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# STUDY ON ANTI-BACTERIAL AND ANTIOXIDANT EFFICACY OF *Solanum Virginianum* EXTRACTED SOLVENTS

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## ABSTRACT

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. *Solanum virginianum* is a key component of Ayurveda's "Dasmul Asava," used to treat inflammation, respiratory, and gastric problems. *S. virgininaum* 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. 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. The current research was designed to perform a comparative assessment of *in vitro* anti-bacterial, and antioxidant potential of plant extracts prepared in four solvents.. 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. 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. 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, *Solanum virginianum*.

## INTRODUCTION

As per WHO (World Health Organization) records, globally 80% people depends on plant based medicines for their primary medical care entails.[1-2] The golden veracity in the use of herbal treatments is that it is suitable for people of any age and gender. 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.[3-5] 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.[6-8]

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.[9] 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.[10-14] The therapeutic properties of the plant is due to the presence of many phytocompounds especially steroidal alkaloids.[15-17] Modern pharmacological properties of the plant have also claimed its traditional uses.[18-21] 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 Saharanpur, Uttar Pradesh, India 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:
MS equation: 1

$$\% \text{ PY} = \frac{\text{Wt. of crude plant extract (g)}}{\text{Wt. of powdered plant material (g)}} \times 100$$

## 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 x 108 CFU/mL) and used for further experiments.

## Disc diffusion assay

Anti-bacterial efficacy of plant extracts was assessed using disc diffusion assay.[29] In brief, nutrient agar plates were impregnated with sterile discs after being inoculated with 100 µL 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.[30] 100 µL of nutrient broth was added to each well of 96-microtiter plate (12x8 size; Tarson) up to twelve wells. 100 µL of plant extract was added in first well to each row and serially diluted up to twelve wells. 10 µL each of bacterial inoculum (1.5 × 108 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.[31] 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:

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

Here, Acontrol is absorbance of control (DPPH + respective solvent); Asample is absorbance of sample (DPPH + plant extract/ standard).

### $IC_{50}$ value

IC50 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 ± SE and *p* value <0.05 was taken as significant statistically. Pearson's correlation coefficient was applied to estimate the interdependence of IC50 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 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%)

### 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 1. ME exhibited highest anti-bacterial activity against *M. smegmatis* (17.8 ± 0.13) followed by *C. violaceum* (16.9 ± 0.10) and *P. aeruginosa* (14.7± 0.15). Least inhibitory activity was observed against *K. pneumoniae* (10.5 ± 0.28). AqE like ME, is more effective against *M. smegmatis* (13.8 ± 0.16) followed by *C. violaceum* (13.1 ± 0.16) and *P. aeruginosa* (11.1 ± 0.16). AqE exhibited least inhibitory activity against *E. coli* (8.1 ± 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 ± 0.26) followed by *C. violaceum* (12.5 ± 0.28) and *B. subtilis* (11.4 ± 0.34). Least inhibitory potential was recorded against *S. aureus* (10.4 ± 0.34). BE is highly

active against *P. aeruginosa* (12.4 ± 0.29) and *B. subtilis* (11.8 ± 0.20). BE like, CE also shows least anti-bacterial potential against *S. aureus* (9.9 ± 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 1. For ME, least MIC (3.125 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 mg/mL against all the tested bacterial strains. CE and BE exhibited lower MIC (3.125 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 mg/mL) followed by *S. aureus* (0.0125 mg/mL) and *E. coli* (0.025 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 2. The results showed that ME has highest antioxidant activity with % inhibition values ranging from 22.95±0.99 to 93.66±0.36% and is comparable to reference compound (42.13±0.61 to 94.19±0.24%). BE had the lowest antioxidant activity, with % inhibition values ranging from 12.35±0.28 to 33.23±0.29%. % inhibition of plant extracts was found to be conc. dependent.

**Table 1: MIC of *S. virginianum* plant extracts and positive control against seven bacterial strains.**

| Plant extracts and positive control | Bacterial strains | | | | | | |
|---|---|---|---|---|---|---|---|
| | *B. subtilis* | *C. violaceum* | *E. coli* | *K. pneumoniae* | *M. smegmatis* | *P. aeruginosa* | *S. aureus* |
| | 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 |

*$IC_{50}$*

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 2). It was observed that IC50 decreases as antioxidant activity increases and vice versa. ME and BE exhibited lowest (50.67±0.82 µg/mL) and highest (161.81±1.10 µg/mL) IC50, values respectively.

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

The correlation among total phenolic/flavonoid content and antioxidant potential (IC50 values) of plant extracts was calculated using Pearson correlation coefficient and is represented in Table 2.

**Table 2: Correlation between TPC, TFC and IC50.**

| 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 IC50 values of plant extracts. It means that lower that IC50 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 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.[15,16,32,33] 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.[34-37] Quantitative analysis of phytoconstituents revealed that the ME had the highest conc. of total phenols (141.28 ± 0.66 mg GAE/g) and flavonoids (48.73 ± 0.34 mg QE/g) among other extracts. This has been confirmed by the FTIR spectrum, which shows a broad band at 3300 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.[38-40] Since the choice of solvent influences the percentage yield and quantity of phytoconstituents, it also influences the *in vitro* activities of extract.[41,42]

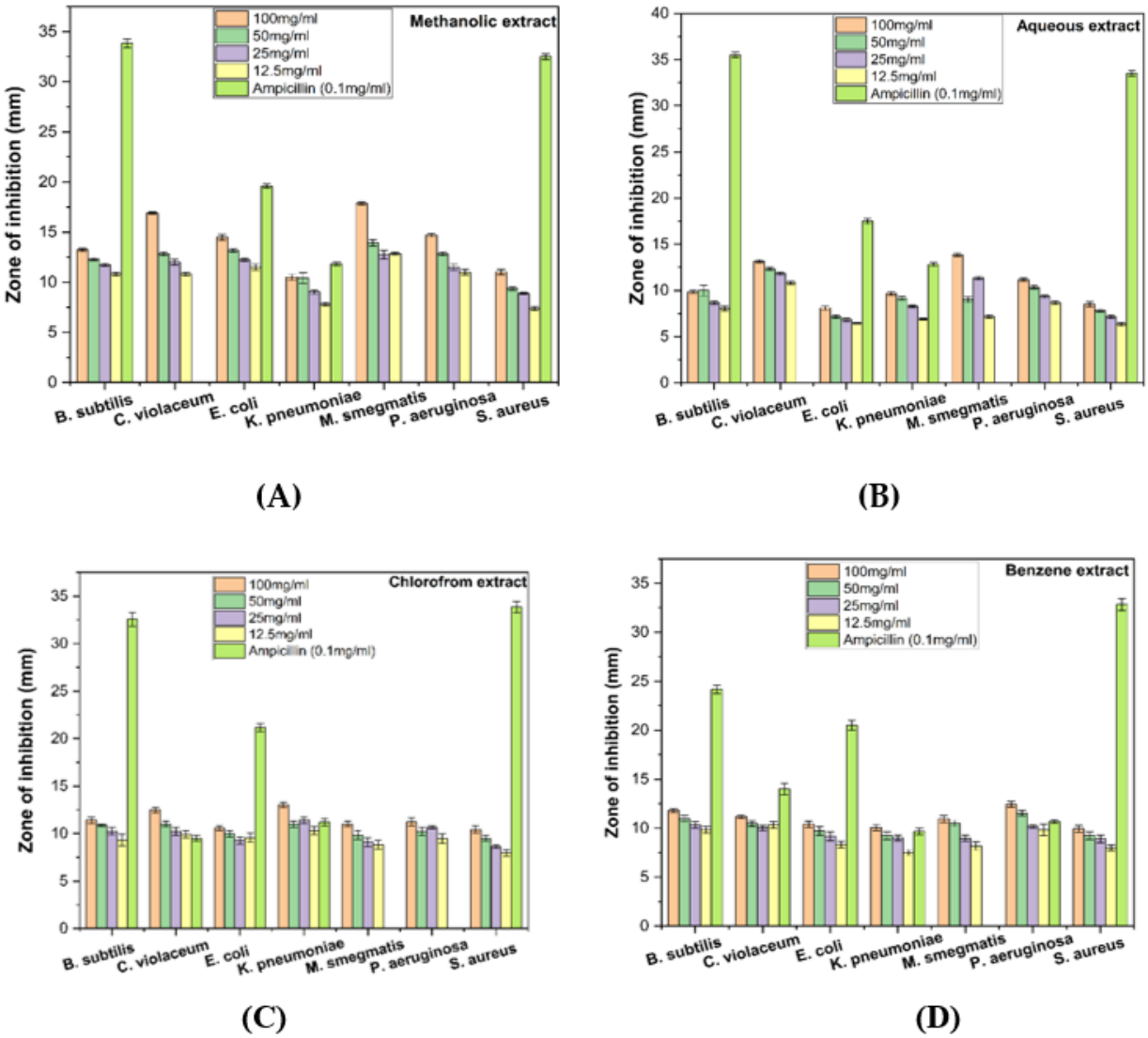


**Figure 1:** ZOI obtained against seven bacterial strains with *S. virginianum* extracts: Methanolic extract (A); Aqueous extract (B); Chloroform extract (C); Benzene extract (D). Values are expressed as Mean ± SE, *n*=3.

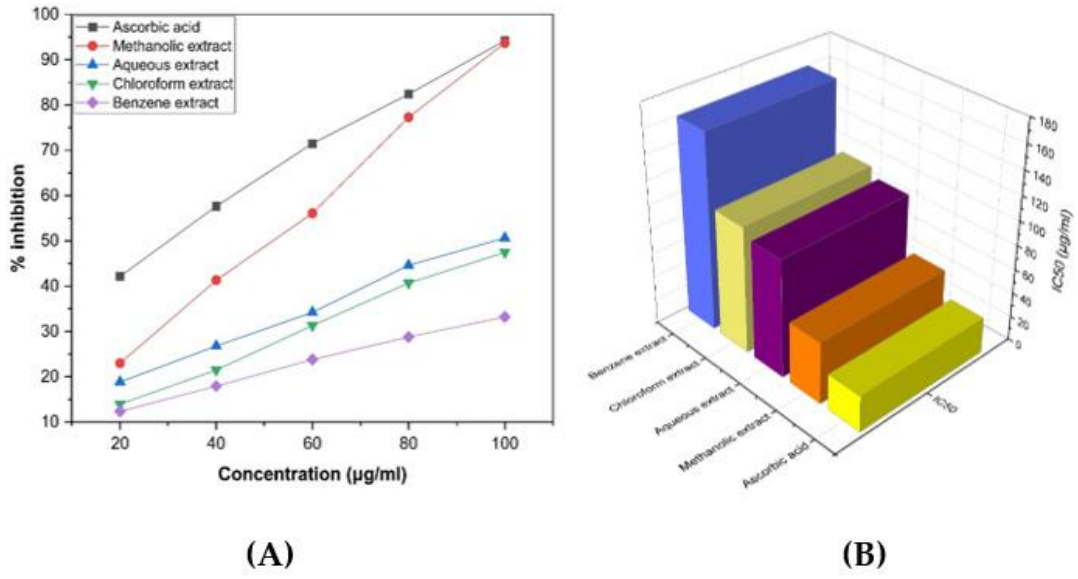


**Figure 2:** Comparison of % inhibition (A) and IC50 values (B) of different extracts of *S. virginianum* with ascorbic acid. Values are expressed as Mean ± SE, *n*=3.

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.[43-47]

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.[16,33,48] The abundance of phenols and flavonoids in ME may contribute to high antioxidant potential of plant.[49] 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*. 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 phytocompounds responsible for these activities and their therapeutic mode of action.

## REFERENCES

1. Jain SP, Verma DM, Singh SC, Singh JS, Kumar S. Flora of Haryana. Lucknow: Central Institute of Medicinal and Aromatic Plants. Lucknow, India; 2000.
2. Mazid, Ta K, Mohammad F. Role of secondary metabolites in defense mechanisms of plants. Rev Artic Biol Med [Internet]. 2011;3(2):232-49.
3. Manase MJ, Mitaine-Offer AC, Pertuit D, Miyamoto T, Tanaka C, Delemasure S, *et al*. *Solanum incanum* and *S. heteracanthum* as sources of biologically active steroid glycosides: confirmation of their synonymy. Fitoterapia. 2012;83(6):1115-9. doi: 10.1 016/j.fitote.2012.04.024, PMID 22579841.
4. Laurila J, Laakso I, Väänänen T, Kuronen P, Huopalahti R, Pehu E. Determination of solanidine- and tomatidine-type glycoalkaloid aglycons by gas chromatography/ mass spectrometry. J Agric Food Chem. 1999;47(7):2738-42. doi: 10.1021/jf981009 b, PMID 10552556.
5. Choi JR, Lee CM, Jung ID, Lee JS, Jeong Il YI, Chang JH, *et al*. Apigenin protects ovalbumin-induced asthma through the regulation of GATA-3 gene. Int Immunopharmacol. 2009;9(7-8):918-24. doi: 10.1016/j.intimp.2009.03.018, PMID 19345747.
6. Heinrich M, Kufer J, Leonti M, Pardo-de-Santayana M. Ethnobotany and ethnopharmacology—interdisciplinary links with the historical sciences. J Ethnopharmacol. 2006;107(2):157-60. doi: 10.1016/j.jep.2006.05.035, PMID 16876349.
7. Tekuri SK, Pasupuleti SK, Konidala KK, Amuru SR, Bassaiahgari P, Pabbaraju N. Phytochemical and pharmacological activities of *Solanum surattense* Burm. J Appl Pharm Sci. 2019;9(3):126-36. f-A [review].
8. Nithya M, Ragavendran C, Natarajan D. Anti-bacterial and free radical scavenging activity of a medicinal plant *Solanum xanthocarpum*. Int J Food Prop. 2018;21(1):313-27. doi: 10.1080/10942912.2017.1409236.
9. Chen Z, Miller AR. Steroidal alkaloids in solanaceous vegetable crops. Hortic Res (Am Soc Hortic Sci). 2010:171-96.
10. Borgato L, Pisani F, Furini A. Plant regeneration from leaf protoplasts of *Solanum virginianum* L. (Solanaceae). Plant Cell Tissue Organ Cult. 2007;88(3):247-52. doi: 10. 1007/s11240-006-9196-x.
11. Petrovska BB. Historical review of medicinal plants' usage. Pharmacogn Rev. 2012;6(11):1-5. doi: 10.4103/0973-7847.95849, PMID 22654398. Available from: / pmc/articles/PMC3358962.
12. Roy MK, Swargiary A. Phytochemical, antioxidant and trace element analysis of *Hypericum japonicum* Thunb. Pharmacogn Res. 2023;15(2):338-46. doi: 10.5530/pre s.15.2.036.

13. Pal SK, Shukla Y. Herbal medicine: current status and the future. Asian Pac J Cancer Prev. 2003;4(4):281-8. PMID 14728584.
14. Pouliot M. Relying on nature's pharmacy in rural Burkina Faso: empirical evidence of the determinants of traditional medicine consumption. Soc Sci Med. 2011;73(10):1498-507. doi: 10.1016/j.socscimed.2011.08.037, PMID 21992735.
15. Aziz MA, Adnan M, Khan AH, Shahat AA, Al-Said MS, Ullah R. Traditional uses of medicinal plants practiced by the indigenous communities at Mohmand Agency, FATA, Pakistan. J Ethnobiol Ethnomed. 2018;14:1-6.
16. Chan K. Some aspects of toxic contaminants in herbal medicines. Chemosphere. 2003;52(9):1361-71. doi: 10.1016/S0045-6535(03)00471-5, PMID 12867165.
17. Muhammad H, Gomes-Carneiro MR, Poça KS, De-Oliveira AC, Afzan A, Sulaiman SA, et al. Evaluation of the genotoxicity of *Orthosiphon stamineus* aqueous extract. J Ethnopharmacol. 2011;133(2):647-53. doi: 10.1016/j.jep.2010.10.055, PMID 21044879.
18. Sen S, Chakraborty R. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: importance, challenges and future. J Tradit Complement Med. 2017;7(2):234-44. doi: 10.1016/j.jtcme.2016.05.006, PMID 28417092.
19. Govindan S, Viswanathan S, Vijayasekaran V, Alagappan R. A pilot study on the clinical efficacy of *Solanum xanthocarpum* and *Solanum trilobatum* in bronchial asthma. J Ethnopharmacol. 1999;66(2):205-10. doi: 10.1016/s0378-8741(98)00160-3 , PMID 10433479.
20. Kumar S, Pandey AK. Medicinal attributes of *Solanum xanthocarpum* fruit consumed by several tribal communities as food: an *in vitro* antioxidant, anticancer and anti-HIV perspective. BMC Complement Altern Med. 2014;14(1):1-8.
21. Javaid U, Javaid S, Ashraf W, Rasool MF, Noman OM, Alqahtani AS, *et al*. Chemical profiling and dose-dependent assessment of fear reducing and memory-enhancing effects of *Solanum virginianum* in rats. Dose Response. 2021;19(1):1559325821998486. doi: 10.1177/1559325821998486, PMID 33746655.
22. Kokate CK, Purohit AP, Gokhale SB. Text book of Pharmacognosy; 2003.
23. Khandalwal KR, Sethi VK. Practical Pharmacognosy techniques and experiments; 2008.
24. Parekh J, Chanda S. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol. 2007;31(1):53-8.
25. Onwukaeme DN, Ikuegbvweha TB, Asonye CC. Evaluation of phytochemical constituents, anti-bacterial activities and effect of exudate of *Pycanthus angolensis* Weld Warb (Myristicaceae) on corneal ulcers in rabbits. Trop J Pharm Res. 2007;6(2):725-30. doi: 10.4314/tjpr.v6i2.14652.
26. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Springer Science+Business Media; 1998.
27. Evans WC. Trease and Evans pharmacognosy. Hartcourt Brace and Company, Asia Pvt; 1997.
28. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Complement Altern Med. 2010;10(1):21. doi: 10.1186/1472-6882-10-21, PMID 20470421.
29. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493-6. doi: 10.1093/aj cp/45.4_ts.493, PMID 5325707.

30. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based anti-bacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* anti-bacterial screening of phytochemicals. Methods. 2007;42(4):321-4. doi: 10.1 016/j.ymeth.2007.01.006, PMID 17560319.
31. Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos dos TC, Coube CS, *et al*. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. 2001;15(2):127-30. doi: 10.1002/ptr.687, PMID 11268111.
32. Chhajed M, Jain A, Gupta S, Dubey I, Shrivastava AK. Phytochemical investigation and pharmacological evaluation of *Solanum xanthocarpum* Endowed with their m.2018.61005.
33. Saraswathi K, Bharkavi R, Khusro A, Sivaraj C, Arumugam P, Alghamdi S, *et al*. Assessment on *in vitro* medicinal properties and chemical composition analysis of *Solanum virginianum* dried fruits. Arab J Chem. 2021;14(12):103442. doi: 10.1016/j. arabjc.2021.103442.
34. Dhivya K, Kalaichelvi K. Screening of phytoconstituents, UV-vis Spectrum and FTIR analysis of *Micrococca mercurialis* (L.) Benth. Int J Herb Med. 2017;5(6):40-4.
35. Hameed LA, Shah SQ, Pirzado AA, Soomro QA, Talpur RA, Sathio R. FTIR Spectrum, phytochemical assessment and biological properties of *Solanum surrattense*. Ann Rom Soc Cell Biol. 2022;26(01):183-95.
36. Ijarotimi OS, Adeoti OA, Ariyo O. Comparative study on nutrient composition, phytochemical, and functional characteristics of raw, germinated, and fermented *Moringa oleifera* seed flour. Food Sci Nutr. 2013;1(6):452-63. doi: 10.1002/fsn3.70, PMID 24804056.
37. Rohilla P, Jain H, Chhikara A, Singh L, Dahiya P. South African Journal of Botany Anticancer potential of Solanaceae plants: a review. S Afr J Bot. 2022;149:269-89. doi: 10.1016/j.sajb.2022.04.032.
38. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, *et al*. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal. 2014;22(3):296-302. doi: 10.1016/j.jfda.2013.11.001, PMID 28911418.
39. Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* anti-inflammatory activities of *Severinia buxifolia*. J Food Qual. 2019;2019:1-9. doi: 1 0.1155/2019/8178294.
40. Dasari NP, Ganga Rao B, Sambasiva Rao E, Mallikarjuna Rao T, Praneeth D VS. Quantification of phytochemical constituents and *in vitro* antioxidant activity of *Synadium grantii*. Free Radic Antioxid. 2012;2(2):68-72. doi: 10.5530/ax.2012.2.2.12.