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ANTI-OXIDANT, ANTIBACTERIAL AND PHYTOCHEMICAL STUDIES OF LEAVES EXTRACT OF *SAPIUM SEBIFERUM* L.

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ABSTRACT

Plant based medicine has served mankind for centuries and an impressive number of modern drugs have been isolated from natural sources. The last 20-25 years have witnessed renewed in folkloric remedies to fight microbial diseases owing to the emergence of multidrug resistant microorganism. *Sapium sebiferum* L. (family: *Euphorbiaceae*) was evaluated for its anti-oxidant activity and antimicrobial activities. Phytochemical analysis of the extract indicated the presence of sugars, tannins, saponins, alkaloids, flavonoids and glycosides. Petroleum ether, chloroform, acetone and methanol extract of *S. sebiferum* were screened for antibacterial activity by well diffusion method at sample concentration of 40 μg/mL. The result of antibacterial activity revealed that methanolic extract of the plant exhibited maximum activity as compared to petroleum ether, chloroform, and acetone extract. Minimum inhibitory concentration (MIC) of the methanolic extract of the plant was also calculated against the pathogens *Staphylococcus aureus* was the most susceptible bacteria. In the qualitative anti-oxidant assay using DPPH (1, 1-diphenyl-2-picryl hydrazyl) the extract showed free radical scavenging properties. These primary findings suggest that the extract might possess some chemical constituents that are responsible for antimicrobial and antioxidant activity.

Key words: Sapium sebiferum L., Phytochemical activity, Anti-oxidant activity, DPPH, Antibacterial.

INTRODUCTION

In the recent times, the rapid development of multi-resistant bacterial strains of clinically important pathogens fetches the interest of scientist to develop newer broad spectrum antimicrobial agents. The less availability, high cost and greater side effects of new generation antibiotics necessitates looking for the substances from alternative medicines, which claimed antimicrobial activity. A number of herbs with significant antimicrobial activity have been reported in the literature. Now, it is aimed to explore scientifically the antimicrobial potential of traditional plant *Sapium sebiferum* and substantiate the folklore claim[1].

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Sapium sebiferum is commonly known as Chinese tallow tree. It is a tree in the spruge family (*Euphorbiaceae*). At maturity, it typically reac hes a maximum height of 15 m. Its bark is reddish-brown with wide fissures and narrow strips. The branches are typically long and dropping. The twigs are slender and waxy. The leaves are alternate and deciduous, broad rhombic to ovate, 3-8 cm wide and have a smooth margin. *S. sebiferum* is monoecious. The flowers are greenish yellow in terminal spike like inflorescence up to 20 cm long fruits are three lobed, three valved capsules about 1-2 cm long and 2 cm long. As the capsule mature, their colour changes from green to nearly black. The capsule walls fall away and expose three globose seeds with white, tallow containing covering[2]. The Chinese have used the tallow tree extensively in traditional medicines for the past 15 centuries, most generally as a preservative against contagious and infectious diseases (analexiteric), and as a remedy for treating wounds and sores in general (a vulnurary), especially skin ailments[2].

EXPERIMENTAL

Materials and methods

Collection of plant material

The leaves of *Sapium sebiferum* was collected from manduwala forest near the road side Dehradun (Uttarakhand). The plant was well identified by Dr. S.K. Srivastava, Botanical Survey of India, Dehradun. The leaves were shade dried and powdered using mortar pestle.

Extraction of plant material

The powdered plant material (250 g) was loaded in soxhlet assembly and extracted in five different solvents i.e. petroleum ether, chloroform, acetone, methanol and water for 72 hrs by successive method. At the end of each extract, it was passed through Whatman filter paper No. 40 and the filtrates were evaporated under reduced pressure. They were then weighed. All the extracts were kept in refrigerator at $4^{o}C$ for further use.

Chemicals and instruments

Quercetin, DPPH and ascorbic acid were obtained from Hi Media Labs, Punjab. Aluminium chloride was purchased from Research lab Punjab. All organic solvents were of analytical grade and supplied from Research Lab, Punjab. UV-Visible Spectrophotometer (JascoV-530) was used for antioxidant activity determination by DPPH method and for total flavonoid content determination.

Phytochemical screening of the plant

Phytochemical screening was carried out of the plant material for the presence of bioactive components such as alkaloids, flavonoids, glycosides, steroids, saponins, tannins, phenolics, amino acid and carbohydrates.[3]

Table 1: Preliminary phytochemical screening of leaves extract of *Sapium sebiferum L.*

S. No.	Test	Petroleum ether extract	Chloroform extract	Acetone extract	Methanol
1	Alkaloids	-	+	+	+
2	Carbohydrate	-	+	+	+
3	Proteins	-	-	-	+
4	Glycoside	+	-	-	+
5	Flavonoids	-	+	+	+
6	Tannins and phenolic compounds	-	-	+	+
7	Fixed oil	-	-	-	-
8	Saponins	-	+	+	+

Micro-organism

The micro-organisms used in this study are: *Staphylococcus aureus MTCC902, Pseudomonas aeruginosa MTCC424, Salmonella typhi MTCC733, E. coli MTCC443,* were provided by Department of Biotechnology, S.G.R.R.I.T.S, Dehradun (Uttarakhand) and checked for the purity by convention biochemical methods. The bacterial cultures were stored on nutrient agar at 4°C.

Antimicrobial activity

Antimicrobial activity of the crude extract was carried against four microbial strains by agar well diffusion method[4]. The *in vitro* antimicrobial activity was carried out against 24 hr culture of four bacterial strains viz; Gram positive *Staphylococcus aureus*, *Pseudomonas aeruginosa* and Gram negative, *Salmonella typhi* and *Escherichia coli*. The extracts were tested at 40 μg/mL concentration against bacterial strain. DMSO was used as a

vehicle. Ciprofloxacin (40 µg in 100 µL) were used as standard drug for the comparison of antibacterial activity. The zone of inhibition was compared with standard drug after 24 hr of incubation at 37°C for antibacterial activity.

Table 2: Zone of inhibition (mm)

Extract	Antibacterial strains			
	Staphylococcus aureus	***Pseudomonas aeruginosa***	***Salmonella typhi***	***Escherichia coli***
Petroleum extract	22.9 ± 0.06	20.23 ± 0.15	23.9 ± 0.06	22.13 ± 0.19
Chloroform extract	23.98 ± 0.12	21.13± 0.19	22.3± 0.17	23.97 ± 0.15
Acetone extract	23.93 ± 0.12	22.13 ± 0.09	23.17 ± 0.17	23.03 ± 0.09
Methanol extract	24.13 ± 0.19	23.19 ± 0.09	23.03 ± 0.09	23.0 ± 0.21
Ciprofloxacin	24.0 ± 0.19	23.1 ± 0.06	24.0 ± 0.0.09	24.13 ± 0.06

Values are mean ± S.D of the triplicate *P < 0.05; **P < 0

Standard drug : Bacteria-ciprofloxacin (40 µg in 100 µL)

Control : DMSO (Dimethyl sulphoxide)

Sample used : Crude extract

Extract : (40 µg in 100 µL)

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration is defined as the lowest concentration of the test samples that result in a complete inhibition of visible growth. MIC of all extracts was determined by a micro dilution method.[5] The respective clinical strain was spread separately on the medium. The wells were created using a stainless steel sterilized cork borer under aseptic conditions. The extracts at different concentration viz. 10, 20, 30, 40, 50 µg was dissolved respectively in 25, 50, 75, 100, 125 µL of DMSO and later loaded into corresponding wells. The standard drug ciprofloxacin (40 µg in 100 µL) were used as standard drug for the comparison of antibacterial activity. The zone of inhibition was compared with the standard drug after 24 hr of incubation at 37°C for antibacterial activity. The results are recorded in Table 2.

Table 3: Minimum inhibitory concentration (MIC)

Extract	MIC (µg/ µL) Antibacterial strains			
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Escherichia coli*
Petroleum extract	30.1 ± 0.06	30.0 ± 0.06	30.1 ± 0.06	40.03 ± 0.03
Chloroform extract	30.1 ± 0.06	40.03 ± 0.03	30.1 ± 0.06	29.97 ± 0.03
Acetone extract	30.03 ± 0.09	40.03 ± 0.03	30.1 ± 0.06	40.03 ± 0.03
Methanol extract	30.1 ± 0.06	30.1 ± 0.06	29.97 ± 0.03	40.03 ± 0.03

Values are mean ± S.D of the triplicate *P < 0.05; **P < 0.01

Standard drug : Ciprofloxacin (40 µg in 100 µL)

Control : DMSO (Dimethyl sulphoxide)

Sample used : Crude extract

Total flavonoid content determination

5 mL of 2% aluminium chloride ($AlCl_3$) in methanol was mixed with the same volume of *sapium sebiferum* leaves (methanol) extract (0.02 mg/mL). Absorption readings at 415 nm were taken after 10 minutes against a blank sample without $AlCl_3$. The total flavonoid content was determined using a standard curve of quercetin (0.01-0.1 mg/mL). The mean of three readings was used and expressed as mg quercetin equivalent (QE)/100 g extract.[6]

Table 4: Absorbance by sample for total flavonoid content

S. No.	Sample	Concentration (mg/mL)	Absorbance
1	Standard	0.01	0.1060
2	Standard	0.02	0.2172
3	Standard	0.04	0.4306
4	Standard	0.06	0.7028
5	Standard	0.08	0.7906
6	Standard	0.1	0.9912
7	Test (alc. Extract)	1	0.6107
8	Test (water Extract)	1	0.4245

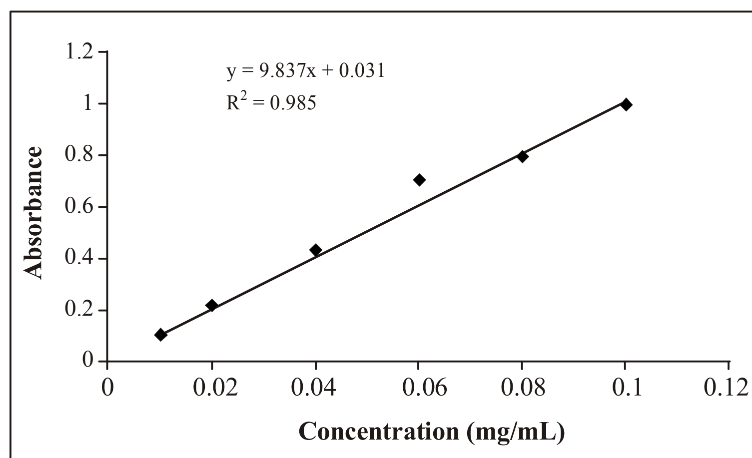

Fig. 1

Antioxidant activity

Free radical scavenging activity of compounds was determined using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. Briefly, 2 mL extract and standard of various concentrations (10-100 µg/mL) were added to 2 mL of 100 µM DPPH solution[7]. After 20 minute incubation at room temperature, the absorbance was read against a blank at 517 nm. The change in absorbance with respect to the control (containing DPPH only without sample, expressed as 100% free radicals) was calculated as percentage scavenging using following the equation.

$$(A517_{blank} - A517_{sample}) / A517_{blank} \times 100\%.$$

The reading was taken in triplicate and mean used for calculation of IC_{50}. The IC_{50} (mean ± SEM) stand for the concentration required for 50% inhibition of DPPH radicals and was calculated from ORIGIN PC version 6.0 software.

Table 5: % Radical scavenging activity of methanolic extract

S. No.	Conc. µg/mL	Standard (Ascorbic acid)		Test (Methanolic extract)	
		Absorbance ± SEM	% RSA ± SEM	Absorbance ± SEM	% RSA ± SEM
1	10	0.8636 ± 0.034	37.92 ± 1.36	0.9835 ± 0.010	29.26 ± 0.89
2	20	0.8131 ± 0.042	46.95 ± 9.02	0.9153 ± 0.007	34.16 ± 1.02

Cont…

S. No.	Conc. µg/mL	Standard (Ascorbic acid)		Test (Methanolic extract)	
		Absorbance ± SEM	% RSA ± SEM	Absorbance ± SEM	% RSA ± SEM
3	30	0.7405 ± 0.028	46.15 ± 1.49	0.8551 ± 0.020	38.50 ± 0.65
4	40	0.6913 ± 0.016	49.72 ± 0.67	0.8051 ± 0.012	42.10 ± 0.51
5	50	0.6300 ± 0.015	54.18 ± 0.74	0.7647 ± 0.009	45.00 ± 0.58
6	60	0.5892 ± 0.010	57.15 ± 0.33	0.7092 ± 0.007	48.99 ± 0.60
7	70	0.5394 ± 0.016	60.77 ± 0.85	0.6322 ± 0.022	54.55 ± 0.79
8	80	0.4888 ± 0.017	64.45 ± 0.89	0.6064 ± 0.008	56.39 ± 0.42
9	90	0.4340 ± 0.026	68.44 ± 1.57	0.5475 ± 0.012	60.63 ± 0.05
10	100	0.3874 ± 0.029	71.84 ± 1.84	0.4945 ± 0.005	64.43 ± 0.49
11	Blank	1.3907 ± 0.031		1.3907 ± 0.031	

Table 6: Antioxidant activity expressed in IC_{50}

Sample	DPPH scavenging activity IC_{50} (± SEM) (µg/mL)[a]
Test (Methanolic extract)	61.72 ± 1.07
Standard (Ascorbic acid)	39.95 ± 4.42

The results are expressed as IC_{50} ± SEM (n=3) (µg), the concentration of the test compound that provides 50% scavenging of the DPPH radicals already available in the solution.

RESULTS AND DISCUSSION

The phytochemical screening of the plant extract showed the presence of alkaloids, flavonoids, glycosides, amino acids, steroids, saponins, tannins and phenolics (Table 1). Alkaloids was present in chloroform, acetone and methanolic extract of the plant whereas absent in petroleum ether extract. Methanolic extract of the plant possess the presence of maximum constituents. Phytochemical screening of the crude extracts of *Sapium sebiferum L*. revealed the presence of tannins, saponins, phenolics, flavonoids and alkaloids. These compounds have potentially significantly application against human pathogens.[8] The zone of inhibition of the petroleum ether, chloroform, acetone and methanol extract (40 µg/mL) from

the *S. sebiferum L.* plant against *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi and E. coli* are shown in (Table 2). The methanolic extract showed the maximum zone of inhibition against all bacteria followed by acetone, chloroform and petroleum ether. The methanolic extract is highly effective against all pathogens because more organic compounds were leached in this solvent.

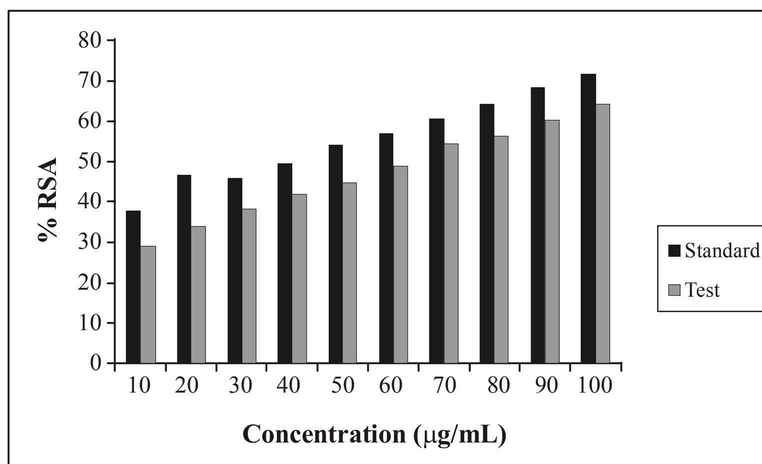

Fig. 2

The zone of inhibition formed by petroleum ether extract is least effective. The methanolic extract was highly active against *S.aureus* (24.13 mm) followed by *Pseudomonas aeruginosa* (23.19 mm), *Salmonella typhi* (23.03 mm) and *E. coli* (23.0 mm) as compare to others. Acetone extract showed best activity against *S. aureus* (23.93 mm) and *Salmonella typhi* (23.19 mm). Chloroform extract is more effective in comparison to petroleum ether extract. Chloroform extract showed maximum inhibition against *Staphylococcus aureus* (23.98 mm) followed by *E. coli* (23.97 mm) *Salmonella typhi* and *Pseudomonas aeruginosa*. Petroleum ether extract was found most effective against *Salmonella typhi* (23.9 mm) followed by *Staphylococcus aureus* (22.9 mm), *E. coli* (22.13 mm) and *Pseudomonas aeruginosa* (20.2 mm). The basis for their difference in susceptibility might be due to constituents present in the extract. The leaves of *S. sebiferum L* have considerable antibacterial activity against *E. coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus* and *bacillus subtilus*. The *in vitro* antioxidant activity of methanolic extract by DPPH Radical scavenging activity was determined. The experiments were performed in triplicates and mean values of Antioxidant activity of the plant extract were determined. The methanolic extracts showed antioxidant activity with IC50 values of 61.72 μg/mL. The known antioxidant ascorbic acid exhibited IC50 value of 39.95 μg/mL as

shown in Table 6. Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in extraction procedure. Traditional healers use primarily water as the solvent[9] but in our studies, we found that plant extracts in organic solvents (methanol) provided more consistent antimicrobial activity. These observations can be rationalized in terms of the polarity of the compound being extracted by each solvent and in addition to their intrinsic bioactivity by their ability to dissolve or diffuse in the media used in the assay. The observed antibacterial effects on the isolates are believed to be due to the presence of flavonoids, alkaloids and tannins, which have been shown to posse's antibacterial properties.

CONCLUSION

In conclusion, the extract of *S. sebiferum L.* plant has high potential as antioxidant and antibacterial agent. This finding has validated the use of these medicinal plants for the treatment of microbial infection. It seems important to recommend that further studies using isolated constituents instead of whole extract must be done in this field.

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