Journal of Research in Ecology

An International Scientific Research Journal
ISSN Online: 2319-1562, Print: 2319-1554

DRAFT COPY

Phytochemical and antimicrobial activities of *Solanum nigrum* and *Solanum trilobatum*

Authors:

Muthulakshmi S., Iruthaya Kalai Selvam, and Sagya Rani C

Institution:

PG and Research Centre of Zoology, Jayaraj Annapackiam College for Women (Autonomous), (Periyakulam, Theni District – 625 601. Tamil Nadu.

ABSTRACT:

The present investigation has been undertaken to find out the antibacterial activities of two plants namely Solanum nigrum and Solanum trilobatum. Sensitivity tests were performed by agar-well diffusion method. Qualitative phytochemical screening as done by the standard method. The following bacterial strains are used to evaluate antibacterial activity Vibrio cholera, Pseudomonas aeruginosa, E. coli, Aeromonas and Staphylococcus aureus. Phytochemical analysis result showed that tannins present in methanol extract, ethanol extract and chloroform extract of S. nigrum but carbohydrates, flavonoids, alkaloids and steroids were absent in all the three solvent extract. Glycosides and saponins were present in methanol extract of S. nigrum. Qualitative analysis phytochemical results showed that carbohydrates and tannins were present in methanol extract, ethanol extract and chloroform extract of S. trilobatum. Alkaloids were present in ethanol extract only. Flavonoids, glycosides and steroids were absent in methanol extract, ethanol extract and chloroform extract of S. trilobatum. Saponins test showed positive result of methanol extract and chloroform extract of S. trilobatum. Methanol extract of S. trilobatum leaf exhibited maximum activity against Aeromonas followed by E. coli and Vibrio cholera. The maximum inhibition zone was obtained against P. aeruginosa and Staphylococcus aureus. Ethanol extract of Solanum trilobatum leaf exhibited maximum activity against Vibrio cholera, E. coli, P. aeruginosa and minimum activity against Aeromonas and S. aureus. Chloroform extract of S. trilobatum leaf exhibited minimum activity against E. coli and no activity against V. cholera, Aeromonas, P. aeruginosa and S. aureus. The test plants offer potential as an alternative to antimicrobial as a means of controlling pathogens. The obtained results may provide a support to use of the plants in traditional medicine. The results from this study form a basis for further studies of the potent plants so as to isolate the compounds responsible for the antimicrobial activity.

Corresponding author:

S. Iruthaya Kalai Selvam

Email Id:
kalai.akila@gmail.com

Keywords: *Solanum trilobatum*, *S. nigrum*, phytochemical screening and antimicrobial activity.

Article Citation:
Journal of Research in Ecology (2016) 4(1): 158-171

Dates:
Received: 12.09.2016 **Accepted:** 18.10.2016 **Published:** 19.11.2016

Web Address:
http://jresearchbiology.com/documents/EC0111.pdf

Introduction

Today, nearly 88% of the global populations turn to plant derived medicines as their first line of defense for maintaining health and combating diseases. Currently, people of Asia especially India are utilizing plants as part of their routine health management (Samy *et al.*, 2008). Medicinal properties of plants are hinged on the presence of bioactive principles such as alkaloids, phenols, tannins, glycosides and essential oils amongst others (Karou *et al.*, 2006). The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. The resistance of the organisms increased due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious disease. This situation forced the researchers to search for new antimicrobial substance from various sources including medicinal plants (Bauer *et al.*, 1996). Gram negative bacterium such as *E. coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia (Benhassaini *et al.*, 2003). Different antibiotics exercise their inhibitory activity on different pathogenic organisms (Chanda and Rakholiya, 2011). Medicinal plants are believed to be important source of new chemical substance s with potential therapeutic effects. These compounds possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities (Bidlack *et al.*, 2000). The expanding bacterial resistance to antibiotics has become a growing concern worldwide (Gardam, 2000). Intensive care physicians consider antibiotic-resistant bacteria a significant or major problem in the treatment of patients (Lepape *et al.*, 2009). Increasing bacterial resistance is prompting a resurgence in research of the antimicrobial role of herbs against resistant strains (Alviano and Alviano, 2009). A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds (Mahady, 2005). Medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat (Iwu *et al.*, 1999). Several medicinal plants have been tried against pathogenic microorganisms (Haraguchi *et al.*, 1999; Sashikumar *et al.*, 2003). *Solanaceae* is a large plant family containing two thousand and three hundred species, nearly half of which belong to a single genus *Solanum*. Herbs are safe, less, economical and a reliable key natural resource of drugs all over the world.

In the present study, methanol, ethanol and chloroform extracts of *S. nigrum* and *S. trilobatum* were screened for antimicrobial activity against *V.cholera, E.coli, P. aeraginosa, Aeromonas and S. aureus* and qualitative phytochemical analysis was carried out.

Materials and Methods

1. plant
2. plant extract
3. antimicrobial activity
4. std amikacin

Determination of antimicrobial activity

Sensitivity tests were performed by agar-agar-good diffusion method (Cole, 1994; Espinel Ingroff *et al.*,1995; Okeke *et al.*,2001). The test bacterial strains were inoculated in to Mueller Hinton agar medium. Different concentrations of leaf extract were poured in the wells. After holding the plates at room temperature for one hour to allow diffusion of the extract in to the agar, they are incubated for 24 hours at 37°C. For *Streptococcus* sp. the incubation was performed in micro aerophilic conditions. After 24 hours, zone of inhibition was observed and recorded. The tests were performed in duplicates for each microorganism evaluated and the final results were presented as arithmetic average.

Phytochemical analysis of *Solanum nigrum* and *Solanum trilopatum*

Plant collection and extraction

Collect the leaf and flower of plant and thoroughly washed with distilled water. Kept it in the room temperature at 27°C for two weeks. The dried plants sample to make fin powder. The plants powder was stored air sealed polythene bags at room temperature. 3gram powder soaked in 15ml of methanol, ethanol and chloroform solution for 2 days. Extract filtered for filter paper and stored for further studies. The preliminary qualitative phytochemical analysis was carried out in crude dry powder of two plants; while total phenol and flavonoid content was estimated in methanol, Ethanol and chloroform extracts of two plants.

Carbohydrates

Five hundred milligram of powdered sample was taken and dissolved in 5ml of distilled water and then filtered. Filtrate was added with few drops of Molisch's reagent. Followed by addition 1ml of Conc. H_2SO_4 by the side of the test tube. After two minutes 5ml of distilled water was added. Red and dull violet color formation at the interphase of the two layers was taken as Positive result (Sofowora, 1993).

Alkaloids

100mg of powdered sample was dissolved in 5ml of methanol and then filtered. The 2ml of filtrate was mixed with 5ml of 1% aqueous HCL. One milliliter of mixture was taken separately in two test tubes. Few drops of Dragendorff 's reagent were added in one test tube and orange or red precipitate was taken as positive result. To the second test tube Mayer's reagent was added and appearance of buff colored precipitate was taken as positive test for the presence of alkaloid (Sofowora, 1993).

Steroids

200mg of powder sample was dissolved in 2ml of acetic acid separately; Solutions were cooled again add few drops of concentration H_2SO_4 Violet and blue or bluish-green was taken positive test steroidal ring (Sofowora, 1993).

Saponins

One gram of powder sample was boiled in 10ml of distilled water and then filtered. 3ml of distilled water was added to the filtrate and shaken vigorously for about 5min. Formation of foam after shaking was taken as a confirmation for the presence of saponins (Sofowora, 1993).

Flavonoids

Five hundred milligram of sample was dissolved in 5ml of ethanol slightly warmed and then filtered. Few pieces of magnesium chips were added to the filtrate followed by addition few drops of Conc. HCL. A pink and orange or red to purple coloration was taken the presence of flavonoid (Trease and Evans, 2002).

Tannins

500mg of powdered sample was mixed with 10ml of distilled water and then filtered followed by the addition of few drops of 1% ferric chloride solution. Occurrence of a blue - black green or blue -green precipitate indicates the presence of tannins (Trease and Evans 2002).

Glycosides

Keller Kiliani test was performed to assess the presence of cardiac glycosides. The crude dry powder of each plant was treated with 1 ml of $FeCl_3$ reagent (mixture of 1 volume of 5% $FeCl_3$ solution and 99 volumes of glacial acetic acid). To this solution a few drops of concentrated H_2SO_4 was added. Appearance of greenish blue color within a few minutes indicated the presence of cardiac glycosides (Ajaiyeobu, 2002).

Result

The result of antibacterial activity of *S. nigrum* and *S. trilobatum leaf* using methanol, ethanol and chloroform solvent extract in various fraction by disc diffusion method against bacteria viz. *V. cholera, P. aeruginosa, E. coli, Aeromonas,* and *S. aureus* are given in Table (1 - 7).

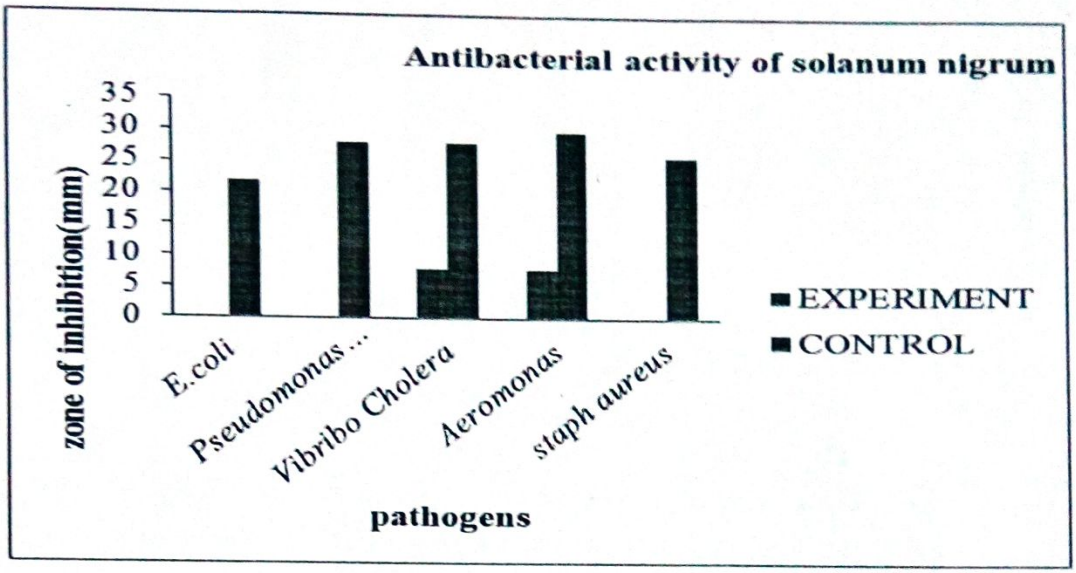
For the pharmacognostic identification, the morphological characters and microscopic characters of the two plants were studied. The anatomical characters of the two plants were studied in stem and leaf. For fluorescence characters the plant powders and their extracts were analysed in UV and visible light.

The quantitative characters such as total ash, insoluble ash, sulphated ash and water solvable ash have been carried out. The qualitative determination of inorganic elements present in plant ash contains chloride, sulphate, phosphorous and iron have been reported in both species. Calcium, magnesium, cobalt, copper and potassium are not observed any of these taxa.

Table1. Antibacterial activity of *S. nigrum* leaf methanol extract (M1)

S.no	Pathogen	Zone of inhibition (mm)	
		Experiment	Control
1.	*E. coli*	-	22 mm
2.	*P. aeruginosa*	-	28 mm
3.	*V. cholera*	8 mm	28 mm
4.	*Aeromonas*	8 mm	30 mm
5.	*S. aureus*	-	26 mm

Figure :1 Antibacterial activity of *S. nigrum* leaf methanol extract.

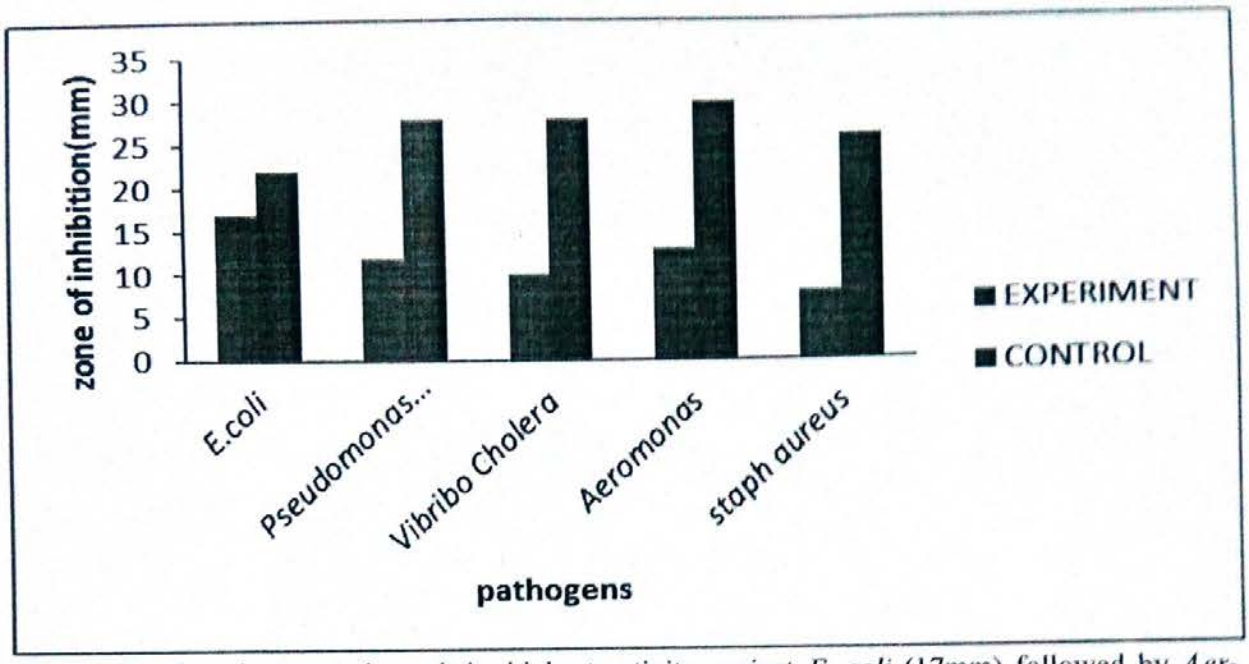


The result revealed that the crude methanol extract showed the highest activity against (8mm) the *V. cholera* and *Aeromonas* in Table1, Figure 1(Plate 3, 4) and no activity against *E. coli, P. aeruginosa* and *S. aureus* (Table1, Figure 1 and Plate 1, 2, 5). Among the step gradient extract of five pathogen tested maximum inhibition zone were obtained against *V. cholera* and *Aeromonas*.

Table. 2 Antibacterial activity of *S. nigrum* leaf ethanol extract (E1)

S.No	Pathogens	Zone of inhibition (mm)	
		Experiment	Control
1	*E-. coli*	17 mm	22 mm
2	*P. aeruginosa*	12 mm	28 mm
3	*V. cholera*	10 mm	28 mm
4	*Aeromonas*	13 mm	30 mm
5	*S. aureus*	08 mm	26 mm

Figure 2. Antibacterial activity of *S. nigrum* leaf ethanol extract

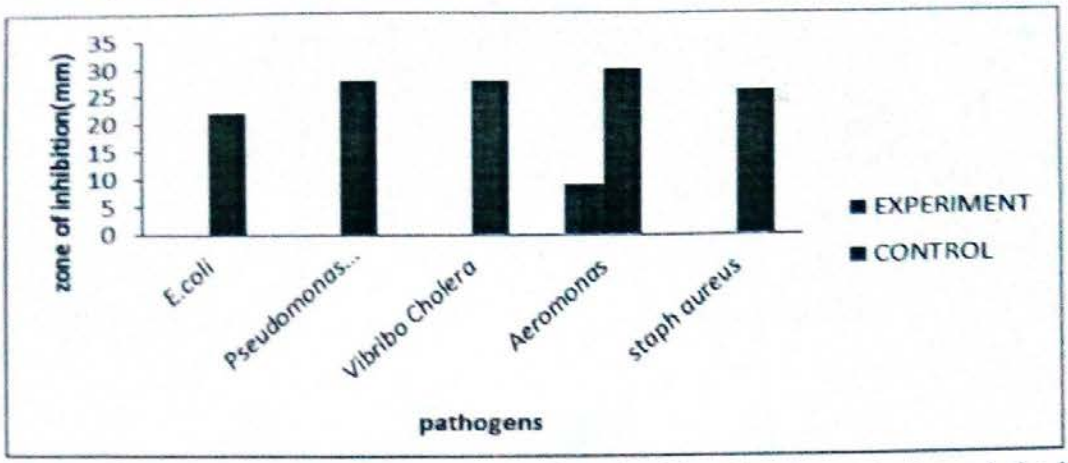


The crude ethanol extract showed the highest activity against *E. coli* (17mm) followed by *Aeromonas* (13mm) and *P. aeruginosa* (12mm) and lowest activity was observed against *S. ureus* (8mm) (Table 2, Figure 2 and Plate 1, 2, 3, 4, 5).

Table :3 Antibacterial activity of *Solanum nigrum* leaf chloroform extract (C1)

S.no	Pathogen	Zone of inhibition (mm)	
		Experiment	Control
1.	*E-. coli*	-	22 mm
2.	*P. aeruginosa*	-	28 mm
3.	*V. cholera*	-	28 mm
4.	*Aeromonas*	9 mm	30 mm
5.	*S. aureus*	-	26 mm

Figure 3. Antibacterial activity of *S. nigrum* leaf chloroform extract

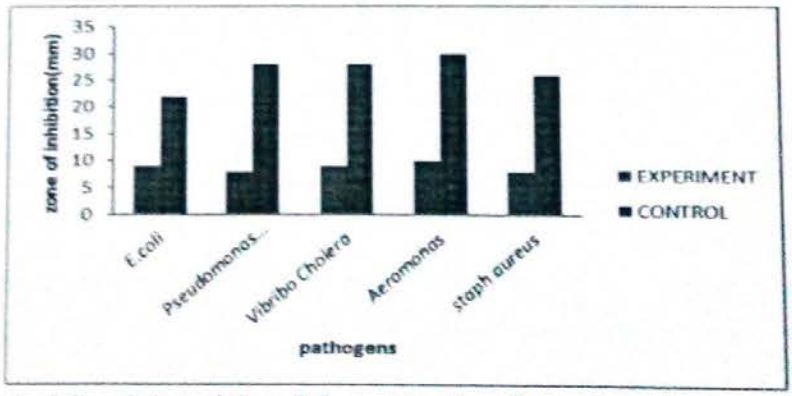


The antibacterial activity of *S. nigrum* leaf crude chloroform extract showed the highest activity against *Aeromonas* (9mm) (Table 3 and Plate 4). No activity were observed against *E. coli, P. aeruginosa, V. cholerae* and *S. aureus.* (Table 1 and Plate 1, 2, 3, 5).

Table: 4 Antibacterial activity of *S. trilobatum* leaf methanol extract (M2)

S.no	Pathogen	Zone of inhibition (mm)	
		Experiment	Control
1.	*E-. coli*	9 mm	22 mm
2.	*P. aeruginosa*	8 mm	28 mm
3.	*V. cholera*	9 mm	28 mm
4.	*Aeromonas*	10 mm	30 mm
5.	*S. aureus*	8 mm	26 mm

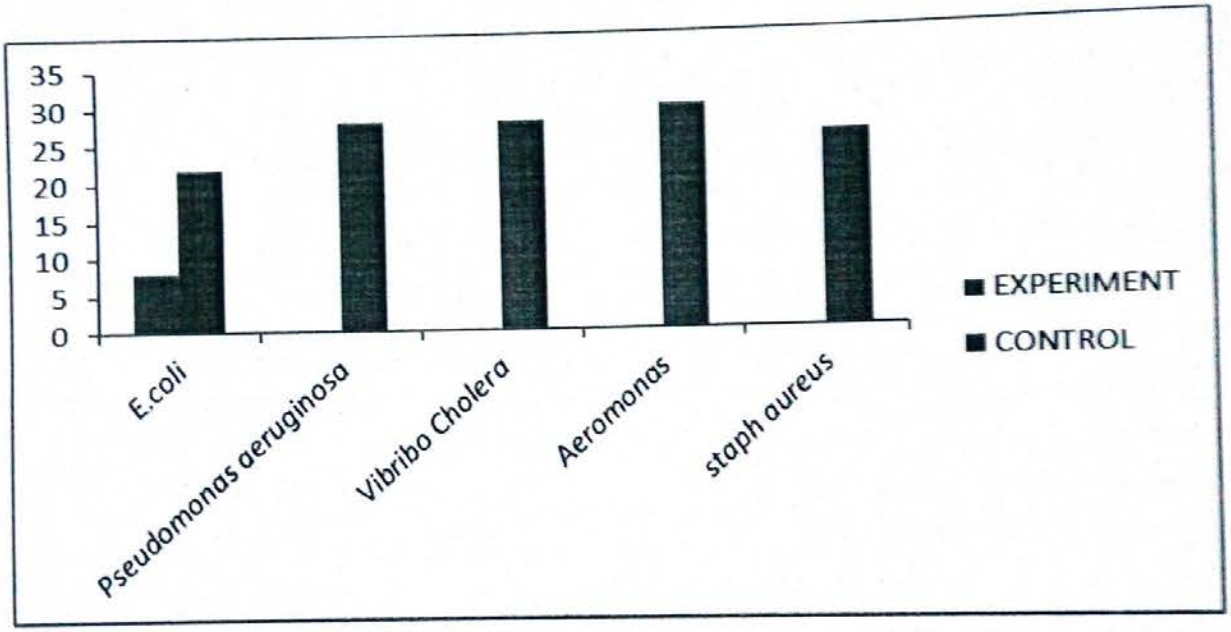
Figure: 4 Antibacterial activity of *S. trilobatum* leaf methanol extract



The antibacterial activity of *S. trilobatum* leaf crude methanol extract showed the highest activity against the *Aeromonads* (10mm), followed by E. coli (9mm) and *V. cholera* (9mm) in Table 4, Figure 4 (Plate 1, 3, 4) and lowest activity against *P. aeruginosa* (8mm) and *S. aureus* (8mm) (Table 4 Figure 4 & Plate 2, 5). Among the step gradient extract of five pathogen tested maximum inhibition zone were obtained against *E. coli, V. cholera* and *Aeromonas.*

Table 6. Antibacterial activity of *S. trilobatum* leaf chloroform extract (C2)

S. No	Pathogens	Zone of inhibition (mm)	
		Experiment	Control
1.	*E-. coli*	8 mm	22 mm
2.	*P. aeruginosa*	-	28 mm
3.	*V. cholera*	-	28 mm
4.	*Aeromonas*	-	30 mm
5.	*S. aureus*	-	26 mm

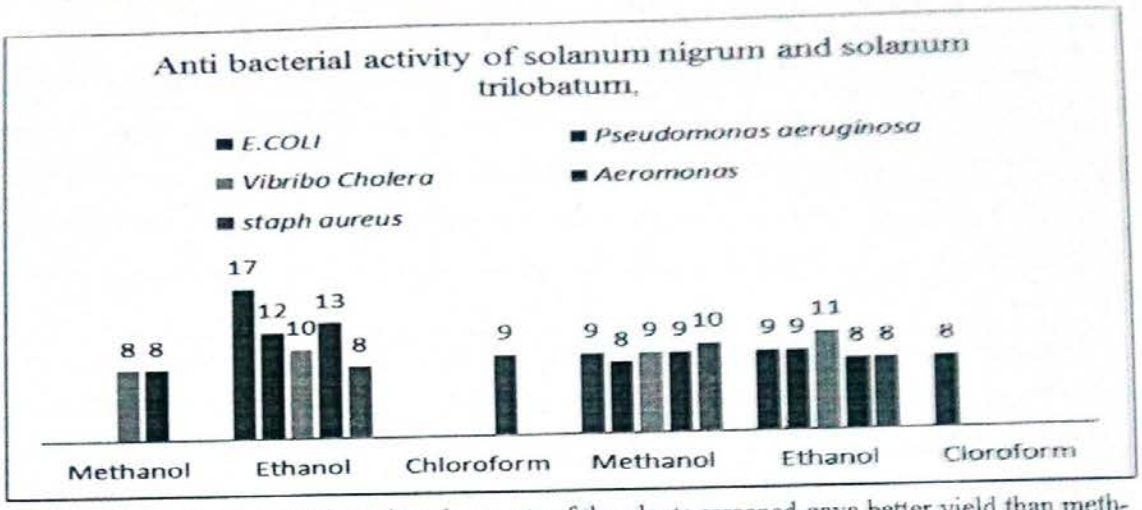


The antibacterial activity of *S. trilobatum* leaf crude chloroform extract showed the highest activity against E. coli (8 mm) Table 6 and no activity against *V. cholera, Aeromonas, P. aeruginosa* and *S. aureus* (Table 6, Figure 6 Plate 2, 3, 4, 5).

Table 7. Antibacterial activity of different solvent extract of *S. nigrum* and *S. trilobatum*

Pathogen	Extract of *S. nigram*			Extract of *S. trilobatum*		
	Methanol	Ethanol	Chloroform	Methanol	Ethanol	Chloroform
V. cholera	8	10	-	9	11	-
Aeromonas	8	13	9	10	8	-
P. aeruginosa	-	12	-	8	9	-
E. coli	-	17	-	9	9	8
S. aureus	-	8	-	8	8	-

Figure 7. Antibacterial activity of different solvent extract of *S. nigrum* and *S. trilobatum* leaf



The results showed that ethanol extracts of the plants screened gave better yield than methanol and chloroform extracts and Gram positive microorganisms were more sensitive to the plant extracts than the Gram-negative microorganisms *(S. aureus)*. Very lowest activities were observed in chloroform extracts (Table 7 & Figure 7.). Antibiotics showed highest activity (Table 1 - 6) when compared the activity with three solvent extracts of *S. nigrum* and *S. trilobatum* leaf.

Table :8 Phytochemical analysis of *S. nigram*

S .no	Phytochemical	Methanol extract	Ethanol extract	Chloroform extract
1.	Carbohydrates	-	-	-
2.	Alkaloids	-	-	-
3.	Glycosides	+	-	-
4.	Steroids	-	-	-
5.	Saponins	+	-	-
6.	Tannins	+	+	+
7.	Flavonoids	-	-	-

Phytochemical analysis result showed that tannins present in methanol extract, ethanol extract and chloroform extract of *S. nigrum* but carbohydrates, flavonoids, alkaloids and steroids were absent in all the three solvent extract. Glycosides and saponins were present in methanol extract of *S. nigrum* (Table 8.).

Table 9. Phytochemical analysis of *S. trilobatum*

S.No	Phytochemical	Methanol extract	Ethanol extract	Chloroform extract
1.	Carbohydrates	+	+	+
2.	Alkaloids	-	+	-
3.	Glycosides	-	-	-
4.	Steroids	-	-	-
5.	Saponins	+	-	+
6.	Tannins	+	+	+
7.	Flavonoids	-	-	-

Qualitative analysis phytochemical results showed that carbohydrates and tannins were present in methanol extract, ethanol extract and chloroform extract of *S. trilobatum*. Alkaloids were present in ethanol extract only. Saponins test showed positive result of methanol extract and chloroform extract of *S. trilobatum* (Table 9.).

The total ash which is the measurement of the amount of the residual substances not volatilized when the drug sample is ignited by heat. Ash may be derived from the plant tissue itself (i.e. physiological ash) or from the extraneous matter, especially sand and soil adhering to the surface of the drugs (i.e. non-physiological ash), and this kinds of ash are determined together, therefore it is referred to as total ash (African Pharmacopoeia, 1986).

A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the drug or drug combinations for marketing (Chandel et al., 2011).

In this work, the total ash value is higher in the powder of stem and leaf with compare to root for both of the studied plants. Higher total ash value is noticed in aerial parts of C. klotzchianus and the value is.47%. Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicate addition of exhausted material, adulteration or incorrect processing during drying or storage (Chandel et al., 2011).

Water soluble ash is that part of the total ash content, which is soluble in water. It is a good indicator of the water-soluble salts in the drug (Mukherjee, 2002).

The determination of acid-insoluble ash is a method intended to measure the amount of silica especially sand and silicious earth present in the drugs (Uduak Essiett and Ikoedem Unung, 2013).

Figure 6. Antibacterial activity of *S. trilobatum* leaf chloroform extract

Discussion

The use of antimicrobial agents is critical to the successful treatment of infectious diseases. Although there are numerous classes of drugs that are routinely used to treat infections in humans, pathogenic microorganisms are constantly developing resistance to these drugs (Al Bari *et al*., 2006) because of indiscriminate use of antibiotics (Gibbons 1992; Rahman *et al*., 2001).

The use of higher plants and preparations made from them to treat infections is a longstanding practice in a large part of the population, especially in the developing countries, where there is dependence on traditional medicine for a variety of ailments (Ahmad and Mohammad 1998). Interest in plants with antimicrobial properties increased because of current problems associated with the antibiotics (Emori and Gaynes 1993; Pannuti and Grinbaum 1995).

Recently, the antimicrobial effects of various plant extracts against certain pathogens have been reported by a number of researchers (Ahmed and Beg 2001; Erasto *et al*. 2004; Nair et al. 2007b; Carneiro *et al*. 2008; Liasu and Ayandele 2008; Parekh and Chanda 2006; Chanda *et al*. 2009). Disc diffusion method is the most widely used procedure for testing antimicrobial susceptibility (Sambath Kumar *et al*. 2006). The disc diffusion procedure (Kirby Bauer method) has been accepted by the Food and Drug Administration (FDA) and as a standard by the National Committee for Clinical Laboratory Standards (Barry and Thornsberry 1985; NCCLS 2003).

In the present study the antibacterial activity of *S. nigrum* and *S. trilobatum* leaf were evaluated by using methanol, ethanol and chloroform solvent extract in various fraction by disc diffusion method against bacteria isolates via. *V. cholera, P. aeruginosa, E. coli, Aeromonas*, and staph aureus. Methanol extract showed the highest activity against (8mm) the *V. cholera* and *Aeromonas* in Table 1, Figure1 (Plate 3, 4) and no activity against *E. coli, P. aeruginosa* and *S. aureus* (Table 1 (plate 1, 2 ,5). The crude ethanol extract showed the highest activity against *E. coli* (17mm) followed by *Aeromonas* (13mm) and *P. aeruginosa* (12mm) and lowest activity was observed against *S. aureus* (8mm). (Table 2, Figure 2 and Plate 1, 2, 3 4, 5). Chloroform extract showed the highest activity against *Aeromonas* (9mm) (Table 3 and Plate 4). No activity was observed against *E. coli, P. aeruginosa, V. cholerae* and *S. aureus*. (Table 1 and Plate 1, 2, 3, 5).

The antibacterial activity of *S. trilobatum* leaf crude methanol extract showed the highest activity against the *Aeromonas* (10mm), followed by *E. coli* (9mm) and *V. cholera* (9mm) and lowest activity against *P. aeruginosa* (8mm), *S. aureus* (8mm) (Table 4 Figure 4 & plate 2, 5). Ethanol extract showed the highest activity against the *V. cholera* (11mm) followed by *E. coli* (9mm), *P. aeruginosa* (9mm) in (Table 5 and Figure 5 & Plate 1, 2, 3). The lowest activity against *Aeromonas* (8mm) and *S.*

aureus (8mm) (Table 5, Figure 5 and Plate 4). Chloroform extract showed the highest activity against *E. coli* (8mm) and no activity against *V. cholera*, *Aeromonas*, *P. aeruginosa* and *S. aureus* (Table 6, Figure 6 and Plate 2, 3, 4, 5).

Antimicrobial activities of various herbs and spices in plant leaves, flowers, stems, roots or fruits have been reported by many workers in different solvent extracts (Mau *et al*., 2001; Uz Zaman *et al*., 2006; Al Bayati and Sulaiman 2008 and Nair *et al*., 2009).

From the results it can be concluded that ethanol extracts of the plants screened gave better yield than methanol and chloroform extracts. It is evident that the Gram positive microorganisms were more sensitive to the plant extracts than the Gram negative microorganisms. (Table 7 & Figure 7). These findings are in agreement with other researchers (Oboh *et al*. 2007; Nair and Chanda 2007; Costa *et al*., 2008; Khan *et al*., 2008). The susceptibility of Gram positive bacteria may be due to their cell wall structure which is of a single layer while the Gram negative cell wall is a multi-layered structure and quite complex (Essawi and Srour 2000). All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested. Antibiotics showed highest activity (Table 1-6) than three solvent extracts of *Solanum nigrum* and *Solanum trilobatum* leaf. These plants may be a source of new antibiotic compounds.

In recent years, secondary plant metabolites (phytochemicals) with antibacterial potency have been actively investigated as alternatives to and or in combination with antibiotics in the therapy of bacterial infections (Sato *et al*., 1995; Liu *et al*., 2001). The preliminary qualitative phytochemical screening of the crude powder of *S. nigrum* and *S. trilobatum* was done to assess the presence of bioactive components. Presence of alkaloids (Dragendroff, Mayer, Wagner), flavonoids, tannins, steroids, saponins and cardiac glycosides was determined (Table 8 and 9). From the results of antimicrobial screening, methanol extract of *S. nigrum* and *S. trilobatum* showed potent antimicrobial activity, which might be due to the higher content of total phenolics. Similar results were reported by Salah *et al*. (2006).

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant (Pulok, 2002). Medicinal plant can be poisonous if wrong plant parts or wrong concentrations are used (FROHNE 1999). Some compounds from plants may be toxic in higher doses. Plants containing pyrrolizidine alkaloids could be toxic for man or livestock (KOCH *et al*., 1994).

The antibacterial activity was greater in *S. nigrum* and *S.trilobatum*. Antibacterial activity was detected in most of the pathogen

tested it is responsible to assume that multiple factors are responsible for antibacterial activity. In the present study the inhibitory effect of plant extract strongly antagonistic to pathogenic bacteria. The test plants offer potential as an alternative to antimicrobial as a means of controlling pathogens. The obtained results may provide a support to use of the plants in traditional medicine. The results from this study form a basis for further studies of the potent plants so as to isolate the compounds responsible for the antimicrobial activity.

Conclusion

In the present study the inhibitory effect of plant extract strongly antagonistic to pathogenic bacteria. Phytochemical analysis result showed that tannins present in methanol extract, ethanol extract and chloroform extract of *S. nigrum* but carbohydrates, flavonoids, alkaloids and steroids were absent in all the three solvent extract. Glycosides and saponins were present in methanol extract of *S. nigrum*.

Qualitative analysis phytochemical results showed that carbohydrates and tannins were present in methanol extract, ethanol extract and chloroform extract of *S. trilobatum*. Alkaloids were present in ethanol extract only. Flavonoids, glycosides and steroids were absent in methanol extract, ethanol extract and chloroform extract of *S. trilobatum*. Saponins test showed positive result of methanol extract and chloroform extract of *S. trilobatum*. The test plants offer potential as an alternative to antimicrobial as a means of controlling pathogens. The obtained results may provide a support to use of the plants in traditional medicine. The results from this study form a basis for further studies of the potent plants so as to isolate the compounds responsible for the antimicrobial activity.

Bibliography

Ahmad ZM, Mohammad F (1998) Screening of some Indian medicinal plants for their antimicrobial properties. Journal of Ethnopharmacology 62: 183-193.

Ajaiyeobu EO (2002) Phytochemical and antibacterial activity of Parkia biglobosa and Parkia bicolor leaf extracts. African Journal of Biomedical Research 5: 125-129.

Al-Bari MA, Sayeed MA, Rahman MS, Mossadik MA (2006) Characterization and antimicrobial activities of a phenolic acid derivative produced by Streptomyces bengladeshiensis a novel species collected in Bangladesh. Respiratory Journal of Medical Sciences 1: 77-81.

Alviano, D.S. and C.S. Alviano. 2009. Plant extracts: search for new alternatives to treat microbial diseases. Curr. Pharm. Biotechnol. 10:106-121.

Barry AL, Thornsberry C (1985) In: Manual of Clinical Microbiology, Lennette EH (Ed.), American Association for Microbiology, Washington pp. 978-987.

Bauer AW, Kirby WM, Sherris JC, Turck M (1996). Antibiotic susceptibility testing by standardized single disc method. Am. J. Clin. Pathol. 44: 493-496.

Benhassaini. H, and enabderrahmane. K and Chi. K (2003). Contribution to the assessment of the antiseptic activity of essential oils and oleoresin of Pistacia tial Atlas on some microbial sources: Candida albicans (ATC 20027), Candida albicans (ATCC 20032) and s accharomyces cerevisiae: ethnopharmacology, February (30): 38-46

Bidlack, W.R., S.T. Omaye, M.S. Meskin, and D.K.W. Topham, 2000. Phytochemicals as Bioactive Agents. CRC press, Boca Raton, FL.

Chanda. S and Rakholiya. K (2011). Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. Science against microbial pathogens: communicating current research and technological advances A. Méndez-Vilas (Ed.).

Gardam, M.A. 2000. Is methicillin-resistant Staphylococcus aureus an emerging community pathogen? A review of the literature. Can. J. Infect. Dis. 11:202-211.

Gibbons A (1992) Exploring new strategies to fight drug resistant microbes. Science 257: 1036 -1038.

Haraguchi H, Kataoka S, Okamoto S, Hanafi Mand & Shibata K,1999. Antimicrobial triterpenes from Ilex integra and the mechanism of antifungal action. Phytotherapia Residence, 13, 151-156.

Karou, D., Savadogo, A., Canini, A., Yameogo,S. and Montesano, C., 2006. Antibacterial activity of alkaloids from Sida acuta. Afr. J. Biotechnol., 5(2): 195-200.

Lepape, A., D.L. Monnet on behalf of participating members of the European Society of Intensive Care Medicine (ESICM). 2009. Experience of European potential sources of antimicrobial and resistance modifying agents. Afr, J. Biotechnol. 6:2886-2896.

Mau JL, Chen CP, Hsieh PC (2001) Antimicrobial effect of extracts from Chinese chive, cinnamon, and corni fructus. Journal of Agriculture and Food Chemistry 49: 183-188.

Oboh IE, Akerele JO, Obasuyi O (2007) Antimicrobial activity of the ethanol extract of the aerial parts of Sida acuta Burm.f. (Malvaceae). Tropical Journal of Pharmaceutical Research 6: 809-813.

Parekh J, Chanda S (2006) In vitro antimicrobial activities of extracts of Launaea procumbens Roxb. (Labiateae), Vitis vinifera L. (Vitaceae) and Cyperes rotundus L. (Cyperaceae). African Journal of Biomedical Research 9: 89-93.

Rahman MM, Wahed MII, Biswas MH, Sadik GM, Haque ME (2001) In vitro antibacterial activity of the compounds of Trapa bispinosa Roxb. Science 1: 214-216

Salah KBH, Mahjoub MA, Ammar S, Michel L, Millet-Clerc J, Chaumont JP, Mighri Z, Aouni M (2006) Antimicrobial and antioxidant activities of the methanolic extracts of three Salvia species from Tunisia. Natural Product Research 20: 1110-1120.