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Spermadictyon suaveolens: A potential natural antimicrobial and antioxidant source

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Abstract

The present work is an effort to discover ethnopharmacological effects, such as anti-microbial, MIC, antioxidant assay, viz; total antioxidant, total phenolic contents, ferric reducing antioxidant power (FRAP) and DPPH analysis. The crude extracts of bark and leaves of plant *Spermadictyonsuaveolens*Roxb. was obtained in polar and nonpolar solvents viz; petroleum ether, chloroform, methanol and distilled water. The physical and chemical properties of plant extracts were determined like colour, texture, chemical nature and percentage yield. *S. suaveolens*bark extract in water exposed maximum yield, i.e. 2.3% The pathogenic bacterial and fungal strains used were including two gram positive bacteria (*Staphylococcus aureus*and *Bacillus subtilis*), three gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*and*Klebsiellapneumoniae*) and three fungal strains(*Aspergillusniger*, *Aspergillusoryzae* and *Fusariumsolani*).Maximum zone of inhibition of *S. suaveolens*was leaf methanolic extract against *S. aureus*, i.e. 32±9.60mm whereas petroleum ether extract of leaf displayed maximum zone against *F. solani*, 29±0.72mm. Methanolic extract of leaf of*Spermadictyonsuaveolens* showed minimum inhibitory concentration (MIC) against gram positive *S. aureus* 0.03±0.0.01 at 0.9g/mL.Maximum percentage scavenging by DPPH assay was shown by the bark methanolic extract of *S. suaveolens* showed 78.317% at 500µg/mL.IC_{50} values methanolic extract of bark of *S. suaveolens*25.81µg/mL.Highest antioxidant activity was displayed by methanol extract of bark of *S. suaveolens*, i.e0.896±0.13. Leaf chloroform extract of *S. suaveolens* showed maximum FRAP value, i.e. 211µg/mL.

Keywords: antimicrobial, antioxidant assay, MIC, DPPH assay, IC50.

Introduction

Plants are essential part of our life. They are used as food, fuel, fodder and medicines. About 7000 plants species are used as food. Plants as traditional medicines are used to treat and cure different diseases by 80% of the world and World Health Organization has listed about 2100 medicinal plant species around the world[1].

The establishment of conventional medicinal system built by the plants plays an important role in the health care of people. The traditional drugs are referred to health and prevent illness for the well-being for thousands of years. A large number of populations in non-industrial countries still rely on conventional medicinal traditions to prevent and cure illness [2]. About 80% of inhabitants of developing countries depend on the drugs obtained from plants for their chief health care directly or indirectly [3].

Plants have a very important role in our lives because they are a source of wide range with biochemical of great biological importance. Plants perform numerous executions in the daily life of human beings. The usefulness of plants is due to the fact that they accommodate a range of accessory metabolites. These metabolites perform necessary functions in keeping up the life concerned activities [4]. Majority of the herbs are a source of phytochemicals like primary metabolites and secondary metabolites for their metabolic activities. The secondary metabolites play important role in defense mechanism and also have therapeutic value [5]. The medicinal value associated with plants is due to the variety of chemical substances present in them that produce biological action on human body [6].

About 70% of the incident histories from the US hospitals, the victims had bacterial infections and that bacterial strains had attained resistance to some extent. In this regard, the most infectious of all was *Staphylococcus aureus*. It is gram positive bacterium having methicillin resistance. This bacterial strain was present in 50% samples extracted from recovering people due to its massive propagation. For the wellbeing of mankind, suitable inspections should be applied for the exploration of new antimicrobial agents. With the emergence of resistant bacterial strains, steps must be taken to reduce the effect of these bacteria by understanding the genetic mechanism and introduction of new drugs, either synthetic or natural [7].

Oxygen is essential for life on earth but under certain circumstances it shows detrimental effect on human health. The reactive form of oxygen that is deleterious to health is known as

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Reactive Oxygen Species (ROS). These reactive oxygen species have free floating electrons rather than match pair's which are unstable and reactive [8].

When the cell produces energy, free oxygen and free radicals are produced as by-product in cellular redox reaction. At high concentration ROS induces oxidative stresses which harm and distract the basis of cellular structure, i.e. lipids, proteins and DNA. The human body has a check mechanism on oxidative stresses by generating antioxidants either naturally by itself or by food and supplements externally. These antioxidative agents not only repair the cell and prevent injuries caused by ROS but also lower the risk of causing diseases and enhance the immunity against diseases [9].

The presence of antioxidants influences the mental and physical health of human beings. The most important source of antioxidants is present in different parts of plants which are the derivatives of phenolic and flavonoids [10]. A variety of synthetic antioxidants have been introduced in food products, i.e. butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG) and tertbutylhydroquinone (TBHQ). These synthetic antioxidants are not only cheap and efficient but also have lethal effect which damages the health. On these grounds, there is a need to find out and identify the natural compounds which are less detrimental and replace the synthetic antioxidants [11].

SpermadictyonsuaveolensRoxb

Spermadictyonsuaveolens belongs to family Rubiaceae, commonly known as Coffee family. It contains a large number of medicinally, horticulturally and economically important plants like Coffee (*Coffea*), Quinine (*Cinchona officinalis*), Tipecacuanha (*Carapicheaipecacuanha*), Madder (*Rubia*), West Indian jasmine (*Ixora*), Partridge berry (*Mitchella*). These plants are present in warmer tropical regions throughout the world with 611 genera and 13000 species. Rubiaceae family is taken to be at fourth by species number and at fifth by genera. The selected plant species *Spermadictyonsuaveolens*Roxb.is foundin Kashmir region and Northern Areas of Pakistan. It is locally called Ban Champa. It is an under shrub about 1-2m tall with spreading branches. The plant contains scented flowers. Fine velvety leaves are 10-20cm in length which gives unpleasant smell on rubbing [12].

Material and Methods

The selected plant *Spermadictyonsuaveolens* was collected from District Kotli, Azad Jammu and Kashmir in the month of September 2012. The plant were identified and submitted in Dr. Sultan Ahmad Herbarium, Department of Botany, GC University, Lahore with Specimen Voucher No.GC. Herb.Bot. 2287.

Tested microorganism

Bacterial strains

Bacillus subtilis: A gram positive bacterium which produces the enzyme subtilisin cause dermal allergic or hypersensitivity reactions in individuals.

Staphylococcus aureus: It is a gram positive bacterium that causes minor dermal diseases to serious diseases such as this as pneumonia, meningitis, endocarditis and toxic shock syndrome.

Pseudomonas aeruginosa: A gram negative bacterium, causative agent of septic shock, gastrointestinal infections, soft tissues and several skin infections.

Klebsiellapneumonia:A gram negative bacterium causes pneumonia, urinary tract infections and bronchitis.

Escherichia coli: It is a gram negative bacterium causing urinary tract infections, diarrhea, bacteremia and cholecystitis. It also causes anemia and kidney failure.

Fungal strains

Aspergillusniger: It is the profuse fungal species all over the world. It can grow on slight nutrients available. It causes hypersensitivity reactions like asthma and allergic infections in humans. It causes deafness in human.

Apergillusoryzae: It produces mycotoxins that cause pulmonary aspergilloma.

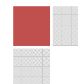
Fusariumsolani: It causes nail infection called onychomycosis. It also causes allergic infection as sinusitis and superficial infections as keratitis. It is plant pathogenic which causes diseases on cereal grains.

The plant parts, i.e. barks and leaves of *Spermadictyonsuaveolens*was separated and subjected to desiccation in shady conditions at room temperature. To avoid the disintegration of phytochemical components of the plant parts, they should not be kept at temperature above 50^0C. The desiccated plant materials were grinded in grinding mill to get fine powder.In this process of maceration, the measured quantity of powered plant material was poured in glass container and the solvent was added in to it. The purpose of using powered plant material for maceration was to ensure that its phytochemical constituents were in proximity to the added solvents. The macerated plant material was placed for about 8-15 days depending on the nature of plant substances and its solubility in the solvent. After the accomplishment of required time period, the material was allowed to filter through filter paper using Whatman filter paper. The solvents applied for maceration were selected according to their polarity indices from non-polar to polar.

Table: Polarity indices of solvents

Solvents used	Polarity indices (P)
Petroleum ether	0.1
Chloroform	4.1
Methanol	5.1
Water	10.2

Eventually the extracts (petroleum ether, chloroform and methanol) were subjected to dehydration and dry out to make it concentrated by using Rotary evaporator on at low temperature. The aqueous extracts obtained from distilled water were desiccated on Lyophilizer. Final concentrated of plant materials stored at 20^0C. The physical examination of plant extract was accomplished by the analysis of its color, texture and % yield before the evaluation of



antimicrobial and antioxidant activities. Formula applied for the calculation of % extraction yield given underneath:

$$\% \text{ Extraction yield} = \frac{\text{Weight of plant extract}}{\text{Weight of initial plant sample}} \quad 100$$

Method

The investigation of bacterial strains was carried out on nutrient agar medium defined by [13] method. The investigation of bacterial strains was carried out on nutrient agar medium defined by [13] method. The fungal strains employed for antifungal investigation was cultured on PDA (potato dextrose agar) medium. The methodology adopted for the preparation of slant defined by Johansen [14].

Bacterial inoculums were adjusted to1.5 10^8 CFU/mL by using McFarland turbidity standards proposed. Spectrophotometer was adjusted to 0.5 McFarland turbidity standards at 625nm, after that the turbidity of bacterial inoculums were measured. 3- 4 colonies of bacterial strains were transferred to 10ml of saline 0.9% $NaCl_2$ solution and incubated at 37 ± 2 ºC temperature for 3-4 hours. The accurate quantity of bacterial cells achieved if the values are 0.8 to 0.13. For fungal inoculums adjustment 4-5 colonies from the fungal culture were transferred to10mL 0.9% $NaCl_2$ solution under aseptic conditions. After3-4 hours incubation the turbidity of inoculums were maintained to 1 10^3 - 5 10^3 CFU/mL [15]. After the calibration 1mL of that sample was used in 1000ml of distilled water to achieve the requisite consistency.

The agar well diffusionmethod was modified by[16] at the University of Washington School Of Medicine for the estimating of zone of inhibition while MIC (Minimum inhibitory concentration) was done by broth dilution assay established by [17].

For the estimation of antioxidant assay stock solution of dried samples of *Spermadictyonsuaveolens*were prepared. 0.02 grams of dried plant material was measured and dissolved in 20ml of methanol to form 1000µg/mL stock solution. From this stock solution various concentrations were prepared as 500µg/ml, 250µg/ml, 125µg/ml and 60µg/ml. four different methods were applied for the investigation of antioxidant potential. DPPH (1, 1, diphenyl-2-picryl hydrazyl) radical scavenging activity was performed by the method of [18],Phosphomolybdenum assay was done by the method of [19] while [20] procedure with some adaption was applied to analyze FRAP. The method of [21] was applied to determine the total phenolic contents of *S. suaveolens*.

Results and Discussion

The selected plant*Spermadictyonsuaveolens*Roxb.leaves and barks (Pl. 1) were dried and extracts were prepared in non-polar and polar solvents for the investigation of antimicrobial and antioxidant potential. The physical properties of plants extracts were observed on the basis of color, appearance and texture (Table 1).

Table 1: Physical properties of extract of *S. suaveolens*

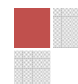
Physical Appearance of Extract of *S. suaveolens*				
Plant Parts	Extract	Color	Appearance	Texture
Bark	Petroleum Ether	Light brown	Sticky	semi-Granular
	Chloroform	Grape Green	Sticky	Smooth
	Methanol	Rust	Non-Sticky	Semi-Granular
	Water	Dark Brown	Non-Sticky	Granular
Leaf	Petroleum Ether	Dark Green	Sticky	Smooth
	Chloroform	Golden Green	Sticky	Smooth
	Methanol	Golden Brown	Sticky	Smooth
	Water	Blackish Brown	Non-Sticky	Granular

Water extract of bark of *S. suaveolens* exhibited maximum percentage yield, i.e. 2.3% whereas chloroform extract displayed 1.9%. Minimum yield of bark was shown by petroleum ether extract (1.4%). Leaf methanol extract of *S. suaveolens* shown maximum yield (2.0%) and chloroform extract displayed 1.8%. Minimum yield was displayed by petroleum ether extract, i.e. 0.9% (Table 2).

For the estimation of antibacterial and antifungal activities, well diffusion method was applied and standard antibacterial / antifungal disc were used against microbes as positive control. The zone of inhibition of the plant extracts were compared with different standard disc against bacterial (Pl. 2) and fungal strains (Pl. 2).

Table 2: % Extraction Yield of Bark and Leaf of *S. suaveolens*

S. suaveolens		
Plant Parts	Solvents	% Yield
Bark	Petroleum ether	1.4
	Chloroform	1.9
	Methanol	1.8
	Water	2.3
Leaf	Petroleum ether	0.9
	Chloroform	1.8
	Methanol	2
	Water	1.7



The entire tested standard discs were resistant against microbes. (Table 3and Table 4)

Table 3: Zone of inhibition produced by standard antibacterial disc (Positive control)

Antibacterial Standard Disc	Conc. (µg)	Bacterial Strains	Zone of Inhibition
Amikacin	30	*B. subtilis*	18
Azithromycin	15	*S. aureus*	13
Erythromycin	15	*K. pneumoniae*	18
Cephradine	30	*E. coli*	24
Ampicillin	10	*P. aeruginosa*	23

Table 4: Zone of inhibition produced by standard antifungal disc (Positive control)

Antifungal standard disc	Conc. (µg)	Zone of inhibition (mm)	
Grisofluvin	100	***A. oryzae***	27±0.5
Voriconazole	100	***A. niger***	40±2.0
Itraconale	100	***F. solani***	10±1.8

Non-polar and polar solvents, in which plant materials were macerated, also tested against all strains of bacteria and fungi as negative control. Petroleum ether was found to be resistant against*K. pneumoniae* and *E.coli* whereas chloroform extract showed inhibition against *K. pneumonia* while other solvents were found to be resistant against bacterial strains (Table 5). Methanol showed resistance against*P. aeruginosa*, *A. oryzae* and *F. solani* (Table 6).

Table 5: Zone of inhibition of solvents against bacterial strains (Negative control)

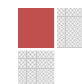
Solvent	Quantity	Zone of inhibition against Bacterial strains (mm)				
		S. aureus	***E. coli***	***P. aeruginosa***	***K. pneumonia***	***B. subtilis***
P. E	1.5	0±0	5.2	0±0	6	0±0
Chloroform	1.5	0±0	0±0	0±0	6.1	0±0
Methanol	1.5	0±0	0±0	5.4	0±0	0±0
Aqueous	1.5	0±0	0±0	0±0	0±0	0±0
Final response		Negligible	Negligible	Negligible	Negligible	Negligible

Table 6: Zone of inhibition of solvents against fungal strains (Negative control)

Solvents	Quantity (mL)	Zone of inhibition (mm)		
		A. niger	***A. oryzae***	***F. solani***
P.E	1.5	0±0	0±0	0±0
Chloroform	1.5	0±0	0±0	11
Methanol	1.5	0±0	9	10
Aqueous	1.5	0±0	0±0	0±0
Final response		Negligible	Negligible	Negligible

The affectivity of the extract of bark of*S. suaveolens* was moderate against gram positive(Table 7). The leaf methanol extract showed inhibitory zone, i.e. 32±9.60mm and 30±0.71mm against *S. aureus* and *B. subtilis*(Pl. 3e) respectively, which were greater as compared to standard disc. Similar results were also listed by [22] while working on *Sporoboluscoromandelianus* and *Echinochloacolona* against gram positive and gram negative bacterial strains. Petroleum ether is a non-polar solvent and the activity showed by the plant was due to the solubility of compounds in non-polar solvent. Both polar solvents obtained from leaf were effective against gram positive bacterial strains.

The bark of *S.suaveolens* was more active against gram negative bacterial strains as compared to gram positive. All the extracts of bark showed inhibition against *E.coli*(Pl. 3), while chloroform extractof bark possess maximum inhibition which was 30±1.41mm (Pl. 3c). Methanolic extract of bark showed25±0.71mm zone against *K. pneumonia* (Pl. 3b). Petroleum ether extract of leaf of *S.suaveolens* showed low resistance against all the gram negative bacterial strains while methanol extract of leaf showed highest inhibition against *K.pneumoniae*, i.e. 32±4.04mm (Pl. 3f). Aqueous extract showed lowest zone 18±0.70mm against *P.aeruginosa* (Table 8) [23]. also experienced similar results while working on *Gynotrochesaxillaris*Blume with different solvent extracts like



petroleum ether, chloroform and methanol. Methanol extract had higher contents of hydrophilic phenolic compounds which become active against gram-negative bacteria. Gram-negative bacterial cell contains lipid-polysaccharide sheet which defend the passage of hydrophobic groups but allows hydrophilic compounds to enter the cell [23].

Table 7: Zone of inhibition produced by *S.suaveolens* against gram positive bacterial strains

Plant part	Solvents	Gram Positive Bacterial strains	
		S. aureus	*B. subtilis*
BARK	P.E	0	17±2.82
	Chloroform	14±0.70	0
	Methanol	17±2.83	20±1.41
	Water	0	0
LEAF	P.E	14±1.14	15±0.70
	Chloroform	16±0.71	0
	Methanol	32±9.60	30±0.71
	Water	20±1.41	30±1.08

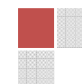
Table 8: Zone of inhibition produced by *S.suaveolens* against gram negative bacterial strains

Plant part	Solvents	Gram Negative Bacterial strains		
		E. coli	*K. pneumoniae*	*P. aeruginosa*
BARK	P.E	20±0.71	15±1.06	13±0.72
	Chloroform	30±1.41	19±2.12	18±1.41
	Methanol	20±1.00	25±0.71	18±0.71
	Water	19±0.72	0	0
LEAF	P.E	0	0	0
	Chloroform	18±1.52	24±2.82	12±0.71
	Methanol	20±0.57	32±4.04	14±0.73
	Water	20±0.71	19±1.41	18±0.70

The bark extract of all solvents showed satisfactory results against *A.niger* except water which was inactive at all. Maximum zone displayed by petroleum ether extract of bark against *A.niger* (Pl. 4) and *F.solani*,i.e.25±1.41mm and 25±2.12mm correspondingly. Methanolic extract of bark of *S.suaveolens* displayed good inhibition against *A. niger* and*A. oryzae* (Pl. 4a and 4c). Such results were listed by [23] during the study of antimicrobial activity of different extracts of leaf and branches of *Daturastramonium*.Among all the extracts of leaf only petroleum ether extract of *S.suaveolens*exposed potential against fungal strains (Pl. 5) while it exhibited maximum zone 29±0.72mm against *F.solani*(Pl. 5b). Aqueous extracts of leaf and bark of *S.suaveolens* showed no resistance against any of the fungal strain (Table 9). [24]also reported similar results while working on stem and bark of *Litchi chinensis* against *B. subtilis*.

Table 9: Inhibitory zone of *S.suaveolens*against fungal strains

Plant part	Solvents	Zone of Inhibition (mm)		
		A. niger	*A. oryzae*	*F. solani*
BARK	P.E	25±1.41	24±0.72	25±2.12
	Chloroform	20±0.71	0	0
	Methanol	24±3.78	23±2.12	22±0.71
	Water	0	0	0
LEAF	P.E	24±2.83	21±0.71	29±0.72
	Chloroform	0	0	0
	Methanol	0	0	0
	Water	0	0	0



MIC results

The extracts macerated in methanol were used for the estimation of minimum inhibitory concentration against bacterial and fungal strains and compared with standards. *S. suaveolens* bark (Fig. 2)and leaf (Figure. 1) showed similar concentration (0.9 g/mL) of extract which resist gram positive strains (Table10). *S. suaveolens* showed minimum inhibition ranged from 0.7g/mL to 1g/mL concentrations against gram negative bacterial strains (Table 11).Terbinafine was used as standard antifungal in MIC. The results obtained were very close to that of standard terbinafine, i.e. 0.069±0.021, 0.062±0.015 and 0.036±0.011against *A. niger*, *A. oryzae* and *F. solani*respectively while all the extracts of plants were found to be resistant against *A.niger*. Bark extract of *S. suaveolens*was also reported to be more sensitive against *A. niger*(Figure. 3)while leaf extract of *S. suaveolens* was effective against *F. solani*(Fig. 4)(Table 12).[24]reported that the antioxidant compounds obtained from plants are active oxygen scavengers. A considerable interest has been increased to explore natural resources having antioxidant potential which can replace synthetic antioxidant.

Table 10: MIC values of *S. suaveolens* against Gram Positive Bacterial strains

Plant part	*S. aureus*		*B. subtilis*	
	Conc.(g/mL)	Abs.	Conc.(g/mL)	Abs.
S. suaveolens Bark	0.8	0.029±0.02	1	0.016±0.03
S. suaveolens Leaf	0.9	0.03±0.0.01	0.9	0.025±0.011
Ciprofloxacin	0.6	0.046	0.6	0.086

Table 11: MIC values *S. suaveolens* against Gram Negative bacterial strains

Plant parts	*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
	Conc.	Abs.	Conc.	Abs.	Conc.	Abs.
S. suaveolens Bark	1	0.025±0.01	0.8	0.052±0.03	0.8	0.091±0.009
S. suaveolens Leaf	0.8	0.019±0.021	0.7	0.122±0.032	0.7	0.118±0.041
Ciprofloxacin	1	0.039	0.6	0.57	1	0.04

Table 12: MIC of *S. suaveolens*againstfungal strains

Plants part	*A. niger*		*A. oryzae*		*F. solani*	
	Conc. (g/mL)	Abs.(nm)	Conc. (g/mL)	Abs. (nm)	Conc. (g/mL)	Abs. (nm)
S. suaveolens Bark	0.6	0.011±0.007	0.3	0.030±0.012	1	0.038±0.010
S. suaveolens Leaf	0.5	0.021±0.010	0.2	0.048±0.011	1	0.014±0.009
Terbinafine	0.7	0.069±0.021	0.1	0.062±0.015	0.1	0.036±0.011

Antioxidant assay results

Free radical scavenging activity of leaf and bark extracts of *S.suaveolens* was done with methanol DPPH reagent (Pl. 6b). The absorbance was measured at 517nm. % scavenging of different extracts of bark of *S.suaveolens* ranged from 78.317% of methanol at 500(µg/mL) to 28.263% water at 125 conc. (µg/mL) (Fig. 1, 2, 3 and 4) whereas % scavenging of different extracts of leaf of *S.suaveolens* ranged from 76.051% of chloroform at 500(µg/mL) to 41.208% of chloroform at 60 conc. (µg/mL) (Fig. 5,6, 7 and 8). Lowest absorbance was measured by methanol extract of bark of *S.suaveolens* 0.201±0.17 having % scavenging 78.317 while chloroform extract of leaf showed minimum absorbance 0.222±0.02 with maximum % scavenging 76.051. Aqueous extract of bark (Fig. 4.20) of *S.suaveolens* showed lowest % scavenging, i.e. 54.584 whereas the other extract showed satisfactory results. Petroleum ether extract obtained from leaves of *S.suaveolens* exhibited maximum absorbance and minimum scavenging among all (Table 13).IC 50 values obtained from different extract was moderate while methanolic extract of bark of *S. suaveolens*25.81µg/mL (Table 14). The same conditions were drawn by [25] during investigation of antioxidant activities of *Rivinahumilis* L.

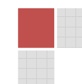


Table 13: Absorbance and free radical scavenging of *S.suaveolens* by DPPH assay

Plant part	Extract	Conc.(µg/mL)	Abs.	% scavenging
Bark	P.E	1000	0.337±0.063	63.646
		500	0.394±0.047	57.497
		250	0.439±0.099	52.642
		125	0.538±0.033	41.963
	Chloroform	500	0.390±0.007	57.928
		250	0.428±0.014	53.829
		125	0.508±0.017	45.199
		60	0.586±0.021	36.785
	Methanol	500	0.201±0.17	78.317
		250	0.281±0.21	69.687
		125	0.343±0.16	62.998
		60	0.519±0.06	44.012
	Water	500	0.421±0.03	54.584
		250	0.543±0.07	41.423
		125	0.665±0.05	28.263
Leaf	P.E	1000	0.329±0.03	64.509
		500	0.397±0.02	57.173
		250	0.451±0.03	51.348
		125	0.513±0.11	44.660
	Chloroform	500	0.222±0.04	76.051
		250	0.306±0.06	66.990
		125	0.483±0.09	47.896
		60	0.545±0.09	41.208
	Methanol	500	0.288±0.02	68.932
		250	0.314±0.07	66.127
		125	0.429±0.04	53.721
		60	0.540±0.05	41.747
	Water	500	0.295±0.03	68.176
		250	0.392±0.07	57.713
		125	0.470±0.03	49.298

Table 14: IC_{50} values of different extracts of *S.suaveolens*

	IC_{50} values (µg/mL)	
	S. suaveolens Bark	*S.suaveolens* Leaf
Petroleum ether	286.139	258.86
Chloroform	337.27	132.42
Methanol	25.81	97.26
Water	418.27	120.83
Standard BHT	12	

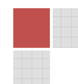


Figure. 1: % scavenging of petroleum ether extract of bark of *S. suaveolens*

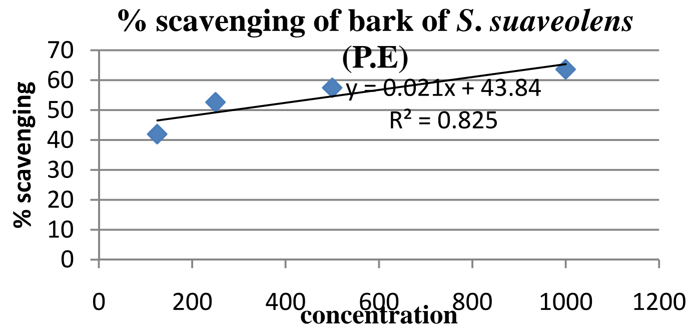


Figure. 2: % scavenging of chloroform extract of bark of *S. suaveolens*

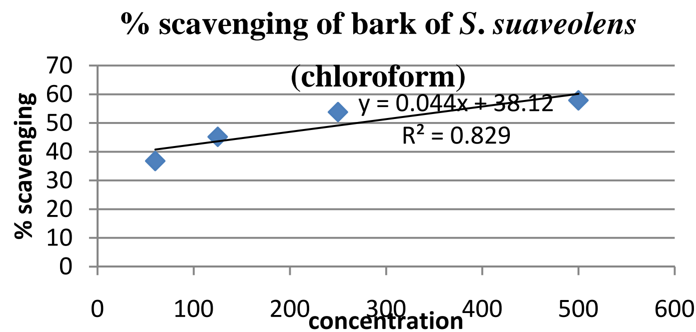


Figure. 3: % scavenging of methanol extract of bark of *S. suaveolens*

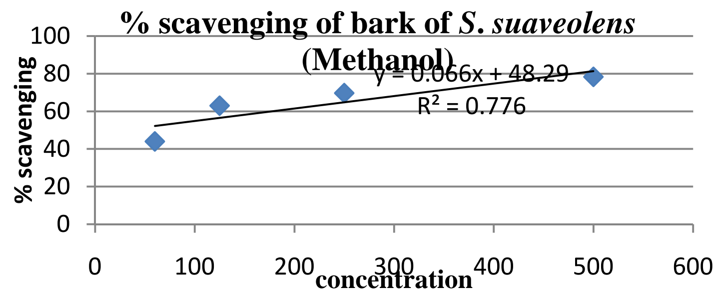
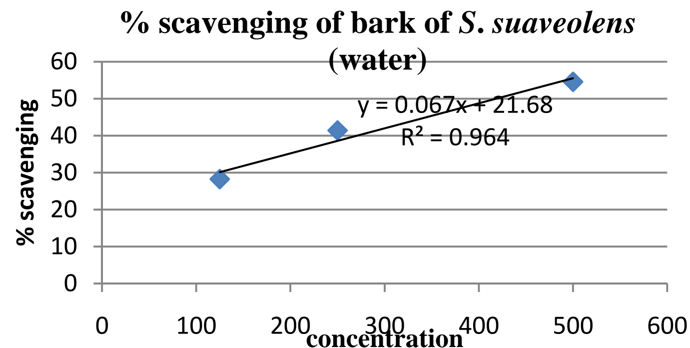


Figure. 4: % scavenging of water extract of bark of *S. suaveolens*



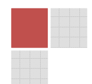


Figure. 5: % scavenging of petroleum ether extract of leaf of *S. suaveolens*

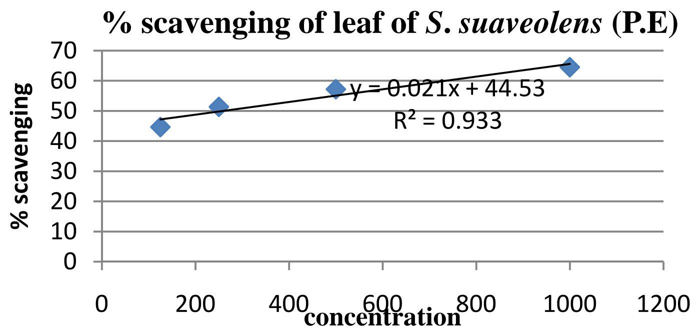


Figure. 6: % scavenging of chloroform extract of leaf of *S. suaveolens*

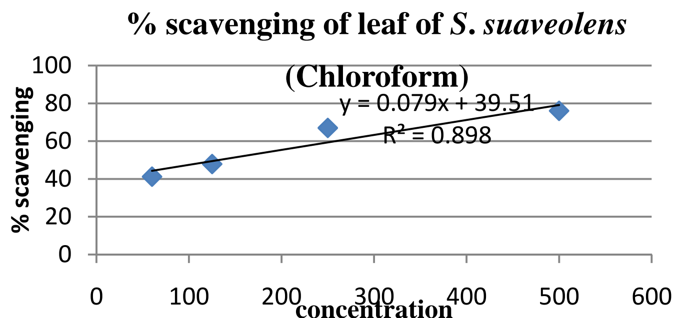


Figure. 7: % scavenging of methanol extract of leaf of *S. suaveolens*

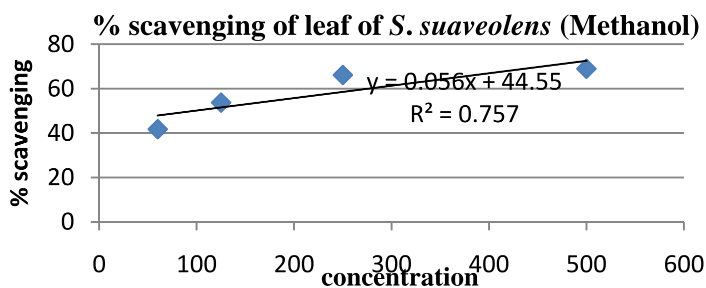
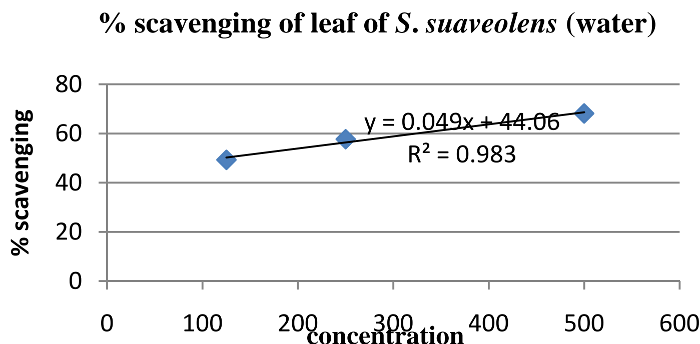
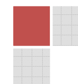


Figure. 8: % scavenging of water extract of leaf of *S. suaveolens*



The total antioxidant potential of *S. suaveolens* (leaf and bark) was evaluated and the maximum results obtained with methanol extract of bark, i.e. 0.898±0.13. The other extract of the plants also showed good potential relative to standard, the minimum activity was showed by bark aqueous extract, i.e. 0.359±0.04 (Pl. 6a). Chloroform extract obtained from the leaves of *S. suaveolens*



showed closest absorbance at 500µg/mL concentration, i.e. 0.840±0.09 while aqueous extract showed minimum absorbance. Other extract showed good to satisfactory results (Table 15). [26]reported similar results while investigating oxidative potential of *Cotinuscoggyria*.

Table 15: Antioxidant activity of *S. suaveolens* by Phosphomolybdenum assay

Plant part	Extract	Conc.	abs.
BARK	P.E	500	0.685±0.01
		250	0.640±0.03
		125	0.549±0.06
	Chloroform	500	0.712±0.02
		250	0.577±0.07
		125	0.496±0.02
	Methanol	500	0.898±0.13
		250	0.697±0.05
		125	0.544±0.03
	Water	500	0.498±0.01
		250	0.418±0.02
		125	0.359±0.04
LEAF	P.E	500	0.636±0.07
		250	0.533±0.04
		125	0.505±0.03
	Chloroform	500	0.840±0.09
		250	0.640±0.02
		125	0.573±0.04
	Methanol	500	0.824±0.14
		250	0.704±0.01
		125	0.621±0.04
	Water	500	0.590±0.12
		250	0.463±0.06
		125	0.435±0.04
BHT	Standard		1.118±0.05

The bark of *S. suaveolens* displayed better ferric reducing ability as compared to the leaf extracts. Chloroform extract of bark of *S. suaveolens* showed maximum results 202TEµM/mL while methanol extract showed minimum, i.e. 70.5TEµM/mL. The leaf extract of the plant also exhibited highest ferric reducing potential whereas water exhibited minimum activity, i.e. 211TEµM/mL and 66TEµM/mL respectively (Table 16). The results obtained by the reaction of FRAP reagent and the plant samples were classified by [27] given underneath:

FRAP value	Remarks
>500µM/100g	Very high
100-500µM/100g	High
50-100µM/100g	Good
10-50µM/100g	Low
<10µM/100g	Very low

In agreement to the classification given above, *S. suaveolens* exhibited good to very high values. The similar results were expressed by [28] during the antioxidant evaluations of *Argyrolobiumroseum*.

S. suaveolens (leaf and bark) was estimated for phenolic contents and obtained satisfactory to poor results. Methanol solution of leaf of *S. suaveolens* displayed 50.5µg/mL of Gallic acid while other extracts showed moderate activity. Leaf extract contained more phenolic compounds as compared to the bark extracts (Table 17).Phenolic compound such as flavonoids, tannins, coumarins and procyanidins present in plants have oxidative activities.Leaf methanolic extract of leaf of *S. suaveolens* exhibited moderate results. The similar observations were also documented by [29] while studying various fractions of *Artemisia incisa*.

Table 16: FRAP Assay of *S. suaveolens*

Plant Parts	Solvents	Concentration (µg/mL) with Abs.		
		500	250	125
BARK	P.E	0.318±0.05	0.288±0.04	0.237±0.05
	Chloroform	0.473±0.06	0.314±0.05	0.211±0.04
	Methanol	0.397±0.02	0.305±0.05	0.21±0.03
	Water	0.452±0.03	0.414±0.02	0.214±0.02
LEAF	P.E	0.339±0.08	0.303±0.06	0.229±0.03
	Chloroform	0.491±0.12	0.407±0.07	0.337±0.04
	Methanol	0.419±0.04	0.289±0.03	0.223±0.06
	Water	0.275±0.11	0.21±0.03	0.201±0.08
Plant Parts		FRAP values(TEµM/mL)		
		500	250	125
BARK	P.E	124.5	109.5	84
	Chloroform	202	122.5	71

	Methanol	164	118	70.5
	Water	191.5	172.5	72.5
LEAF				
	P.E	135	117	80
	Chloroform	211	169	134
	Methanol	175	110	77
	Water	103	70.5	66

Table 17: Total Phenolic Content of *S. suaveolens*

Total Phenolic Content of *S. suaveolens*					
Plant Parts		P.E	Chloroform	Methanol	Water
S. suaveolens(Bark)	Absorbance	0.302±0.05	0.3371±0.06	0.389±0.12	0.198±0.12
	TPC	27.16	33.016	41.66	9.83
S. suaveolens(Leaf)	Absorbance	0.412±0.07	0.394±0.11	0.442±0.02	0.201±0.21
	TPC	45.5	42.5	50.5	10.33

Conclusions

It was observed that there was no relation between % yield of crude extract and antimicrobial potential of *S. suaveolens* under examination. Leaf and bark of plant *S. suaveolens* displayed good results against gram positive and gram negative bacterial strains. The parts of plant *S. suaveolens* showed moderate potential against fungal strains whereas aqueous samples had not displayed any activity against fungal strains. Methanolic extract of leaf and bark of *S. suaveolens* gave highest results against gram positive and gram negative bacterial strains while only petroleum ether extract was active against fungi. The results obtained by the estimation of MIC (minimum inhibitory concentration) of *S. suaveolens* by broth dilution method were similar with the values obtained by zone of inhibition. Different antioxidant assay applied for the estimation of potential capacity of plants. The overall antioxidant capacity of leaf and bark of plants ranged from good to satisfactory.

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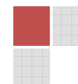

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