EFFICIENT EXTRACTION OF THERMOSTABLE METABOLITES OF *Sesbania aculeata* AND *IN VITRO* EVALUATION FOR THEIR ANTIBACTERIAL POTENTIAL

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Abstract. In the recent times, the trend has been towards the use of natural and safe drugs with less or no side effects and equally effective like synthetic drugs for curing diseases. It leads the research towards finding new drugs from natural sources of which plants seems to be good candidate. So, in this view the present research has been done to investigate the presence of different phytochemicals such as phenols, terpenoids, saponins, tannins, etc. and antibacterial property of *Sesbania aculeata* against different bacteria. The plant is mainly cultivated for soil enrichment, various medicinal purposes, fibre, fuel wood, fodder, paper and dye industry and other agro-forestry use though up to yet not reported in details for its extract's thermostability and antimicrobial property. Methanolic extracts were prepared from different parts of the plant at 65ºC for 10 cycles using Soxhlet apparatus. All the extracts analysed at their natural pH have been found to show antibacterial activity and among them prepared from seeds coat shows best results with average zone of inhibition varies from 26-32 mm in diameter at concentration of 10 mg/ml, by optimising biophysical parameters. The minimum inhibitory concentration of all the extracts ranges from 1-4 mg/ml against different bacterial strains which shows that the extracts even act at low doses. Extracts checked for thermostability at the range of temperature (60-100ºC) and found stable up to 90ºC with no effects on antimicrobial activity. Among the different strains tested, Gram negative bacteria show higher susceptibility as compare to Gram positive bacteria. The results have shown that the extracts could be the good source of new medicines that may be used to treat various bacterial infections.

Keywords: *Sesbania aculeata*; phytochemicals; plant extract; antibacterial.

INTRODUCTION

Plants are nature's gift to the human being that serves for disease free life. Medicinal plants which are commonly used as home remedies against multiple ailments. A large number of plants have been listed and described in ancient Indian treatise and Ayurveda for possessing not only antimicrobial activity but also for the treatment of other diseases and are used by a large population [2]. According to World Health Organization (WHO), 80% of the population of the developing countries uses traditional medicine. Over the last several decades, researchers have aimed at identifying and validating plant-derived substances for the treatment of various diseases. It is estimated that today more than 25% of the modern medicines are directly or indirectly derived from plants [13]. The medicinal properties of the plants are attributed by the chemical substances called secondary metabolites of which important ones are alkaloids, terpenoids, flavonoids, steroids, phenolic compounds etc. A large number of medicinal plants have been studied for their antimicrobial activity though most of them are wild plants and not cultivated, these mostly belong to different families including *Aizoaceae* [18], *Asteraceae* [3, 27], *Caesalpiniaceae* [12], *Celastraceae* [26], *Combretaceae* [32], *Leguminosae* [8, 10], *Zingiberaceae* [6, 7, 37], *Apocynaceae* [33, 36] and *Rhamnaceae* [4]. A very few cultivated plants have been studied for antimicrobial potential [20]. Among the families studied, *Leguminosae* family is of choice because the members of this family make a major contribution to the diet of people. Legumes being a rich source of protein are a good choice of plant for studying antimicrobial property. Of the various legume plant species, *Sesbania aculeata* is used for the present study. *S. aculeata* is mainly used as green manure crop but the flowers are consumed as food in some parts of the world. Besides this, many other pharmacological properties have been reported from the genus.

Sesbania aculeata (Pers.) (2n = 12) is commonly known as Dhaincha, a member of the *Fabaceae* family. The plant is though native to India, Pakistan and other Asian particularly South Asian countries but found throughout the world [15]. It is a fast-growing annual sub shrub crop reaching a height of about 3.5 m within a few months and is known to fix nitrogen annually up to 500 to 600 kg/hectare [29]. Its root is alexeteric, antihelmintic and diuretic. It is also useful in snake-bite and disease of eye. Its seeds are useful in ringworm and skin diseases. It is mainly cultivated for soil enrichment, various medicinal purposes, fibre, fuel wood, fodder, paper and dye industry and other agro-forestry uses and has not been reported up to yet in such detail for its extract's thermostability, antimicrobial property and presence of different phytochemicals such as phenols, terpenoids, saponins, tannins, etc.

MATERIALS AND METHODS

Plant materials and Optimisation of biophysical parameters for preparation of extracts

Different parts (leaf, flowers, seeds and seed coat) of *S. aculeata* were collected from the field grown plants. The parts were cleaned, washed, shade dried and powdered for the further study. The extracts were prepared by using methanol as choice of solvent for extraction and various factors affecting the extraction were optimized. Methanolic extracts of different parts of the *Sesbania aculeata* were extracted using Soxhlet apparatus with number of extraction cycles (4-12 cycles) and at different temperature (50-100ºC) maintained in water bath. The obtained solvent extracts

were further filtered with Whatman No 1 filter paper and then allowed to evaporate the solvent at different temperatures (40-100ºC) in the hot air oven. After evaporation, the samples were obtained in the form of powder (concentrated form) and stored at 4°C until further use. The solubility of the powdered samples was checked by dissolving them in water (aqueous) and Dimethyl sulfoxide (DMSO - organic) solvents.

Phytochemical screening

The four extracts thus obtained were analyzed to preliminary qualitative phytochemical screening following the standard protocols (Table 1) [5, 17, 19, 23].

Antibacterial assay

The different extracts of *S. aculeata* were tested for antimicrobial activity by agar well diffusion method against different Gram positive and negative bacteria that are *Bacillus subtilis*, *Klebsiella pneumoniae*, *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and plant pathogen *Agrobacterium tumefaciens*. Stock solutions of crude plant extracts with natural pH 5.0 as well as of different pH (3 - 9) by using HCl or NaOH were prepared at the concentration of 100 mg/ml by dissolving them in water. Muller Hinton Agar medium was used to cultivate human pathogenic bacteria and YEM medium for plant pathogenic bacteria. Fresh overnight grown culture of each strain was swabbed uniformly onto the individual plates using sterile cotton swabs and then made 5 wells of 6 mm in diameter on each plate. Then extracts of different parts of plant were poured into each well on all plates at concentration of 10mg/ml (100µl from each stock). The commercial drug cefotaxime was used as positive control (10 mg/ml) and were incubated for 24 h at 37°C. After incubation the different levels of zonation formed around the well was measured.

Thermostability

Stock solutions of the extracts were prepared and exposed to different temperatures (60-100ºC) in water bath for a period of one to twelve hours keeping one set at room temperature as control. After each incubation period, extracts were checked for their antibacterial activity. Thermostability was measured as percentage loss in the activity by comparing the results of test samples with the control.

Minimum Inhibitory Concentration

Different extracts were subjected to minimum inhibitory concentration by using agar dilution method [31]. Stock solution of concentration 100mg/ml was used for preparing a range of different concentrations (0.1-10 mg/ml) of extracts which were then dispensed in cooled agar medium. Then the medium was plated and was swabbed with active bacteria. Swabbed plates were incubated at 37ºC for 24 hours. Minimum inhibitory concentrations were evaluated for each extract for each bacterium by looking for the minimum concentration at which no visible growth appears. The test was performed in triplet along with positive (cefotaxime 1mg/ml) and negative control (water).

Time-Kill Assay of Aqueous Extract

Viable cell count method was used for assaying the rate of killing of the bacteria by the extracts dissolved in distilled water [35]. Mixed suspension of individual bacteria was prepared, by mixing the serially diluted (10^{-3}) cultures of 4 hour grown activated bacterium with equal amount of extract (10 mg/ml) and were incubated at 37ºC. A control was also taken in which the extract was replaced with distilled water. Later 100 µL of the mixed suspension was poured on suitable agar plates at different time intervals (1-24 hours) and incubated at 37ºC for 24 hours. After incubation, bacterial colonies were counted and compared with that of control. All the experiments were performed in triplet.

Table 1. Methodology for phytochemical screening of different compounds

Test for	Methodology	Predictive Observation	
		For Presence	For Absence
Alkaloids	To the extract (1 ml) add 1 ml of Wagner's reagent (2 g iodine and 6 g of potassium iodide in 100 ml distilled water)	Reddish brown precipitate	No precipitate
Saponins	About 5 ml of diluted extract was shaken vigorously and kept for 5 min	Foamy layer	No Foam
Tannins	To the 5 ml extract, add few drops of 1 % ferric chloride solution	Green colour precipitate	No precipitate
Glycosides	To 2 ml of the concentrated extracts, add 10 ml of 50% H_2SO_4 and kept in water bath shaker. After 15min add 2 ml of Fehling's solution and boil.	Brick-red precipitate	No precipitate
Flavonoids	To 2 ml of extracts, add few drops of sodium hydroxide solution and diluted sulfuric acid	Colourless solution	Yellow colour
Sugars	About 10 ml of extract was boiled with 3-4 drops of Fehling's A and B solutions for 2 minutes in water bath	Red colour	No red colour
Proteins	To extract add 1 ml of Biuret reagent (40% NaCl & 1% $CuSO_4$)	Violet colour	Blue colour
Terpenoids	To 5 ml of extract, add 2 ml of chloroform and 3 ml of concentrated sulfuric acid	Reddish brown colour	No reddish brown colour
Phenols	To the extract, add 3-4 drops of 5% ferric chloride solution	Blackish colour	No colour at interface

RESULTS

Optimization of Physiochemical Parameters

Extraction Cycles Number

There was a progressive increase in the activity of extracts with increase in the number of cycles of Soxhlet extraction. There was no significant increase in the activity of the extracts observed after 10^{th} cycle showing that it is optimal for complete extraction of the compounds from different parts of the *Sesbania aculeata* plant (Fig. 1).

Effect of Extraction Temperature

Different temperature range (50-100ºC) was used for Soxhlet extraction in this study. The maximum antibacterial activity of the extract was found to be at 65ºC. Above the 70ºC temperature there was slightly decrease in the activity of the extracts. There was significant difference in the activity below the temperature 60ºC with very less antibacterial activity in extractions at lower temperatures. This may be due to the fact that the boiling temperature for methanol is around 65ºC and at this temperature all the compounds dissolve in it completely. The inhibition zone of seed coats extract at different temperature of extraction has been shown in Fig. 2.

Effect of Temperature on Preparation of Powdered Extract

To obtain the extract in the powdered form, it is necessary to concentrate the methanolic extract obtained from Soxhlet by evaporating the solvent. For the same, optimum temperature should be selected to avoid any loss in activity due to the effect of temperature. The solvent was evaporated at different temperature range of 40-100ºC and the activity of the dry extract obtained was checked. It was found that the maximum antibacterial activity was obtained when the solvent was evaporated at 50ºC with no significant difference up to 70ºC (Fig. 3).

Phytochemical Screening

The results for phytochemical tests have shown the presence of almost all the tested components in the methanolic extracts of the seeds and seed coats while some compounds were found absent in the extracts of other parts of the plant. These differences in metabolites composition may be responsible for the differences in the activities of these extracts against different pathogenic bacteria. The results for the phytochemical screening for different parts of *S. aculeata* are shown in Table 2.

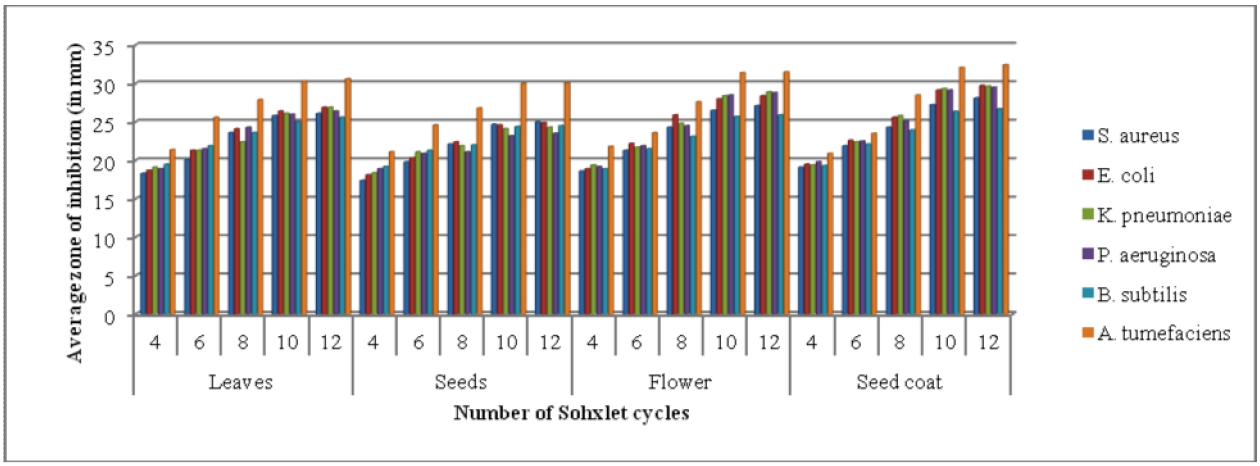

Figure 1. Effect of number of Soxhlet cycles on antibacterial activity of different extracts of *Sesbania aculeata* on different bacteria strains

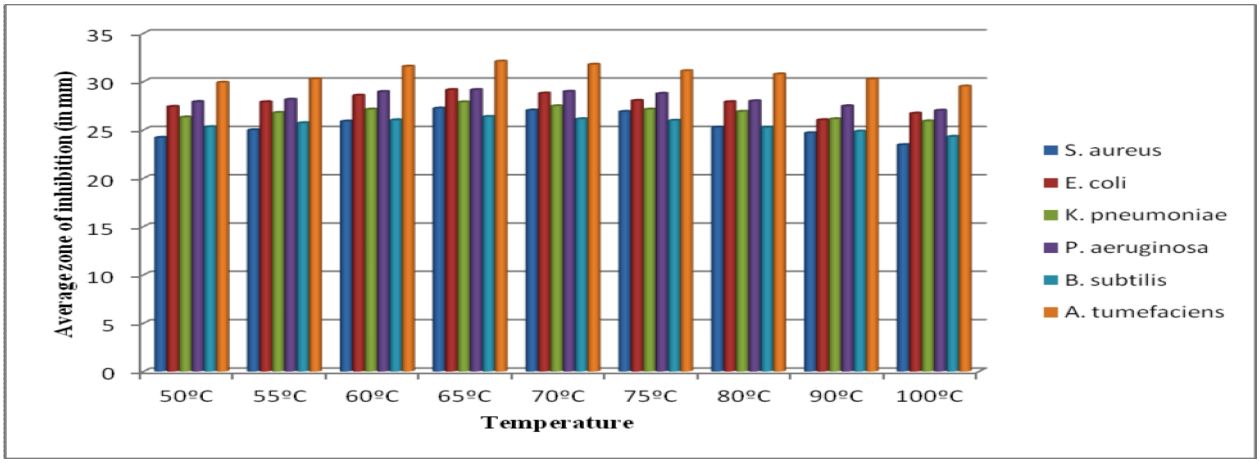

Figure 2. Effect of different temperatures of extraction on antibacterial activity of seed coat extracts of *Sesbania aculeata* on different bacteria strains

Antibacterial Screening

The antibacterial activity checked for the extracts obtained from different parts of the *S. aculeata* and all were found to possess significant inhibitory activity on all selected strains of bacteria. Among the different parts of the plant, seed coat extract showed the highest antibacterial properties against all strains selected. The inhibition zones of the extracts against the bacteria tested were found to be in range of 26-32mm in diameter which was close to the drug cefotaxime (29-34mm) used as a positive control. It was also found that the extracts showed more inhibitory activity against the Gram negative bacteria than the Gram positive bacteria. Moreover, a highly significant inhibitory activity was found against the plant pathogen *A. tumefaciens* than the human pathogens. Zone of inhibition of extracts of different parts of plants against different bacteria are given in Table 3 (Fig. 4).

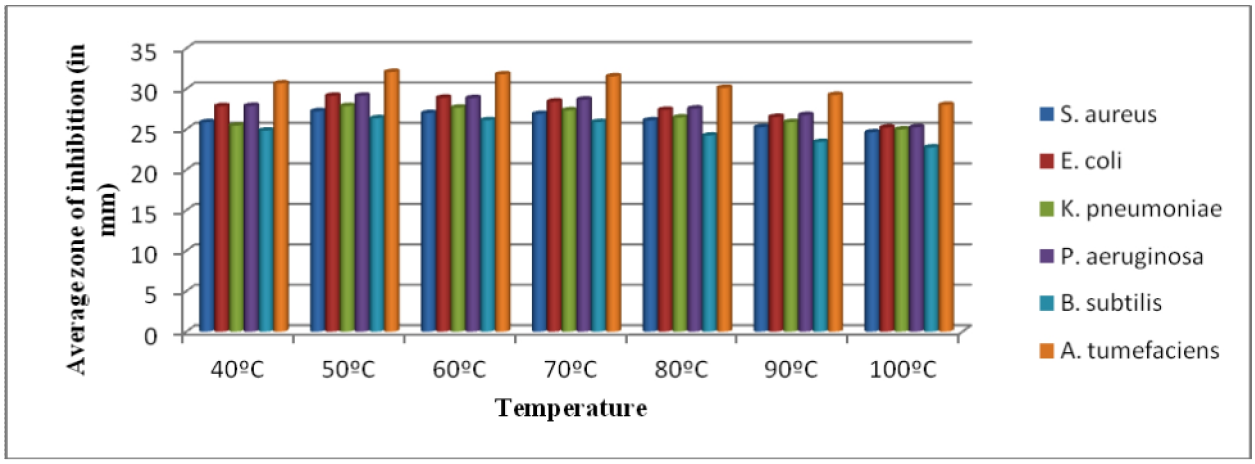

Figure 3. Effect of different temperature used for preparing powdered extract on antibacterial activity of seed coat extract of *Sesbania aculeata* on different bacteria strains

Table 2. Results of the phytochemical screening tests done for different plant parts

	Alkaloids	Flavonoids	Saponins	Glycosides	Sugars	Protein	Tannins	Terpenoids	Phenols
Seeds	+	+	+	+	+	+	+	+	+
Leaves	+	+	-	+	+	+	+	-	+
Flower	+	+	-	+	+	+	+	+	+
Seed Coat	+	+	+	+	-	-	+	+	+

Table 3. Antibacterial activity of different extracts of *S. aculeata* against different bacteria

Bacterial Stains	Leaves	Seeds	Flowers	Seed Coat	Cefotaxime
S. aureus	25.8±0.45	24.7±0.34	26.5±0.42	27.23±0.42	29.3±0.31
E. coli	26.4±0.54	24.6±0.39	28.0±0.34	29.14±0.34	32.2±0.46
K. pneumoniae	25.6±0.38	25.7±0.28	27.0±0.29	27.87±0.29	30.5±0.33
P. aeruginosa	26.0±0.63	25.2±0.52	28.5±0.47	29.14±0.47	31.1±0.41
B. subtilis	25.0±0.26	24.4±0.31	25.7±0.32	26.36±0.32	29.7±0.30
A. tumefaciens	30.3±0.57	30.0±0.43	31.4±0.48	32.08±0.48	34.2±0.51

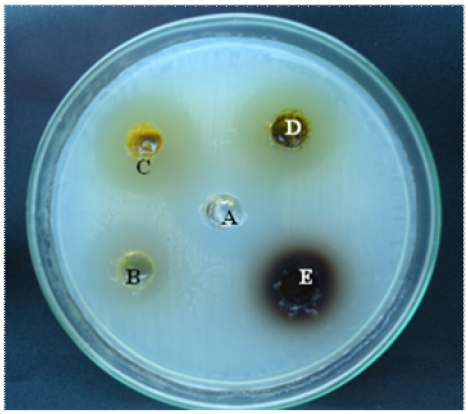

Figure 4. Antibacterial activity of extracts of *S. aculeata* against *A. tumefaciens* on YEM agar plate. **A:** positive control (Cefotaxime), **B:** Seeds, **C:** Flower, **D:** Leaves, **E:** Seed coat

Effect of pH

Among the extracts of different pH, there was slightly increase in activity observed when increasing from natural pH up to 7 against Gram negative bacteria as evident from the inhibition zones. Whereas the Gram positive bacteria showed no or very less sensitivity towards change in pH up to 7.0 as compare to natural pH on which showing maximum zone of inhibition. Above the pH 7.0 there was significantly decrease in the activity observed in both the cases. Similarly, on decreasing the pH less than the 4.0 was found to be affecting decrease in activity. The inhibition zone of seed coat extract at different pH has been shown in Fig. 5.

Thermostability Studies

All the extracts studied were found to show some thermostability when treated at 60-100ºC temperature for one to twelve hours prior to their use. Loss in the activity of different extracts against different bacteria was found in the range between 8.41-23.48% at 100ºC temperature while negligible loss in activity was found up to 90ºC temperatures when incubated for twelve hours (Fig. 6). No significant decrease in activity was noted when incubated at different temperatures for less than twelve hours (data not shown).

Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) of different extracts was found in the range between 1- 4 mg/ml when tested against different bacteria (Fig. 7). Seed coat extract was found to demonstrate the lowest MIC value of 1mg/ml against the Gram negative bacteria while it was 2 mg/ml for Gram positive. Extracts of flowers and seeds showed MIC values 1- 2 mg/ml for Gram negative and 2-3 mg/ml for Gram positive bacteria. In case of leaves, the MIC value of 2mg/ml was obtained for Gram negative whereas it was 3 – 4 mg/ml for Gram positive bacteria.

Viable Cell Count Studies

In viable Cell Counts studies all the extracts show bactericidal property with killing rate ranging from 0-8 hours and 2-20 hours for seed coat and other plant sources, respectively. A steady decline was found in the viable cell count over the period of time of incubation for different bacteria. Of the bacterial strains studied, *E. coli* was instantly killed as no cell was found at 0 hours making it the most sensitive bacterium. Another Gram negative bacterium *A. tumefaciens* took 1 hour for complete killing where as Gram positive bacteria took longer time intervals for complete killing ranging from 2- 8 hours using seed coat extracts (Fig. 8).

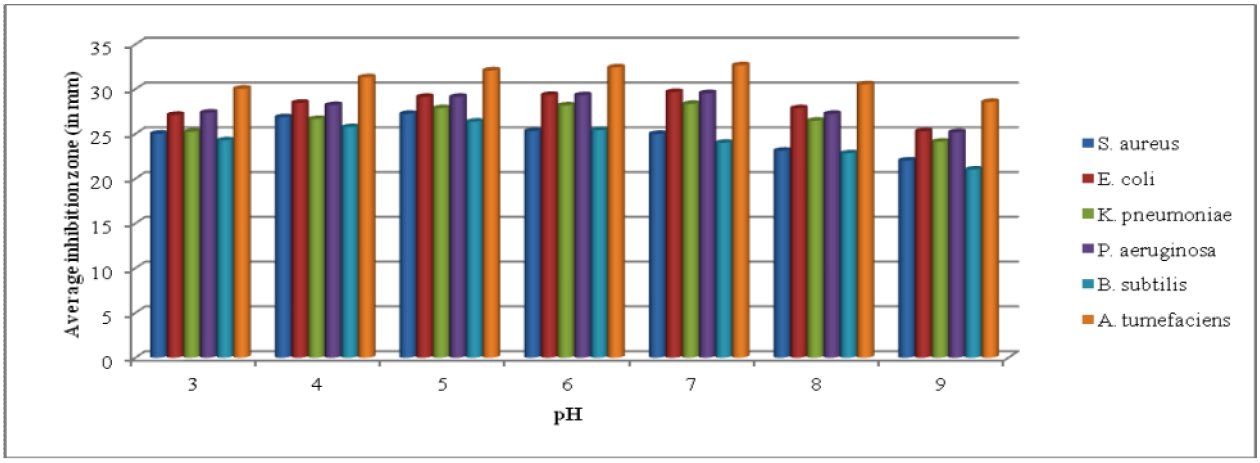

Figure 5. Effect of different pH on antibacterial activity of seed coat extract of *Sesbania aculeata* on different bacteria strains

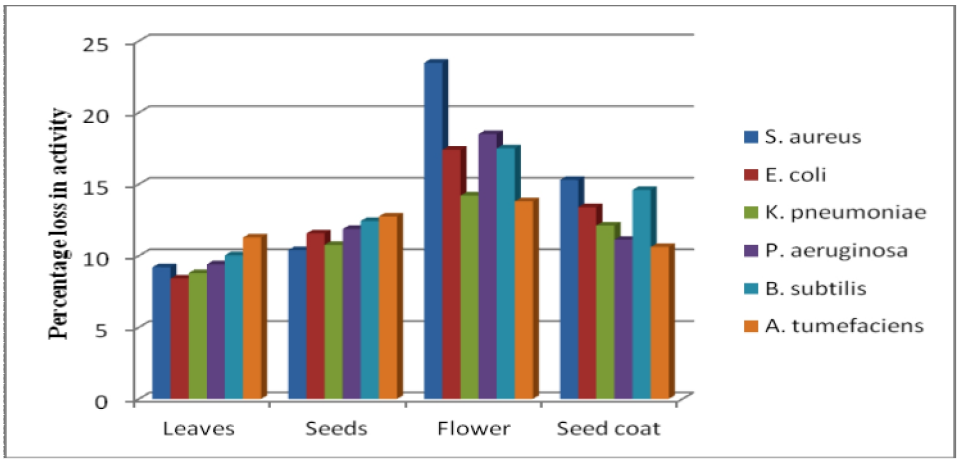

Figure 6. Thermostability of different extracts of *Sesbania aculeata* against different bacteria, at 100ºC temperature incubated for 12 hrs

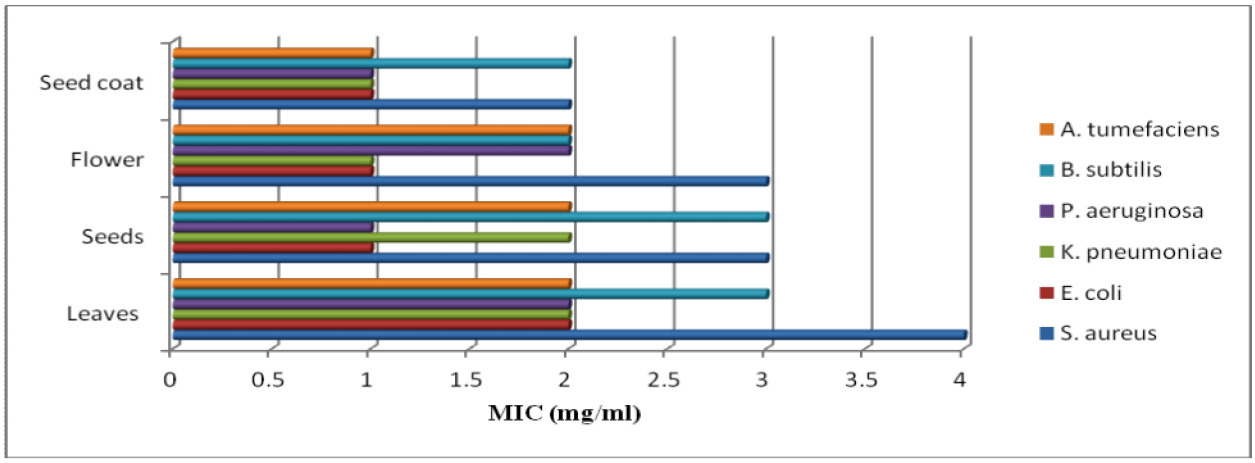

Figure 7. Minimum inhibitory concentration studies of different extracts of *Sesbania aculeata* against different bacteria strains

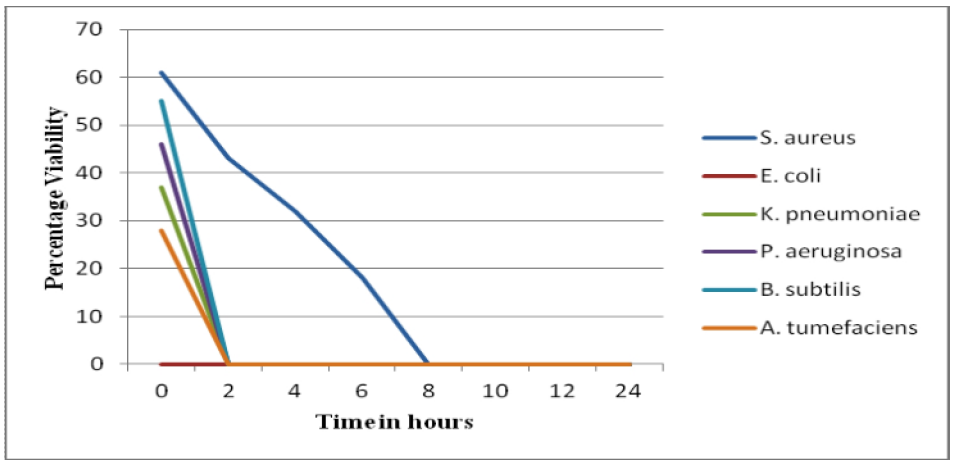

Figure 8. Viable cell count study of seed coat extract of *Sesbania aculeata* against different bacteria strains

DISCUSSION

With the increasing health concerns, the market for the herbal products related to heath has been increased tremendously. With increasing instances of resistance of microbes against the drugs and side effects of synthetic drugs, plant derived drugs have found their importance for curing diseases and therefore we always remain in the hunt of new plant metabolites for effective antimicrobial property. So, in the present study methanolic extracts of different parts of the plant *Sesbania aculeata* were evaluated for the presence of phytochemical and antibacterial screening, for the first time. The extracts were found to contain almost all the phytochemicals tested though vary slightly in composition in different parts of the plant and may be responsible for possessing diverse level of antibacterial activity. As reported earlier also that the differences in metabolite composition of different parts of the plant may be responsible for diverse uses of the *Sesbania* plant parts in Ayurveda [25]. The presence of phenols and its quantity particularly important as it is found to be responsible for antibacterial properties [30]. It has been found to have saponins present in the seeds whereas in the other species of the same genera i.e. *S. grandiflora*, saponins were completely absent or not detected [30].

Among the different pathogenic bacteria tested, the plant has showed a high activity against the plant pathogen *Agrobacterium tumefaciens*. The plant used as a green manure which may help to reduce the population of the plant pathogens in the soil that will protect the next crop from infection as well as could be used in formulations for the control of various plant bacterial diseases. Though the extracts showed broad spectrum of action as evident from potent activity against both Gram positive and Gram negative bacteria but higher activity was found against Gram negative bacteria than Gram positive bacteria which may be due to the difference in some properties of Gram positive and Gram negative bacteria. Thus, this might open new avenues for antibacterial drugs against pathogenic bacteria to treat various diseases in a safer way.

Various biophysical factors such as Soxhlet cycles (10), extraction temperature (65ºC), drying temperature (50ºC) were optimized for extraction of active metabolites, checked by zones of inhibition against the bacteria. There was very significant difference observed with low activity of the extracts up to 6 cycles of Soxhlet extraction as compare to high activity at higher number of cycles maximum up to 10 cycles. More the activity at high number of cycles may be due to the compounds dissolved in the extracting solvent more, as solvent in pure form gets access to the

compounds in the powdered plant material in each cycle [28, 34]. After 10 cycles, no increase in the activity of the extracts observed, which may be due to the fact that almost all compounds that can be extracted has been dissolved in the solvent and been extracted. The extraction temperature of the solvent was found to be at its boiling temperature [24, 38]. The active compounds that are responsible for antibacterial activity may show increased solubility at this temperature. In accordance to earlier reports, at higher temperatures, there is very less effect on extraction as the temperature is above the boiling temperature of the extracting solvent while at temperature lower than 60ºC, the compounds are not extracted completely which may be due to less solubility at lower temperatures [24, 34, 38]. To obtain the extracts in powdered form, slightly lower temperature is required for evaporation of the methanol. A temperature of 50ºC was optimum for drying of the extracts to obtain a fine powder. There was slightly decrease in the activity as the temperature rises. This may be due to either the burning of the compounds or change in properties of active compounds which could be the result of reaction between the compounds at higher temperature [9, 39]. At lower or room temperature, the evaporation rate was very low and a viscous extract (hydrated) was obtained with significantly less activity as compared to the dry extract obtained at higher temperature ranges up to 70ºC. Among the different pH of dissolved extracts, maximum activity was found at their natural pH (5.0), though there was some increase in the activity of the extracts against the Gram negative bacteria when the pH was increased up to neutral pH but at the same time there was decrease in activity against the Gram positive bacteria, in overall the activity was found decreasing on increasing the pH towards alkalinity [14, 16]. According to earlier report in case of *K. pneumoniae* significant increase in the antibacterial activity of the extracts with increasing the pH was observed [1]. But in our study the effect of increasing the pH on sensitivity of *K. pneumoniae* was very less and was up to the 7.0 pH and above that the sensitivity decreased. This may be because of the instability of the active compounds at higher pH. The effectiveness of the extracts was also evaluated by their MIC values against the tested bacteria that ranges in between 1- 4 mg/ml depending on the plant source and bacterial strain, where the Gram negative bacteria were found more sensitive than Gram positive bacteria. Different values of MIC obtained for different plant material may be because of the different qualitative and quantitative content of metabolites [11]. The efficiency of the extracts in terms of rate of killing was checked by viable cell count studies and it has been found that the extracts possess very good bactericidal property with killing rate ranges from few minutes to maximum 20 hours as evident from the killing of *E. coli* with in few minute exposure (0-hour) time interval showing high effectiveness of the extracts. Among the different factors, temperature also plays an important role in isolation and processing of extracts and affects their activity differently. There are reports, where increases in it beyond to a threshold values it lowers the activity [16, 21, 28], as well as in some of the plant species increases in temperature to particular values it increases the activity rather decreasing [14, 22]. In the present study, extracts were thermostable as very less loss in the activity was found up to 90ºC temperature even after 12 hours of incubation which might be significant property of *Sesbania aculeata* extracts that could be used as a valuable prospective for efficient isolation of compounds and purification or other processing at high temperature.

In conclusion, extracts from different parts of *Sesbania aculeata* can be prepared in less time with highly effective metabolites using the above optimized method. The metabolites thus extracted are thermostable and least affected with pH change. Moreover, the extracts exhibit broad spectrum of antibacterial activity by inhibiting the growth of both Gram positive and Gram negative bacteria effectively and in particular highly effective in controlling the growth of plant pathogens. Also, the MIC values are low and time required for complete killing of bacteria is less, showing the effectiveness of the extracts with low dose and short duration of exposure. Therefore, *Sesbania aculeata* plant extract being having broad spectrum potent antibacterial property, which could be exploited in future to formulate the new drugs that are safe, cheap and efficient to treat the bacterial infections.

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REFERENCES

[1] Adeshina, G., Okeke, C., Onwuegbuchulam, N., Ehinmidu, J., (2009): Preliminary studies on antimicrobial activities of ethanolic extracts of *Ficus sycomorus* Linn. and *Ficus platyphylla* Del. (*Moraceae*). International Journal of Biological and Chemical Sciences, 3: 147-151.

[2] Agarwal, V.S., (1986): Economic plants of India. Botanical Survey of India. Kailash Prakashan, India, 414 p.

[3] Aliyu, A.B., Musa, A.M., Abdullahi, M.S., Ibrahim, H., Oyewale, A.O., (2011): Phytochemical screening and antibacterial activities of V*ernonia ambigua*, V*ernonia blumeoides* and V*ernonia oocephala* (*Asteraceae*). Acta Poloniae Pharmaceutica ñ Drug Research, 68(1): 67-73.

[4] Ammar, R.B., Kilani, S., Bouhlel, I., Skandrani, I., Naffeti, A., Boubaker, J., Sghaier, M.B., Bhouri, W., Mahmoud, A., Chekir-Ghedira, L., Ghedira, K., (2007): Antibacterial and cytotoxic activities of extracts from (Tunisian) *Rhamnus alaternus* (*Rhamnaceae*). Annals of Microbiology, 57: 453.

[5] Brindha, P., Sasikala, B., Purushothaman, K.K., (1981): Pharmacognostic studies on. Merugan Kizhangu. Bulletin Medicinal and EthanoBotanical Research, 3(1): 84-96.

[6] Chan, E.W.C., Lim, Y.Y., Omar, M., (2007): Antioxidant and antibacterial activity of leaves of *Etlingera* species (*Zingiberaceae*) in Peninsular Malaysia. Food Chemistry, 104(4): 1586-1593.

[7] Chan, E.W.C., Ng, V.P., Tan, V.V., Low, Y.Y., (2011): Antioxidant and antibacterial properties of *Alpinia galanga*, *Curcuma longa*, and *Etlingera elatior* (*Zingiberaceae*). Pharmacognosy Journal, 3(22): 54-61.

[8] Chanda, S., Dudhatra, S., Kaneria, M., (2010): Antioxidative and antibacterial effects of seeds and fruit rind of nutraceutical plants belonging to the *Fabaceae* family. Food Function, 1: 308-315.

[9] Cheng, Y., Xu, Q., Liu, J., Zhao, C., Xue, F., Zhao, Y., (2014): Decomposition of five phenolic compounds in high temperature water. Journal of Brazilian Chemical Society, 25(11): 2102-2107.

[10] Chew, Y.L., Chan, E.W.L., Tan, P.L., Lim, Y.Y., Stanslas, J., Goh, J.K., (2011): Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of *Leguminosae* medicinal plants in Peninsular Malaysia. BMC Complementary and Alternative Medicine, 11: 12.

[11] China, R., Mukherjee, S., Sen, S., Bose, S., Sanjukta Datta, S., Koley, H., Ghosh, S., Dhar, P., (2012): Antimicrobial activity of *Sesbania grandiflora* flower polyphenol extracts on some pathogenic bacteria and growth stimulatory effect on the probiotic organism *Lactobacillus acidophilus*. Microbiological Research, 167: 500-506.

[12] De Britto, J., Gracelin, D.H.S., Sebastian, S.R., (2011): Antibacterial activity of a few medicinal plants against *Xanthomonas campestris* and *Aeromonas hydrophila*. Journal of Biopesticides, 4(1): 57-60.

[13] de Silva, T., (1997): Industrial utilization of medicinal plants in developing countries, Non-wood Forest Products II: Medicinal plants for forest conservation and healthcare, FAO, Rome, Italy, pp. 34-44.

[14] Doughari, J.H., Manzara, S., (2008): *In vitro* antibacterial activity of crude leaf extracts of *Mangifera indica* Linn. African Journal of Microbiology Research, 2: 67-72.

[15] Duke, J.A., (1981): Handbook of legumes of world economic importance. Plenum Press, New York, pp. 216-217.

[16] Durairaj, S., Srinivasan, S., Lakshmanaperumalsamy, P., (2009): *In vitro* antibacterial activity and stability of garlic extract at different pH and temperature. Electronic Journal of Biology, 5(1): 5-10.

[17] Edeoga, H.O., Okwu, D.E., Mbaebie, B.O., (2005): Phytochemical constituents of some Nigerian plants. African Journal of Biotechnology, 4(7): 685-688.

[18] El-Amier, Y.A., Haroun, S.A., El-Shehaby, O.A., Al-hadithy, O.N., (2016): Antioxidant and antimicrobial properties of some wild *Aizoaceae* species growing in Egyptian desert. Journal of Environmental Sciences, 45(1): 1-10.

[19] Evans, W.C., (1994): Trease, Text Book of Pharmacognosy, ELBS, 3rd ed, pp. 177- 179, 247, London.

[20] Farnsworth, N.R., Morris, R.W., (1976): Higher plants: the sleeping giant of drug development. American Journal of Pharmacy Society, 148: 46-52.

[21] Ginovyan, M.M., (2017): Effect of heat treatment on antimicrobial activity of crude extracts of some Armenian herbs. Procedings of the Yerevan State University Chemistry and Biology, 51(2): 113-117.

[22] Hoquea, M.M., Barib, M.L., Junejac, V.K., Kawamotob, S., (2008): Antimicrobial activity of cloves and cinnamon extracts against food borne pathogens and spoilage bacteria, and inactivation of *Listeria monocytogenes* in ground chicken meat with their essential oils. Report of National Food Research Institute, 72: 9-21.

[23] Kokate, C.K., Purohit, A.P., Gokhale, S.B., (2009): Pharmacognosy, 17th edition, Nirali Prakashan, pp. 99, 231, 185, 271, 445, Pune.

[24] Lawson, O.S., Oyewumi, A., Ologunagba, F.O., Ojomo, A.O., (2010): Evaluation of the parameters affecting the solvent extraction of soybean oil. ARPN Journal of Engineering and Applied Sciences, 5(10): 51-55.

[25] Malviya, R., Sharma, R., Mishra, A., Rama Murthy, A., (2013): Agasthya (*Sesbania grandiflora* Linn.): Ayurvedic approach. Universal Journal of Pharmacy, 02(04): 1-5.

[26] Mokoka, T.A., McGaw, L.J., Mdee, L.K., Bagla, V.P., Iwalewa, E.O., Eloff, J.N., (2013): Antimicrobial activity and cytotoxicity of triterpenes isolated from leaves of *Maytenus undata* (*Celastraceae*). MC Complementary and Alternative Medicine, 13: 111.

[27] Naili, M.B., Alghazeer, R.O., Saleh, N.A., Al-Najjar, A.Y., (2010): Evaluation of antibacterial and antioxidant activities of *Artemisia campestris* (*Asteraceae*) and *Ziziphus lotus* (*Rhamnaceae*). Arabian Journal of Chemistry, 3(2): 79-84.

[28] Olivares-Tenorio, M.L., Ruud Verkerk, R., van Boekel, M.A.J.S., Dekker, M., (2017): Thermal stability of phytochemicals, HMF and antioxidant activity in cape gooseberry (*Physalis peruviana* L.). Journal of Functional Foods, 32: 46-57.

[29] Onim, J.F.M., (1986): Multiple use of pigeonpea. In: Proceedings of ICRISAT Consultative Group Meeting for Eastern and Central African Regional Research on Grain Legumes held at ILCA, Addis Ababa, Ethiopia, 8-10 December 1986, pp. 115-120.

[30] Ouattara, M.B., Konaté, K., Kiendrébéogo, M., Ouattara, N., Compaore, M., Meda, R., Millogo-Rasolodimby, J., Nacoulma, O.G., (2011): Antibacterial potential and antioxidant activity of polyphenols of *Sesbania grandiflora*. Current Research Journal of Biological Sciences, 34: 351-356.

[31] Parekh, J., Chanda, S., (2006): *In vitro* antimicrobial activities of extracts of *Launaea procumbens* Roxb. (*Labiatae*), *Vitis vinifera* L. (*Vitaceae*) and *Cyperus rotundus* L. (*Cyperaceae*). African Journal of Biomedical Research, 9: 8993.

[32] Shinde, S.L., Junne, S.B., Wadje, S.S., Baig, M.M., (2009): The diversity of antibacterial compounds of *Terminalia* species (*Combretaceae*). Pakistan Journal of Biological Sciences, 12(22): 1483-1486.

[33] Steenkamp, V., Fernandes, A.C., van Rensburg, C.E.J., (2007): Antibacterial activity of Venda medicinal plants. Fitoterapia, 78(7–8): 561-564.

[34] Suwari, Kotta, H.Z., Buang, Y., (2017): Optimization of soxhlet extraction and physicochemical analysis of crop oil from seed kernel of Feun Kase (*Thevetia peruviana*). AIP Conference Proceedings 1911, 020005.

[35] Suzuki, H., Okubo, L., Yamazaki, S., Suzuki, K., Mitsuya, H., Toda, S., (1989): Inhibition of the infectivity and cytopathic effect of the human immunodeficiency virus by water soluble lignin in an extract of the culture medium of *Lentinus edodes* mycelia (LEM). Biochemical and Biophysical Research Communications, 60: 367-373.

[36] Vital, P.G., Rivera, W.L., (2011): Antimicrobial activity, cytotoxicity, and phytochemical screening of *Voacanga globosa* (Blanco) Merr. leaf extract (*Apocynaceae*). Asian Pacific Journal of Tropical Medicine, 4(10): 824-828.

[37] Voravuthikunchai, S.P., Limsuwan, S., Supapol, O., Subhadhirasakul, S., (2006): Antibacterial activity of extracts from family *Zingiberaceae* against foodborne pathogens. Journal of Food Safety, 26(4): 325-334.
[38] Wilga, J., Kot- Wasik, A., Namiesnik, J., (2007): Comparison of extraction techniques of robenidine from poultry feed samples. Talanta, 73: 812-819.
[39] Xu, G., Ye, X., Chen, J., Liu, D., (2007): Effect of heat treatment on the phenolic compounds and antioxidant capacity of citrus peel extract. Journal of Agricultural and Food Chemistry, 55(2): 330-335.

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