Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6571

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (3):** 65-69 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Screening of Antibacterial and Antifungal Properties of *Sambucus wightania* Leaves

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Received: 16.05.2018 | Revised: 14.06.2018 | Accepted: 20.06.2018

ABSTRACT

Multi drug resistance issue has driven focus towards use of natural products against treatment of microbial infections. The current study investigated antibacterial and antifungal properties of Sambucus wightiana leaves. Antibacterial and antifungal assays revealed marked sensitivity against 10 bacterial and 8 fungal strains. The petroleum ether extract showed significant zones of inhibition (P<0.05) against four micro-organisms i.e. two bacterial strains (E.coli, Serratia marcescens) and two fungal strains (Candida albicans and Aspergillus flavus)with inhibition zones as 14±0.7mm, 10 ± 1.1mm, 7±0.5mm and 8.5±0.9 mm respectively. The methanol extract showed significant antimicrobial activity (P<0.05) against 6 pathogenic bacterial strains with different zones of inhibition. MIC has showed that methanolic extract has displayed strong antimicrobial activity as compared to standard ampicillin.

Key words: *Sambucus wightania, Antimicrobial activity, Inhibition zone.*

INTRODUCTION

Multi drug resistance (MDR) due to commercial antibiotics is considered to be a major health issue globally. MDR has aggravated from the recent past due to frequent and widespread use of chemical based synthetic drugs to treat different infections. The emergence of resistant bacterial and fungal strains has posed human life at a very critical juncture to evade from multiple infections, such as caused by methicillin-resistant *Staphylococcus aureus* (MRSA).

The medicinal and aromatic plants could act as natural biosynthetic laboratories for lead bioactive molecules which could be utilized for therapeutic applications.

Cite this article: Mir, M.A., Dar, Z.A., Mani, P., Dar, N., Bhat, W., Rao, M.V., Screening of Antibacterial and Antifungal Properties of *Sambucus wightania* Leaves, *Int. J. Pure App. Biosci.* **6(3):** 65-69 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6571

The diverse traditional medicinal use of the plants has led thorough scientific investigations to find out the bioactive constituents responsible for these medicinal uses against different diseases[1]. A growing interest towards plant derived pharmaceutically valuable molecules is very evident as various pharmaceutical companies such as Boehringer, Syntex, Merck, Glaxo and CIBA have in-house dedicated facilitites for studying new drugs from natural sources[2]. Hence, plant derived bioactive molecules are seen as a promising alternatives to tackle multi drug resistant microbial strains safely, effectively and economically[3]. Further, strong scientific evidences indicated that plant derived small molecules could act more safer and effective to treat and prevent different types of health ailments including cancer, inflammation, infections etc.[4] *Sambucus wightiana* is an ethno-medicinal plant with beautiful orange-reddish, edible berries which are attracting ecotourists[5]; it grows from Afghanistan to Himalayan regions of Pakistan and India. Different parts of this plant species are used for ethno-medicinal remedies such as emetic, diuretic, to treat skin and stomach diseases. The various important phytocompounds found in different parts of *Sambucus* species are phenolics, anthocyanins, quercetin, kaempferol etc[6], which could be responsible for its various bioactivities. Therefore, the current investigation was carried out to test antibacterial and antifungal properties of *S. wightiana* leaves against various pathogenic microbes.

MATERIAL AND METHODS

Plant material collection

Sambucus wightiana leaves were collected from Ahribal region of Kashmir, India (2,266 m above sea level) and authenticated at Centre for Biodiversity and Taxonomy, University of Kashmir herbarium (KASH) under voucher specimen number KASH-1732. Leaves were allowed for shade drying, grind into fine powder, cleared via mesh and kept at 5^0C for further processing.

Biological materials

Ten Bacterial and 8 fungal strains were mostly procured from National Microbial repository (MTTC, Chandigarh, India). The bacterial strains were as *Escherichia coli* (MTCC-77), Proteus mirabilis (MTCC-425), Pseudomonas aeruginosa (MTCC-424), Staphylococcus aureus (MTCC-3160), Serratiamarcescens (MTCC-7103), Bacillus cereus (MTCC-1272), Klebsiella pneumoniae (7028), Enterococcus species (9728), Citrobacterspps., Serratiaplymuthica. Fungal strains used were as Malassezia furfur (MTCC-1374), Candida albicans (MTCC-1637), Stachybotrys chartarum (MTCC-2146), Trichophyton rubrum (MTCC-7859), Aspergillus niger (MTTC-478), Aspergillus flavus (MTTC-9606), Tricholosporumviolaceum and Penicillium marneffei. The bacterial strains were grown in the respective media overnight at 37°C and maintained on agar slants at 40^0C till further analysis while as fungal strains were incubated for 5 days in Potato dextrose agar (PDA) medium at 25^0C.

Phytochemical extraction

The powder form of leave sample was subjected to phytochemical extraction using two solvents i.e. Petroleum ether (PE) and methanol (MeOH) through Soxhlets extraction. Crude extracts so obtained were filtered using Whatman No. 1 filter paper and dried using rotary evaporator. The dried extracts were subjected to further bioactivity assays[7, 8].

Determination of *In-vitro* antibacterial and antifungal activities

The extracts obtained were subjected to agar well diffusion to assess antimicrobial properties[8]. Briefly, 25 ml of Mueller Hinton Agar (MHA) medium and potato dextrose agar (PDA) medium for bacterial and fungal culture were respectively transferred into each sterilized petri plate in a laminar air flow, plates were allowed for solidification at room temperature. The wells of 8 mm diameter were made using sterile borer and the respective bacterial and fungal cultures were inoculated by swab plating technique. Extracts were maintained at concentration 15mg/ml using DMSO as solvent. 50 µl of each extract was used in each well, while as plain DMSO acted as negative control. Incubation of plates was done for 24hrs at 37°C (Bacteria) and 3-5 days at room temperature (Fungi). Post incubation,

zone of microbial inhibition was recorded (mm) for each well.

The extracts which showed initial positive antimicrobial results were subject minimum inhibitory concentration (MIC) using agar well diffusion method[9]. The various concentrations of extracts were used i.e. 20, 40, 60, 80,100µg/ml, dissolved in DMSO. Two commercial antibiotics namely Gentamycin and ampicillin (10 µg/ml) were used (Positive control) for antibacterial activity, while as Ketoconazole (30 µg/ml) served antifungal positive control agent. The proportion index (PI) of each extract was determined[10].

$$\text{Proportion Index (PI)} = \frac{\text{Number of positive results obtained for individual extract}}{\text{Total number of tests carried out for each extract}}$$

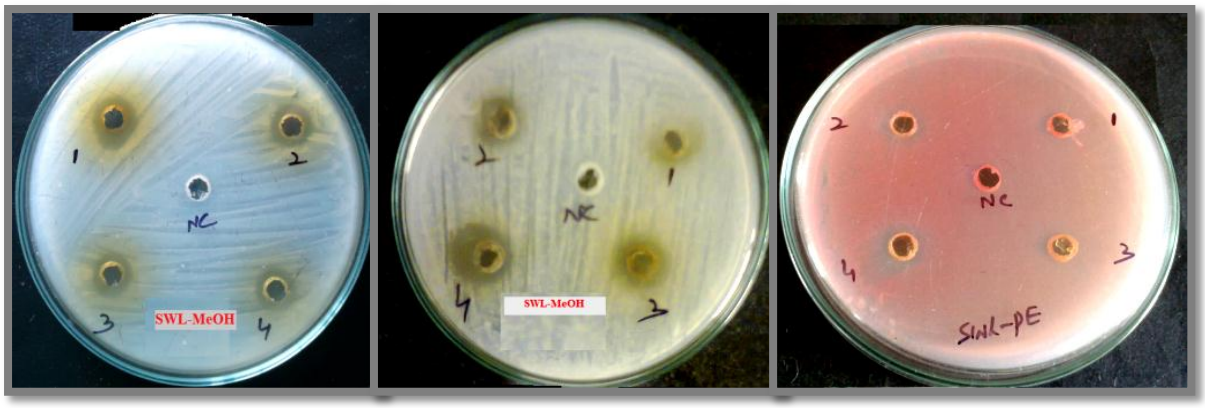
RESULTS

The results derived from antibacterial & antifungal *In-vitro* assays of *Sambucus wightania* extracts against10 bacterial and 8 fungal strains have demonstrated that highest inhibitory zone of 16±2.6 mm was showed by *Citrobacter spps* (Table 1, Fig.1). The petroleum ether extract showed significant zones of inhibition ($P<0.05$) against four microorganisms i.e. Two bacterial strains (*E.coli, Serratia marcescens)* and two fungal strains *(Candida albicans and Aspergillus flavus)* with inhibition zones as 14±0.7mm, 10 ± 1.1mm, 7±0.5mm and 8.5±0.9 mm respectively. The methanol extract showed significant antimicrobial activity ($P<0.05$) against 6 pathogenic bacterial strains with different zones of inhibition against *Citrobacter spps., E. coli, Serratia marcescens, Proteus mirabilis, Staphylococcus aureus & Enterococcus spps*. Nevertheless, the methanol extract did not show inhibitory zones against any fungal isolate. The higher proportion index (PI) was shown by methanol extract (0.33) while as lower proportion index was found in petroleum ether extract (0.22). The pathogenic microorganisms which showed antimicrobial activities were subjected for determination of Minimal Inhibitory Concentrations (MIC). Although, ampicillin showed resistance against *Enterococcus spps.and Citrobacter spps.*, however methanolic extract of *S. wightania* have showed activity against both the microbes with inhibitory zones as 7.3±1.1 and 8.2±1.5 mm, therefore displaying their strong antimicrobial activity as compared to standard ampicillin. Also, MIC value of petroleum ether was found higher than ketokonazole against *Aspergillus flavus*, a lower but significant zone of inhibitionwas found against *Candida albicans (*6.1±0.3 mm).

Table 1: *In-vitro* antimicrobial activities of *Sambucus wightania* Wall. leaf extracts

S.No.	Microorganisms	PE	MeOH
1	*E. coli* (MTCC-077)	14±0.7[a]	14.0±1.5[a]
2	*Proteus mirabilis* MTCC-425	NI	10.0±0.3[b]
3	*Serratia marcescens* (MTCC-7103)	10.0±1.1[b]	12.0±1.2[c]
4	*Staphylococcus aureus* (MTCC-3160)	NI	8±0.6[d]
5	*Pseudomonas aeruginosa* (MTCC-424)	NI	NI
6	*Bacillus cereus* (MTCC-1272)	NI	NI
7	*Klebsiella spps.* (MTTC-7028)	NI	NI
8	*Enterococcus spps.* (MTTC-9728)	NI	8.0±0.5[d]
9	*Citrobacter spps.*	NI	16.0±2.6[e]
10	*Serratia plymuthica*	NI	NI
11	*Malassezia furfur* (MTTC-1374)	NI	NI
12	*Candida albicans* (MTTC-1637)	7.0±0.5[c]	8.5±1.2[d]
13	*Stachybotrys chartarum* (MTTC-2146)	NI	NI
14	*Trichophyton rubrum* (MTCC-7859)	NI	NI
15	*Aspergillus niger* (MTTC-478)	NI	NI
16	*Aspergillus flavus* (MTTC-9606)	8.5±0.9[d]	NI
17	*Tricholosporum violaceum*	NI	NI
18	*Penicillium marneffei*	NI	NI
	Proportion Index (PI)	**0.22**	**0.33**

Note: PE-Petroleum ether, MeOH-Methanol, AQ-Aqueous. [*Inhibition zone, NI-No inhibition].Values are mean ± SD, n=3; and values with different letters shows significant differences at $P<0.05$ level).



A **B** **C**

Fig. 1: Inhibitory zones of *Sambucus wightania* extracts against selected bacterial pathogenic microorganisms A) *Enterococcus species B) P.aeruginosa C) S.marcescens.*
(Values are mean ± SD, n=3, P<0.05)

DISCUSSION

The current study has demonstrated significant levels of antimicrobial properties of *S. wightania* leaf extracts. Difference in the antimicrobial activities of different extracts is based on extracts chemical composition, metabolism and permeability of microbial membranes[11]. The presence of different phytocompounds found in the leaf portion of this species could be responsible for such antimicrobial activities, thus validating the traditional claim to treat different disease such as stomach disorders, skin diseases and also foot and mouth disease in cattle. Furthermore, it is also reported that leaves of American elder plant *Viburnum opulus*were traditionally used as a disinfectants to wash sores to prevent infection and also to treat upper respiratory tract infections[12]. These medicinal properties could be due to their antimicrobial properties against microbes responsible for such diseases such as *Staphylococcus aureus* (Skin infections). There is multiple drug resistance (MDR) and side effects of commercial antibiotics due to their indiscriminate use, this has become a major global concern to treat various infectious diseases such as methicillin-resistant *Staphylococcus aureus* (MRSA) which is responsible for skin and soft tissue infections[13]. For this reason, the researchers are keen for herbal based lead antimicrobials which could be used against multiple drug resistance (MDR) microbial strains or to control infections globally[14] and it has been found that naturally occurring oils, spices, herbs etc could be used against food spoiling pathogens such as *Bacillus cereus and E.coli* or to treat skin causing infections.The presence of various plant based secondary metabolites that have been found to possess antimicrobial properties such as tannins, flavonoids, alkaloids, terpenoids, quinines, coumarins, lectins, polypeptides & polyacetylenes etc. which makes medicinal plants safer, cost effective and clinically effective alternative to synthetic antibiotics.The presence of volatile oil compounds could be responsible for the antifungal activities which has been found against *A. flavus*[15]. The existence of antimicrobial properties of *S. wightania* leaf extracts towards various bacterial and fungal strains indicate its potential to treat various drug resistant pathogenic microorganisms and could act as a source of broad spectrum antimicrobial agents. Conclusively, the results of this study provide justification for some of its ethno-medicinal properties. The existence of novel, functional and biologically active constituents could give new dimensions towards its usage as a medicinal plant rather than just being considered as menace of invasive plant.

STATISTICAL ANALYSIS

All the measurements were done in triplicates (n=3) and results are expressed in mean ± SD. Prism Pad was used for analysis and graph creation.

CONCLUSION

Based on the results of this study, it is concluded that the leaf portion of *S. wightania* has potential to prevent and treat different pathogenic bacterial and fungal infections, and as such it may be used as an alternative therapy to treat microbial infections.

REFERENCES

1. Mandal, S., Patra, A., Samanta, A., Roy, S., Mandal, A., Mahapatra, T. D., ... & Nandi, D. K. Analysis of phytochemical profile of Terminalia arjuna bark extract with antioxidative and antimicrobial properties. *Asian Pacific journal of tropical biomedicine*, **3(12):** 960-966 (2013).
2. Rates, S.M.K., Plants as source of drugs. *Toxicon.* **39:** 603–613 (2001).
3. Rosina Khan, Barira Islam, Mohd Akram, Shazi Shakil, Anis Ahmad, et al., Antimicrobial activity of five herbal extracts against multidrug resistant (MDR) strains of bacteria and fungus of clinical origin. *Molecules*, **14:** 586-597 (2009).
4. Jigna Parekh, and Sumitra Chanda, Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *Afr. J. Biomed. Res*. **10:** 175–181 (2007).
5. Kaul, M.K., Medicinal Plants of Kashmir and Ladhak. *Indus PublisihningCo, New Delhi*, 144 (1997).
6. Ballabh, B., Chaurasia, O.P., Ahmed, Z., Singh, S.B., Traditional medicinal plants of cold desert Ladakh—Used against kidney and urinary disorders. *J Ethnopharmacol*, **118:** 331–339 (2008).
7. Harborne, J.B., Phytochemical methods: A guide to modern technique of plant analysis. *Chapman and Hall*, London (1998).
8. Perez C., Paul M., Bazerque P., Antibiotic assay by agar-well diffusion method. *Acta Biologiae Medicine Experimentalist*, **15:** 113–115 (1990).
9. Gautam, S.S., Navneet, Kumar, S., Prabhat, Screening of antibacterial activity of nepetaciliaris benth. Against respiratory tract pathogens. kathmandu university *Journal of science, engineering and technology*. I, 100-103 (2012).
10. Gopalakrishnan, S., Rajameena, R., Vadivel, E., Antimicrobial activity of the leaves of Myxopyrum serratulum AW Hill. *Int. J. Pharm. Sci. Drug Res.* **1:** 31-34 (2012).
11. Ramkumar, K. M., Rajaguru, P., Ananthan, R., Antimicrobial properties and phytochemical constituents of an antidiabetic plant Gymnema montanum. *Adv Biol Res*. **1:** 67-71 (2007).
12. Charlebois, D., Byers, P.L., Finn, C.E. and Thomas, A.L., Elderberry: Botany, Horticulture, and Potential as a Food and Medicinal Crop. *Horticultural Reviews.* 37 (2010).
13. Middleton, E.Jr. and Kandaswami, C., Effects of flavonoids on immune and inflammatory cell functions. *Biochem Pharmacol.* **43:** 1167-1179 (1992).
14. Mir, M.A., Rajesh, T.S., Rameashkannan, M.V., Pala, RA., Balaji. M.R., A comparative study of Phytochemical and Antimicrobial properties of Stigmas and Stamens of Saffron (*Crocus sativus* L.). *Adv. biotech*. **6:** 35-38 (2011).
15. Feizbakhsh, A., Pazoki, H., Mohammadrezaei, V. and Ebrahimzadeh, M., Effect of Phytohormones on the Composition of Sambucus ebulus Leaf Essential Oil. *Trop J Pharm Res*. **13:** 581-586 (2014).