

***In vitro* evaluation of phytochemical and antibacterial activity of wild species of *Solanum* L.**

Pooja Singh, Prashant Singh*, Anjana Singh and M. P. Singh

Department of Botany, Udai Pratap (Autonomous) College, Varanasi – 221002

Mahatma Gandhi Kashi Vidhyapith University, Varanasi - 221001

Corresponding Author: Prashant Singh

Abstract: The potential antimicrobial activity of medicinal plants need identification and screening due to increasing use of synthetic drugs, side effects and antibiotic resistance of pathogenic microorganisms. In-vitro, antimicrobial activity of methanolic extracts of *Solanum nigrum*, *S.villosum*, *S.torvum*, *S.surratense*, *S.sysimbrifolium*, *S.diphyllum* and *S.incanum* against two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and three Gram negative bacteria (*E.coli*, *Salmonella typhi* and *Proteus vulgaris*) have been screened and evaluated phytochemically in this study. The zone of inhibition was determined by agar disc diffusion method varied with plant to plant extract and the organism tested. It reveals that methanolic extract of *S.sysimbrifolium* at 10gm/100ml conc.is effective to all the bacterium, while *S. aureus* with inhibition zone of 23.66±0.72 mm is most susceptible to all extracts. The above results suggested that antimicrobial activities were specific against the growth of bacteria. Moreover, medicinal plant species lead to the development of new drugs, if fully explored phytochemically. Phytochemical screening revealed the presence of carbohydrates, proteins, alkaloids, phenols, flavonoids, glycosides, saponins, tannins and steroids as a major compound in the plants.

Keyword: Wild *Solanum*, Phytochemical screening, Antimicrobial activity, Inhibition zone.

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I. Introduction

The resistance of microbes is a major global health problem and approximately half of all deaths occur in tropics. In this regard plant species play significant roles in the nation's populations health care. *Solanums* are potent and effective in pharmaceuticals because they are rich in alkaloids (Solasodine and other steroids) extracted from the roots and leaves of some species are potent and effective pharmaceuticals (Jennifer and James, 1997).The lack of immediate known use leads to their neglect and subsequent genetic erosion. Depletion of such potentially useful plant resources should be taken care of by paying attention to germplasm exploration and conservation studies due to their medicinal properties. Solanaceous plants are generally annuals, perennial herbs or undershrubs taken in the present study.

***S.diphyllum* L.** is a perennial shrub, up to 2 m tall, branches erect, and younger slightly angled from decurrent leaf bases, older cylindrical leaves in unequal pairs. *S. diphyllum* L. was suggested as a new record by Singh and Singh (2015) from eastern Uttar Pradesh. The genus has an important economic value as sources of edible vegetables and fruits (Muthoni *et al.*, 2012), in addition to several medicinal ones (Pereira *et al.*, 2014 and Rajathi *et al.*, 2015). The plant showed promising toxicity against colon and breast carcinoma cell lines, and could be considered as a potent source of anticancer molecules.

***S.villosum* Miller** is annual or short-lived perennial herb up to 50-60 cm tall, much branched, unarmed; stem rounded to angled, almost glabrous to pubescent with appressed hairs, brittle-stemmed weed. It is often used as an ointment for abscesses, sores and in a douche for leucorrhoea, eczema, nappy rash, wound and cold sore.

***S.nigrum* L.** is a common black nightshade herb found in many disturbed habitats. *S.nigrum* is an important ingredient in traditional Indian medicines. Infusions are used in dysentery, stomach complaints and fever. The plant juice is used in ulcers and skin diseases. The fruits are used as a tonic, laxative, appetite stimulant; and also for treating asthma and "excessive thirst" (Kaushik *et al.*, 2009). Traditionally the plant was used to cure tuberculosis (Jian *et. al.*, 2008).

***S.incanum* L.** is a wooded shrub up to 1.8 m in height with spines on the stem, stalks and calyces and with velvet hairs on the leaves. Throughout tropical Africa a sore throat, angina, stomach-ache, colic, headache, painful menstruation, liver pain and pain caused by onchocerciasis, pleurisy, pneumonia and rheumatism are treated with *S. incanum*.

S.sisimbrifolium Lamk. is sticky nightshade is a densely prickly perennial herb, native to South Africa. It has naturalized in parts of India. Only the fruits are edible and a source of Solasodine. Solasodine is a glycoalkaloid used in the amalgamation of corticosteroids and sex hormones. The mixture is also used in oral contraceptives.

S.surattense Burm. a prickly, diffuse herb. Leaves ovate or elliptic sinuate or sub-pinnatifid glabrescent, with many straight spines. Whole plant used in bronchitis, cough, constipation and in dropsy, decoction used in gonorrhoea and promotes conception.

S.torvum Sw. is a bushy, erect and spiny perennial plant. The fruits of *S. torvum* are edible; decoction of fruits is given for cough ailments and is considered useful in cases of liver and spleen enlargement. The plant is sedative and diuretic and the leaves are used as a haemostatic. The ripened fruits are used in the preparation of tonic and haemopoietic agents and also for the treatment for pain (Kala, 2005). It has antioxidant properties (Sivapriya and Srinivas, 2007). It is intensively used worldwide in the traditional medicine as poison anti-dote and for the treatment of fever, wounds, tooth decay, reproductive problems and arterial hypertension (Ndebia *et al.*, 2007).

II. Material and Methods

Collection and identification of plant material

In the present study mature leaves of *S.nigrum*, *S.vellosum*, *S.torvum*, *S.surattense*, *S.sysimbrifolium*, *S.diphyllum* and *S.incanum* collected from wild. The leaves were initially rinsed with distilled water and dried on paper towels in the laboratory at $(37\pm 1)^{\circ}\text{C}$ for 24 h. The plant specimens were identified with the help of different floras (Dastur, 1970; Jaeger, 1985; Khanna *et al.*, 1999; Mabberley, 2008; Mishra & Verma, 1992; Raizada, 1976; Saini *et al.*, 2010; Hooker, 1872-96; Duthie, 1903-1920). The plant species are preserved in the herbarium of Botany Department of Udai Pratap (Autonomous) College, Varanasi.

Preparation and presentation of plant extract

Preparation of aqueous extract:

Samples of mature leaves of different Solanums viz. *S.nigrum*, *S.villosum*, *S.torvum*, *S.surattense*, *S.sysimbrifolium*, *S.diphyllum* and *S.incanum* was weighed out (10g) and soaked separately in 100 ml of cold water stored in stoppered conical flasks with rubber cork and left for 24hrs. Filtered off using sterile filter papers (whatmann no.1) and subjected to water bath evaporation at boiling temperature of 100°C . The standard extracts were stored in refrigerator.

Preparations of methanol, acetone and n-Hexane extracts:

After drying, the plant materials were ground separately with the help of pestil and mortar in shade. The powdered plant materials (10g) was mixed in methanol, acetone and n-hexane fluid (100 ml) and mixtures were kept in tightly sealed vessels at room temperature for 24 hrs. stirred thoroughly with sterile glass rods several times a day and then the mixtures were filtered thoroughly whatman no.1 filter paper. Residues were adjusted to the required concentration (100mL methanol, acetone and n-Hexane for the residue of 10g of powdered plant material) with the extraction fluid for further extraction. This was repeated thrice, and a colorless supernatant extraction liquid was finally obtained.

Phytochemical analysis

Preliminary phytochemical screening of aqueous and methanolic extracts of seven wild *Solanum* species was done (Kumar *et.al.*, 2006).

Antibacterial assay

The antibacterial activity assay was performed by agar disc diffusion method (Raja *et.al.*, 2011). The suspension was inoculated into petriplates (90mm in diameter) with a sterile nontoxic cotton swab on a wooden applicator. The impregnated discs containing the test sample (100 $\mu\text{g/ml}$) were placed on the agar medium with tested microorganisms. Standard antibiotic discs (Gentamycin 10 $\mu\text{g/disc}$, Neomycin 10 $\mu\text{g/disc}$) and blank discs (impregnated with solvent and water) were used as positive and negative control. The plates were then incubated at 37°C for 24 hrs to allow maximum growth of the microorganisms (Mahindra and Kateryna, 2013). The antibacterial activity of the test samples was determined by measuring the diameter of zone of inhibition expressed in millimeter. The assay was repeated twice and mean of the three experiments was recorded.

Dilution method for minimum inhibitory concentration (MIC):

Out of seven plates tested, the one showed antibacterial activity against the selected pathogens were selected for further tests to calculate their MIC by dilution method. The tests were performed in agar disc diffusion method, the cultures were diluted in Muller-Hinton broth, density adjusted to 0.5 McFarland turbidity.

The final inoculum was 5×10^5 CFU/mL of bacterial colony. Controls with 0.5 mL of culture medium only with plant extract were used in the tests. The 30 μ L of the plant extracts were added to the disc by serial two-fold dilution from the suspension of plant extract stock solution. Each plate was inoculated with 100 μ L of 0.5 McFarland standard bacterial suspensions. The plates were covered, and placed in plastic bags and incubated at 37°C for 24 hrs. In this study, the MIC was the lowest concentration of plant extracts that exhibited the growth of organisms by visual reading.

Statistical analysis

Since the readings of control (distilled water) experiments in the *in vitro* antibacterial studies of the plants were zero, the data were analyzed by simple arithmetic means of the different extracts, and the standard errors were compared with the control.

III. Results and Discussion

Phytochemicals are non-nutritive substances of plants that have disease preventive properties. The phytochemical analysis of seven wild *Solanum* species revealed the presence of alkaloids, carbohydrate, glycoside, phenols, saponin, tannins and protein in different extracts (methanol, acetone, n-hexane and water) (Table 1). The antibacterial activities of the leaf extract of *S.nigrum*, *S.villosum*, *S.torvum*, *S.surratense*, *S.sysimbrifolium*, *S.diphyllum* and *S.incanum* in different solvents (aqueous, methanol, acetone and n-hexane) against *S.aureus*, *B.subtilis*, *E.coli*, *S.typhi*, and *Proteus vulgaris* were shown in table 2. In table 2, methanolic, acetone and n-hexane extract of *S.sysimbrifolium* showed the significant antibacterial property against four pathogens viz., *S.aureus*, *B.subtilis*, *P.vulgaris* and *S.typhi* and least significant against *E.coli*. Out of all the 4 solvent used methanolic extract of all seven *Solanum* species showed maximum antibacterial activity against *S.sysimbrifolium* while the methanolic extract of *S.incanum* show comparatively low degree of antibacterial activity. The inhibition zone were observed 16.33 ± 0.72 *S.aureus*, 15.0 ± 0.47 *B.subtilis*, 16.33 ± 0.72 *P.vulgaris*, 10.0 ± 0.94 *S.typhi*, and minimum *E.coli* 7.33 ± 0.72 in 1,2,3,4,5 pathogens respectively (Fig. 1).

All the bacterial strains were found sensitive to aqueous, methanol, acetone and n-hexane extracts. But, it is evident from the present study that the organic extracts were comparatively more effective than other plants. MIC value of the tested plant extracts against the tested micro-organisms was shown in table 2.

The antibacterial compounds extracted from the plants inhibit bacteria and have therapeutic values as antibacterial agents, due to the presence of different phytochemicals in different solvents. Traditional healers used only water for the extraction but studies have proven the importance of the type of extracting solvent in determining the pharmacological activity of a medicinal plant. All types of extracts showed varied antibacterial efficacies against all the reference bacteria. Eloff (1998) reported that methanol was the most effective solvent for plant extraction than n-hexane, acetone and water. Aqueous extracts showed less activity than n-hexane, acetone and methanol extracts possibly because of the presence of similar active substances were soluble in organic solvents and, therefore, not present in water extracts as suggest by Sharma *et.al.* (2011).

The presence of antimicrobial activity due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavanoids, steroids, saponins etc. (Balandrin and Klocke, 1988). A number of plants have been reported for antimicrobial properties (Olowosulu and Ibrahim, 2006). Antibiotic substances in plant are inhibitory to gram-positive than gram-negative type. The lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer wall of gram-negative bacteria. The outer lipopolysaccharide layer hinders access of most compounds to the peptidoglycon layer of the cell wall. This explains the resistance of gram negative strains to the lytic action of most extracts (Kumar *et. al.*, 2006; Raja *et. al.*, 2011).

The MIC of crude extracts of individual plants varies against different test strains. The relationship between inhibition zone and MIC value may or may not be related. The crude extracts have mixture of phyto-constituents, which may influence the diffusion power of the active constituents. The similar observations by using essential oils or complex mixture from higher plants.

The test strains have different levels of intrinsic tolerance to anti-microbes and thus MIC values differ from isolate to isolate. However, in such cases, the potency of crude extract on the basis of mean MIC values is helpful in defining the relative potency of the extracts. It suggests that these plants be used to discover natural bioactive products that lead to the development of new drugs. The present study has proven that phytochemical compounds can even disrupt the activities of multiple drug resistant (MDR) microbes by influencing various parameters such as efflux pumps, beta-lactamase enzymes, resistance plasmids and bacterial gene transposition (Mahindra and Kateryna, 2006).

IV. Conclusion

Present study suggests the use of these plants in the traditional medicinal systems for the treatment of various infections. From the present study it is concluded that the crude extracts of the plant possess considerable antimicrobial activity, especially against bacterial strains (Gram positive *S. aureus* and *B. subtilis* and Gram negative *E. coli*, *Salmonella typhi* and *Proteus vulgaris*). The plant extract contains potent antimicrobial compounds, effective in the treatment of various bacterial infections. However, further investigation is needed in this direction of pure compound isolation, toxicological studies and clinical trials using the promising compound(s) as an effective anti-microbial agents.

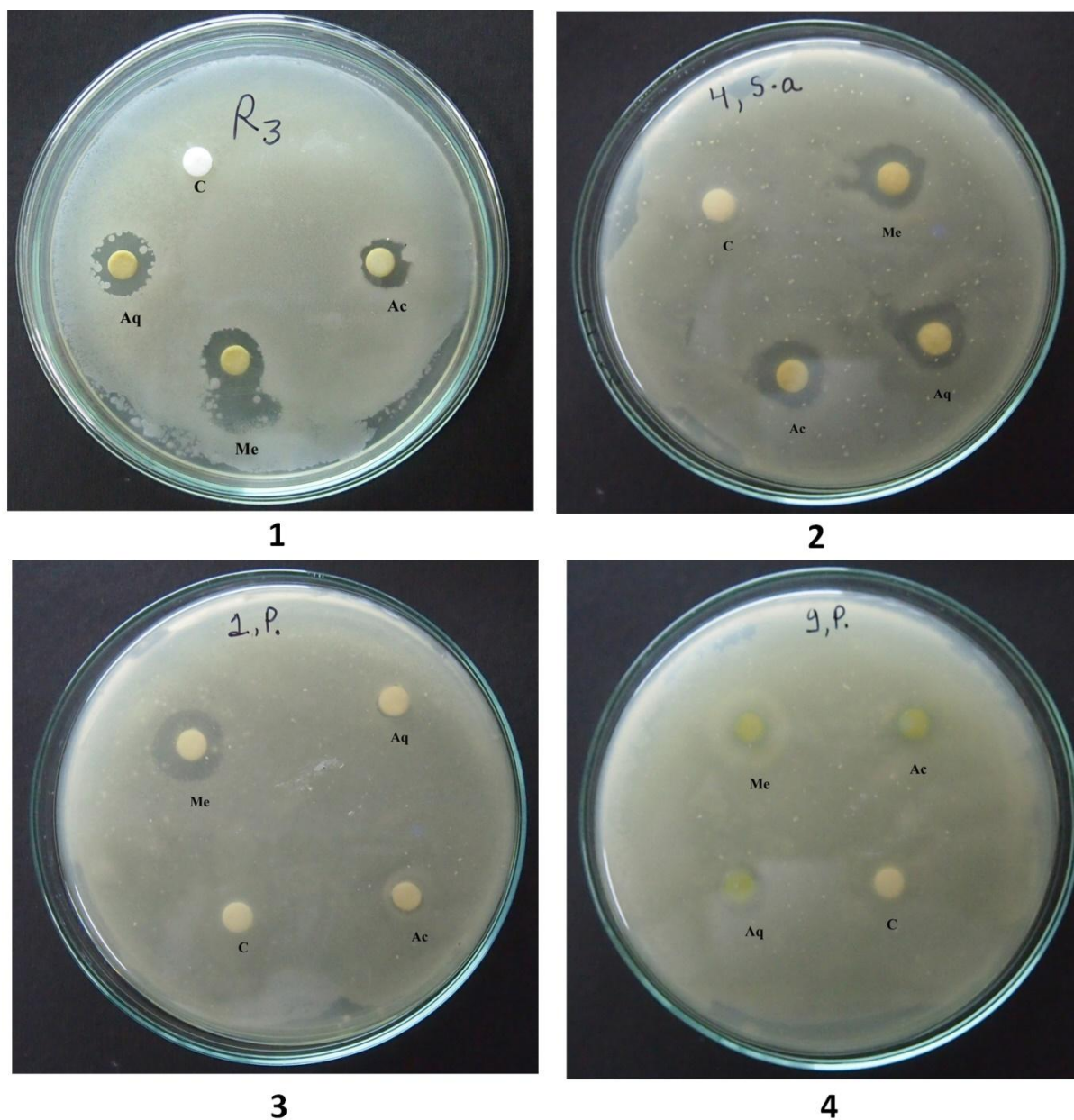


Fig.1. Plates showing the antibacterial activity on different solvents viz. C. Control; Aq. Aqueous; Me. Methanol; Ac. Acetone. 1 *S.sisymbriifolium* against *S.aureus*; 2 *S.incanum* against *S.aureus*; 3 *S.sisymbriifolium* against *E.coli* 4 *S.incanum* against *E.coli*.

Table .1 Phytochemical screening of seven wild *Solanum*L. species

Solvent: Distilled Water								
Plant Name	Alkaloids	Carbohydrate	Glycoside	Phenols	Saponin	Flavonoids	Tannins	Protein
<i>S. diphyllum</i>	++	+	+	-	+	+	-	-
<i>S. incanum</i>	-	-	-	-	-	-	++	-
<i>S. nigrum</i>	+++	+	+	+	+	+	+	+
<i>S. sisymbriifolium</i>	+	+	+	-	+	+	++	+
<i>S. surrantense</i>	++	++	+	-	+	++	+	+
<i>S. torvum</i>	+	+	+	-	+	+	-	-
<i>S. villosum</i>	+++	++	+	+	++	+++	+	+
Solvent: Methanol								
<i>S. diphyllum</i>	++	+	-	-	+	+	+	+
<i>S. incanum</i>	-	-	-	++	-	-	-	-
<i>S. nigrum</i>	++	+	++	-	+++	+++	+	++
<i>S. sisymbriifolium</i>	++	+	-	-	+	+	-	+
<i>S. surrantense</i>	++	++	+	+	+	++	+	+
<i>S. torvum</i>	+	+	-	-	+	+	-	+
<i>S. villosum</i>	+++	++	+	-	++	+++	+	+
Solvent: Acetone								
<i>S. diphyllum</i>	++	+	+	-	+	+	-	-
<i>S. incanum</i>	+	-	-	-	-	-	+	-
<i>S. nigrum</i>	--	++	--	-	+	++	-	--
<i>S. sisymbriifolium</i>	++	+++	+	-	++	-	-	+
<i>S. surrantense</i>	-	-	-	+	+	+	-	+
<i>S. torvum</i>	-	+	-	-	-	++	-	+
<i>S. villosum</i>	++	+	+	++	-	++	+	++
Solvent: n-hexane								
<i>S. diphyllum</i>	++	+	-	-	+	++	-	+
<i>S. incanum</i>	++	-	++	-	+	++	+	+
<i>S. nigrum</i>	+	+	++	-	+++	+	+	++
<i>S. sisymbriifolium</i>	-	-	-	-	-	-	-	-
<i>S. surrantense</i>	-	-	-	+	+	+	-	+
<i>S. torvum</i>	++	+	+	+	+	+	--	+
<i>S. villosum</i>	+++	+	+	++	-	++	+	++

Table 2. Antibacterial activities of specific concentrations (30mg/disc) of aqueous, methanol, acetone and n-hexane extracted of seven wild *Solanum* L. species compared with control distilled water and slandered antibiotics (Gentamycin and Ampicillin -10 µg/disc) (mean±SE)

Plant name	Extraction solvent	Antibacterial activity				
		<i>S. aureus</i>	<i>B. subtilis</i>	<i>E.coli</i>	<i>S.typhi</i>	<i>P.vulgaris</i>
<i>S. diphyllum</i>	Aqueous	13.33 ± 0.72	8.33 ± 0.72	3.33 ± 0.72	6.33 ± 0.27	11.0 ± 0.47
	Methanol	19.0 ± 0.47	16.33 ± 0.72	8.33 ± 0.72	13.33 ± 0.98	13.33 ± 0.72
	Acetone	16.66 ± 0.98	13.0 ± 0.47	5.66 ± 0.98	10.0 ± 0.94	11.66 ± 0.98
	n-hexane	15.0 ± 0.47	11.66 ± 0.72	5.0 ± 0.94	6.66 ± 0.72	9.0 ± 0.47
<i>S. villosum</i>	Aqueous	9.0 ± 0.47	7.66 ± 0.27	11.66 ± 1.36	3.66 ± 0.72	3.66 ± 0.54
	Methanol	17.0 ± 0.81	14.0 ± 0.47	14.66 ± 0.27	6.0 ± 0.47	9.0 ± 0.47
	Acetone	11.0 ± 0.81	13.0 ± 0.47	12.66 ± 0.72	5.0 ± 0.47	6.66 ± 0.54
	n-hexane	10.66 ± 0.72	6.66 ± 0.72	9.33 ± 1.08	3.33 ± 0.72	3.66 ± 0.54
<i>S. nigrum</i>	Aqueous	8.66 ± 0.72	9.0 ± 0.81	6.0 ± 0.47	9.0 ± 0.47	3.0 ± 0.47
	Methanol	17.0 ± 0.47	15.0 ± 0.47	8.0 ± 0.94	16.0 ± 0.47	6.67 ± 0.27
	Acetone	14.66 ± 0.27	12.33 ± 0.98	7.0 ± 0.47	12.0 ± 0.94	5.33 ± 0.54
	n-hexane	10.66 ± 0.54	10.66 ± 1.08	4.0 ± 0.47	10.66 ± 0.54	3.0 ± 0.47
<i>S. incanum</i>	Aqueous	8.66 ± 0.54	10.33 ± 0.72	4.66 ± 0.98	5.33 ± 0.54	10.0 ± 0.94
	Methanol	16.33 ± 0.72	15.0 ± 0.47	7.33 ± 0.27	10.0 ± 0.94	16.33 ± 0.72
	Acetone	16.0 ± 0.47	1.66 ± 0.98	9.0 ± 0.47	9.66 ± 0.72	14.0 ± 0.94
	n-hexane	14.0 ± 0	11.0 ± 0.47	4.33 ± 0.72	6.0 ± 0.47	9.33 ± 0.98
<i>S. sisymbriifolium</i>	Aqueous	17.0 ± 0.46	16.66 ± 0.72	4.33 ± 0.98	8.66 ± 0.54	15.0 ± 0.47
	Methanol	23.66 ± 0.72	22.33 ± 0.98	7.33 ± 1.18	17.0 ± 0.47	21.0 ± 0.94
	Acetone	22.0 ± 0.47	21.0 ± 0.47	5.33 ± 0.98	15.33 ± 0.27	19.67 ± 0.72
	n-hexane	19.66 ± 0.27	17.66 ± 0.27	5.66 ± 0.54	11.0 ± 0.47	12.33 ± 1.18
<i>S. surrantense</i>	Aqueous	11.66 ± 0.98	10.0 ± 0.47	8.66 ± 0.72	10.0 ± 0.94	4.0 ± 0.47
	Methanol	17.0 ± 0.47	17.66 ± 1.18	11.0 ± 1.24	16.33 ± 0.98	8.0 ± 0.94
	Acetone	14.33 ± 0.98	14.33 ± 0.72	9.0 ± 0.47	15.66 ± 0.72	6.66 ± 0.72
	n-hexane	13.0 ± 0.94	13.66 ± 0.98	6.66 ± 0.72	14.0 ± 0.94	4.33 ± 0.72
<i>S. torvum</i>	Aqueous	7.66 ± 0.98	9.33 ± 0.54	4.33 ± 0.98	3.66 ± 0.27	3.0 ± 0.47
	Methanol	16.66 ± 0.72	13.0 ± 1.24	6.66 ± 0.72	7.66 ± 0.27	7.66 ± 0.98
	Acetone	13.0 ± 0.47	10.66 ± 0.72	5.33 ± 0.98	5.0 ± 0.47	6.0 ± 0.47
	n-hexane	8.66 ± 0.98	6.33 ± 0.72	3.33 ± 0.72	3.0 ± 0.47	5.0 ±
Control (D W)		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

- [24]. Rajathi, M., Anandan, R., Sindhu, R. and Logeshwari, M.N. 2015. Screening of *Solanum nigrum* for its phytochemical and antimicrobial activity against respiratory tract pathogen. *Int. J. Pure Appl. Zool.* 3 (3): 210–215.
- [25]. Saini, D.C., Singh, S.K. and Rai, K. 2010. Biodiversity of aquatic and semi aquatic plants of Uttar Pradesh. *Uttar Pradesh State biodiversity board Lucknow*: 230-233.
- [26]. Sharma, C., Aneja, K.R. and Kasera, R. 2011. Screening of *Berberis aristata* DC. for antimicrobial potential against the pathogens causing earinfection. *Int. J. Pharmacol.* 7(4): 536-541.
- [27]. Singh, P. and Singh, M.P. 2015. *Solanum diphyllum* L. (Solanaceae) – A new record for Uttar Pradesh, India, *Indian Forester.* 141 (9): 1001-1002.
- [28]. Sivapriya, M. and Srinivas, L. 2007. Isolation and purification of a novel antioxidant protein from the water extract of Sundakai (*Solanum torvum*) seeds, *Food Chemistry.* 104: 510 – 517.

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