	+
CE	+	ND	+	+	+	+	+	+
BE	+	+	+	+	+	+	ND	+

Note: (+) phytochemical detected; (ND) not detected; ME- Methanolic extract; AqE- Aqueous extract; CE- Chloroform extract; BE- Benzene extract.

anti-bacterial activity against *M. smegmatis* ( $17.8 \pm 0.13$ ) followed by *C. violaceum* ( $16.9 \pm 0.10$ ) and *P. aeruginosa* ( $14.7 \pm 0.15$ ). Least inhibitory activity was observed against *K. pneumoniae* ( $10.5 \pm 0.28$ ). AqE like ME, is more effective against *M. smegmatis* ( $13.8 \pm 0.16$ ) followed by *C. violaceum* ( $13.1 \pm 0.16$ ) and *P. aeruginosa* ( $11.1 \pm 0.16$ ). AqE exhibited least inhibitory activity against *E. coli* ( $8.1 \pm 0.20$ ). ME and AqE showed ZOI even against *C. violaceum*, *M. smegmatis* and *P. aeruginosa* which are found to be resistant to positive control i.e., ampicillin

CE showed highest ZOI against *K. pneumoniae* ( $13.0 \pm 0.26$ ) followed by *C. violaceum* ( $12.5 \pm 0.28$ ) and *B. subtilis* ( $11.4 \pm 0.34$ ). Least inhibitory potential was recorded against *S. aureus* ( $10.4 \pm 0.34$ ). BE is highly active against *P. aeruginosa* ( $12.4 \pm 0.29$ ) and *B. subtilis* ( $11.8 \pm 0.20$ ). BE like, CE also shows least anti-bacterial potential against *S. aureus* ( $9.9 \pm 0.34$ ).

### Microbroth dilution assay

Minimum inhibitory conc. of plant extracts and standard compound was evaluated with 96-well microbroth dilution assay and the results are represented in Table 3. For ME, least

MIC ( $3.125 \text{ mg/mL}$ ) was observed against four bacterial strains i.e. *C. violaceum*, *M. smegmatis*, *P. aeruginosa* and *S. aureus*. For AqE, MIC value obtained was  $12.5 \text{ mg/mL}$  against all the tested bacterial strains. CE and BE exhibited lower MIC ( $3.125 \text{ mg/mL}$ ) for *C. violaceum*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. For ampicillin, least MIC was recorded in case of *B. subtilis* ( $0.00625 \text{ mg/mL}$ ) followed by *S. aureus* ( $0.0125 \text{ mg/mL}$ ) and *E. coli* ( $0.025 \text{ mg/mL}$ ). No inhibitory activity was observed against *C. violaceum* and *P. aeruginosa*, which was in line with the disc diffusion assay outcomes.

### Antioxidant activity

#### DPPH assay

Antioxidant capacity of plant extracts was compared with reference compound i.e., ascorbic acid and results obtained are depicted in Figure 4. The results showed that ME has highest antioxidant activity with % inhibition values ranging from  $22.95 \pm 0.99$  to  $93.66 \pm 0.36\%$  and is comparable to reference compound ( $42.13 \pm 0.61$  to  $94.19 \pm 0.24\%$ ). BE had the lowest antioxidant activity, with % inhibition values ranging from

**Table 2: Bands and functional groups identified in FTIR spectrum of different plant extracts.**

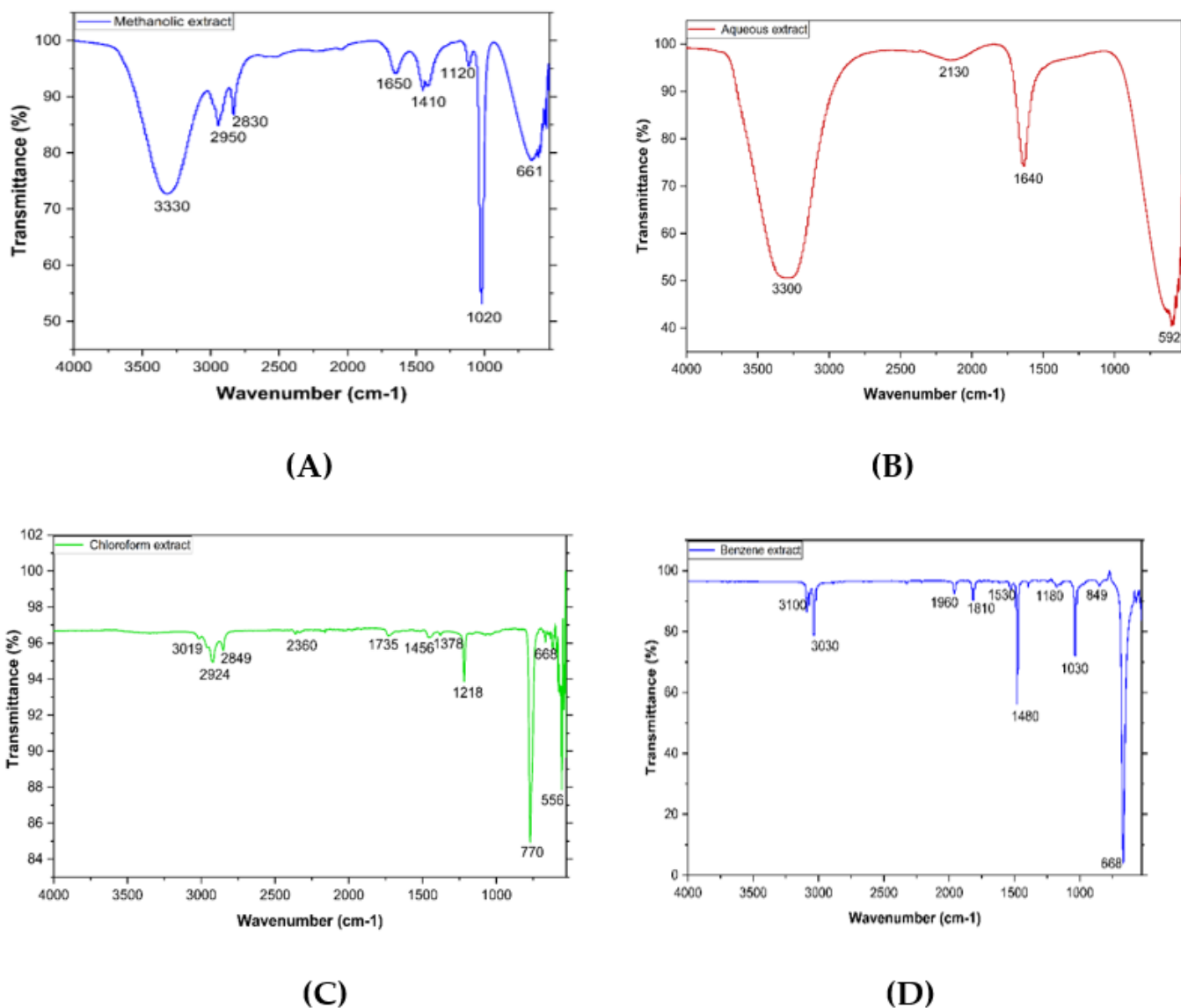
Sl. No.	Absorption bands in ME (cm <sup>-1</sup> )	Functional group	Absorption bands in AqE (cm <sup>-1</sup> )	Functional group	Absorption bands in CE (cm <sup>-1</sup> )	Functional group	Absorption bands in BE (cm <sup>-1</sup> )	Functional group
1	3330	ν(O-H) carboxylic acid	3300	ν(O-H) carboxylic acid	3019	ν(C-H) alkene	3100	ν(C-H) alkene
2	2950	ν(C-H) alkane	2130	ν(C≡C) alkyne	2924	ν(N-H) amine salt	3030	ν(C-H) alkene
3	2830	ν(C-H) aldehyde	1640	ν(C=N) imine/oxime	2849	ν(C-H) alkane	1960	ν(N=C=S) isothiocyanate
4	1650	ν(C=C) conjugated alkene	592	ν(C-Cl) halo compounds	2360	ν(S-H) thiol	1810	ν(C=O) acid halide
5	1410	ν(S=O) sulfonyl chloride			1735	ν(C=O) esters and δ-lactone	1530	ν(N-O) nitro compounds
6	1120	-			1456	ν(N-O) nitro compounds	1480	ν(N-O) nitro compounds
7	1020	ν(C-N) amine			1378	δ(C-H) alkane	1180	-
8	661	ν(C-I) halo compounds			1218	ν(C-O) vinyl ether	1030	ν(S=O) sulfoxide
9					770	δ(C=C) trisubstituted	849	ν(C-Cl) halo compounds
10					668	ν(C-Br) halo compounds	668	ν(C-Br) halo compounds
11					556	ν(C-I) halo compounds		

Note: δ-bending; ν-stretching.

**Table 3: MIC of *S. virginianum* plant extracts and positive control against seven bacterial strains.**

Plant extracts and positive control	Bacterial strains						
	<i>B. subtilis</i>	<i>C. violaceum</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>M. smegmatis</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
	MIC (mg/mL)						
ME	6.25 ± 0.0	3.125 ± 0.0	6.25 ± 0.0	6.25 ± 0.0	3.125 ± 0.0	3.125 ± 0.0	3.125 ± 0.0
AqE	12.5 ± 0.0	12.5 ± 0.0	12.5 ± 0.0	12.5 ± 0.0	12.5 ± 0.0	12.5 ± 0.0	12.5 ± 0.0
CE	6.25 ± 0.0	3.125 ± 0.0	6.25 ± 0.0	3.125 ± 0.0	6.25 ± 0.0	3.125 ± 0.0	3.125 ± 0.0
BE	6.25 ± 0.0	3.125 ± 0.0	6.25 ± 0.0	3.125 ± 0.0	6.25 ± 0.0	3.125 ± 0.0	3.125 ± 0.0
Ampicillin	0.00625 ± 0.0	0.0 ± 0.0	0.025 ± 0.0	0.05 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0125 ± 0.0

Note: MIC- Minimum Inhibitory Conc.; ME- Methanolic extract; AqE- Aqueous extract; CE- Chloroform extract; BE- Benzene extract.



**Figure 2:** FTIR spectrum of *S. virginianum* extracts: Methanolic extract (A); Aqueous extract (B); Chloroform extract (C); Benzene extract (D).

12.35±0.28 to 33.23±0.29%. % inhibition of plant extracts was found to be conc. dependent.

### $IC_{50}$

Half maximal inhibitory conc. of ascorbic acid and plant extracts was calculated using the equation ( $y=mx+b$ ) derived from the calibration graph of conc. versus % inhibition (Figure 4). It was observed that  $IC_{50}$  decreases as antioxidant activity increases and vice versa. ME and BE exhibited lowest ( $50.67\pm 0.82$   $\mu\text{g/mL}$ ) and highest ( $161.81\pm 1.10$   $\mu\text{g/mL}$ )  $IC_{50}$  values respectively.

### Correlation between $IC_{50}$ , TPC and TFC

The correlation among total phenolic/flavonoid content and antioxidant potential ( $IC_{50}$  values) of plant extracts was calculated using Pearson correlation coefficient and is represented in Table 4.

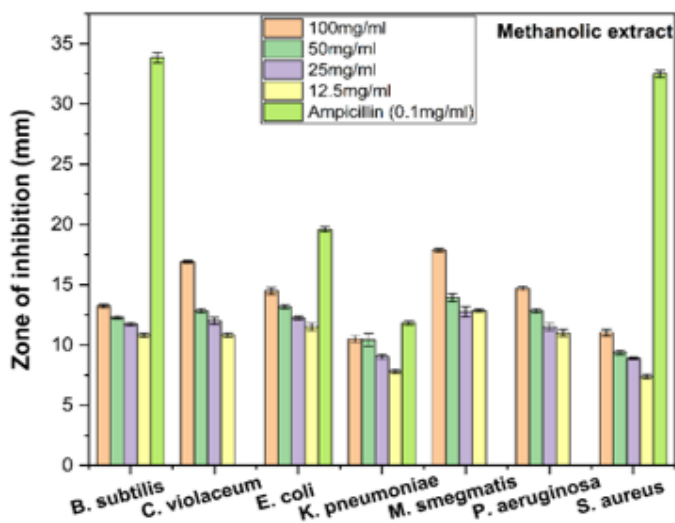
**Table 4:** Correlation between TPC, TFC and  $IC_{50}$ .

Pearson coefficient (r)		
TPC and TFC	TPC and $IC_{50}$	TFC and $IC_{50}$
0.492	-0.913	-0.599

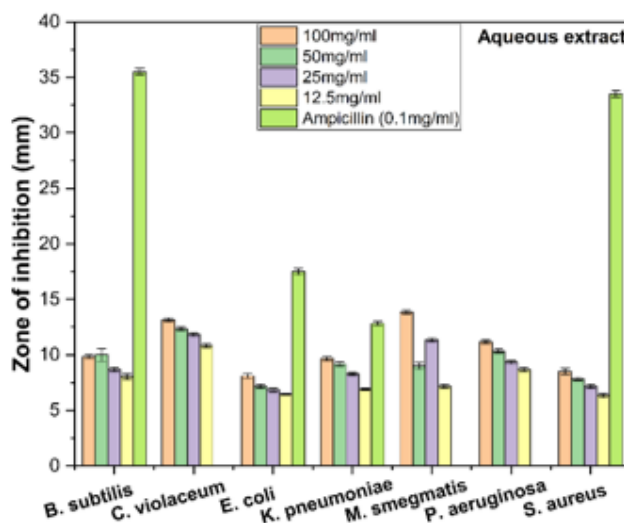
Two variables TPC and TFC were found to be positively correlated and a negative correlation was observed between TPC/TFC and  $IC_{50}$  values of plant extracts. It means that lower that  $IC_{50}$  higher the TPC/TFC and vice-versa.

## DISCUSSION

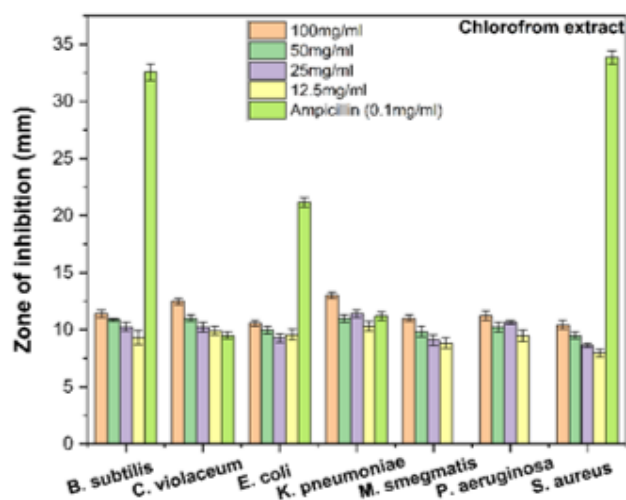
The present investigation was carried out with four extracts (methanol, aqueous, chloroform, benzene) prepared from aerial vegetative parts of *S. virginianum* to understand its medicinal properties. Highest percentage yield was obtained in methanol followed by aqueous, chloroform, and benzene. Preliminary



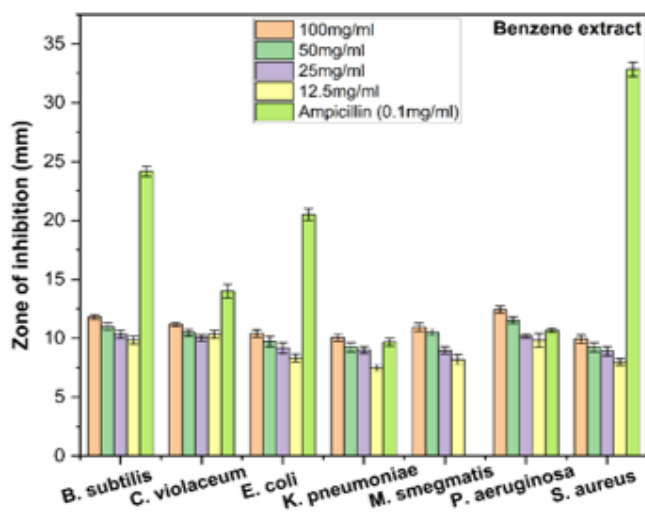
(A)



(B)



(C)

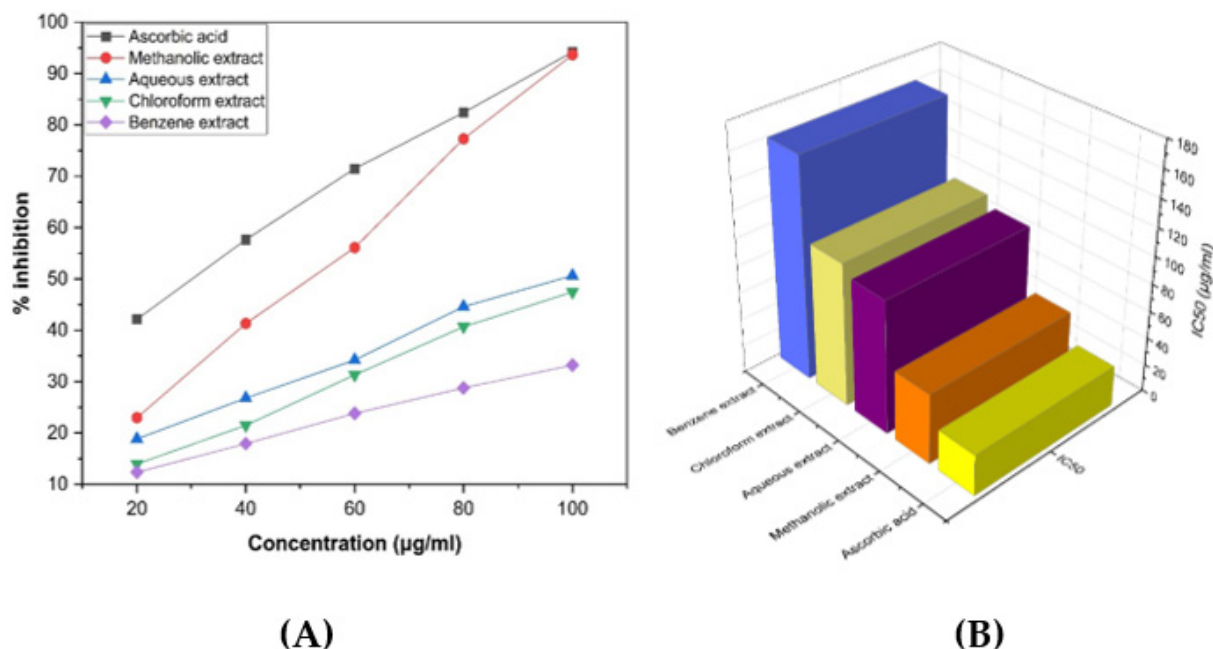


(D)

**Figure 3:** ZOI obtained against seven bacterial strains with *S. virginianum* extracts: Methanolic extract (A); Aqueous extract (B); Chloroform extract (C); Benzene extract (D). Values are expressed as Mean  $\pm$  SE,  $n=3$ .

screening suggests the presence of various botanicals (alkaloids, phenols, flavonoids, tannins, glycosides, terpenoids, saponins and steroids) in all the extracts. Several research groups of the global community reported similar findings.<sup>[15,16,32,33]</sup> The presence of these different phytoconstituents is further supported by FTIR spectroscopy, displaying peaks of chemical entities like O-H stretching of phenols; C-H stretching of aldehyde, alkane and alkene C-Cl/Br stretching of halo compounds, S-H stretching of thiols, N-H and N-O stretching of amine and nitro compounds respectively. These functional groups are likely to be accountable for antioxidant, and anti-microbial potential of the plant. Beside these properties, they are also known for anti-cancer, anti-inflammatory and anti-diabetic activities.<sup>[34-37]</sup>

Quantitative analysis of phytoconstituents revealed that the ME had the highest conc. of total phenols ( $141.28 \pm 0.66$  mg GAE/g) and flavonoids ( $48.73 \pm 0.34$  mg QE/g) among other extracts. This has been confirmed by the FTIR spectrum, which shows a broad band at  $3300\text{ cm}^{-1}$  in ME, indicating the stretching of the O-H functional group. These findings are in support with the percentage yield of extracts, suggesting that polar solvents exhibit better extraction performance and more appropriate for extracting an extensive variety of plant-based constituents.<sup>[38-40]</sup> Since the choice of solvent influences the percentage yield and quantity of phytoconstituents, it also influences the *in vitro* activities of extract.<sup>[41,42]</sup>



**Figure 4:** Comparison of % inhibition (A) and IC<sub>50</sub> values (B) of different extracts of *S. virginianum* with ascorbic acid. Values are expressed as Mean ± SE, n=3.

Disc diffusion assay was used to evaluate *in vitro* anti-bacterial efficacy of extracts against seven bacterial strains. The method is simple, reliable, versatile, affordable and the results are easy to interpret. Although all the tested extracts hindered the growth of bacteria, ME came out as best anti-bacterial agent among others. This is presumably owing to the presence of secondary metabolites like alkaloids, tannins, phenols, and flavonoids etc., which pass through cell wall of microorganism and disturb their ability to survive. Interestingly, plant extracts were found to be effective even against *C. violaceum*, *M. smegmatis*, and *P. aeruginosa*, all of which exhibited resistance to the broad-spectrum antibiotic (ampicillin). It indicates considerable anti-bacterial effectiveness of *S. virginianum*, which could aid in developing new medication especially for antibiotic-resistant strains. Our findings line up with the results of other researchers around the world.<sup>[43-47]</sup>

Free radicals are unstable and extremely reactive substances that trigger cellular damage through oxidative stress and accountable for various kinds of dreadful diseases. Herbal medicine contains biologically active elements that serve as neutralizers of free radicals and aid in treating diseases associated with oxidative stress. Here, DPPH decolorization assay was adopted to determine free radical scavenging capability of plant extracts. ME possessed better antioxidant activities as compared to plant extracts prepared in other solvents. The antioxidant potential of ME was comparable to ascorbic acid which clearly shows the vast medicinal potential the plant. The outcomes are also consistent

with previous reports.<sup>[16,33,48]</sup> The abundance of phenols and flavonoids in ME may contribute to high antioxidant potential of plant.<sup>[49]</sup> These findings support that *S. virginianum* serves as a potent free radical scavenger and anti-bacterial agent, which could be helpful in controlling disorders associated with the production of free radicals and resistance to antibiotics.

## CONCLUSION

The goal of the current research was to look at the phytochemical composition, anti-bacterial and antioxidant potential of *S. virginianum*. Preliminary screening of phytochemicals revealed the presence of alkaloids, phenols, flavonoids, glycosides, tannins, terpenoids, and saponins. Furthermore, FTIR spectrum displays various functional groups which might be responsible for medicinal importance of plant. Quantitative analysis of phytocompounds showed that ME exhibited highest amount of total phenol and flavonoids. Least conc. of total phenols and flavonoids was observed in BE and AqE, respectively. The results demonstrated that ME exhibited highest anti-bacterial activity and antioxidant potential similar to ascorbic acid. The aforementioned results suggest methanol as the best solvent for phytocompounds extraction among the four tested solvents since it provides the highest extraction percentage yield as well as substantial quantity of phenol and flavonoids. Furthermore, studies are required to identify the specific phytochemicals responsible for these activities and their therapeutic mode of action.



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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**µl**: Micro liter; **AqE**: Aqueous extract; **BE**: Benzene extract; **CE**: Chloroform extract; **conc**: Concentration; **ME**: Methanolic extract; **mg/mL**: Milli gram per milli liter; **min**: Minutes; **mm**: Milli meter; **mM**: Milli molar; **TFC**: Total flavonoid content; **TPC**: Total phenolic content; **ZOI**: Zone of inhibition.

## AUTHOR'S CONTRIBUTION

Preety Rohilla: Conceptualization; Methodology; Writing - original draft preparation; Writing - review and editing. Ashmita Chhikara: Organization and editing. Pushpa Dahiya: Supervision.

## SUMMARY

*S. virginianum* is an important medicinal herb of Ayurvedic system of medicine. It is a salient element of herbal formulation 'Dasmul Asava' mainly used to treat respiratory and gastric problems. Thus, the present research focused on phytochemical, anti-bacterial and antioxidant potential of *S. virginianum* extracts prepared in four solvents. The results revealed that methanol is the best solvent for extraction of phytochemicals since highest % yield and maximum concentration of TPC and TFC was observed in ME. Although all the tested extracts have good anti-bacterial and antioxidant efficacy, but ME was best among the tested extracts and found to be a promising free radical scavenger and anti-bacterial agent.

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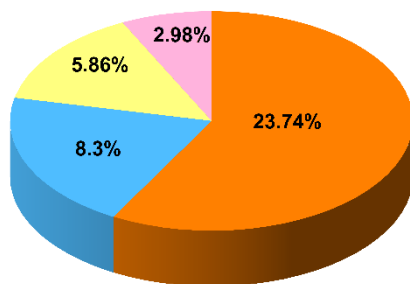
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■ Methanolic extract  
■ Aqueous extract  
■ Chloroform extract  
■ Benzene extract



**Supplementary Figure 1:** % yield of *S. virginianum* extracts in four solvents (at column width)