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Research Article

ANTHELMINTIC, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF DIFFERENT PART OF INDIAN ORIGIN SALVADORA PERSICA

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ABSTRACT

Nowadays, Herbal products are attracting the whole population of the world because of their safety features. The present study aimed to reveal the phytochemical compositions, anthelmintic activity, antioxidant and antimicrobial potential of *Salvadora persica* leaves extract in different extraction solvent systems. Shade dried leaves of *Salvadora persica* were extracted in chloroform, ethyl acetate, methanol, ethanol, and water using the modified fractional maceration method. These extracts were analyzed for their phytochemical, anthelmintic activity, antioxidant and antimicrobial potential. The antioxidant activity was done using DPPH and H_2O_2 radical scavenging method. While the antimicrobial potential was analyzed using the disc diffusion method. Anthelmintic activity was determined against Indian earthworms (*Eiseniafetida*).The highest percentage yield of extract was found in the hydro solvent extraction system. The DPPH radical scavenging was found 67.3% (lowest) and 99.07% (highest), (dose100µg/ml), in SPLEC and SPLEW respectively. The highest antimicrobial activity was found in SPLEE (200µg/ml and 100µg/ml) i.e. 6 mm and 4 mm zone of inhibition against *E.coli* while 5mm and 3mm again B. *subitilis* respectively. All extract fractions of *Salvadora persica* exhibited anthelmintic activity but less than standard drug albendazole. Based on our findings, we were concluded that leaves of *Salvadora persica* have an anthelmintic effect good antioxidant and antimicrobial potential so their consumption may exert a beneficial effect on human and animal health as well.

KEYWORDS: Salvadora persica; Antioxidant; Antimicrobial; Anthelmintic; λ max validation; extract.

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INTRODUCTION

Salvadorapersicalinn belongs to the Salvadoracea family also known as 'MISWAK'. It is also known as kharijal and Pilu[1]. *Salvadorapersica* is an evergreen shrub or small plant that grows with a crooked trunk. It has around one foot in diameter and ten feet in height. Leaves of *Salvadorapersica* are small, ovate to rounded, succulent, thick, and fleshy like 2.0-3.2cm width and 3.8-6.8cm in length, light to dark green[2]. *Salvadorapersica* root and twigs have been used to clean the teeth from ancient times in India[3]. *Salvadorapersica* leaves are eaten as salad and green vegetables in the whole world. Traditionally, *Salvadorapersica* has been used in the preparation of various types of foods, as the source of fuel, and in various formulations of cosmetic products. Paste of *Salvadorapersica* leaves mixed with cow's urine used to remove the hairs *Salvadorapersica* leaves are used as fodder for cows, buffalos, camels, sheep's and goats that improve lactation and body weight[4]. Leaves of *Salvadorapersica* are used as antidotes for poison and anti-rheumatic in India. The juice of *Salvadorapersica* leaves is shown a positive result against scurvy[5]. Various studies reported several pharmacological actions of *Salvadorapersica* leaves extract including antibacterial, antiseptic, carminative, antifungal, diuretic, anticonvulsant, analgesic, hypoglycemic, antiscorbutic, antiplasmodial, antispasmodic, astringent, anticaries, anthelmintic, anti-inflammatory, anti hepaticdisorder, and wound healing properties[1]. Extraction is a process to separate certain components present in crude plant material using different solvents. After removing the solvent, complexes of metabolites were found as an extract in the form of solid, liquid, and semisolid. The solvent used in extract should be low toxic, ease to evaporation, preservative, high physiologic absorption, and unable to cause extract dissociation. The presence of antioxidant substances in medicine, foods, juices, extract, and body compared to standard oxidizable substances to find out their activity and prevent the oxidation of the substrate. Standard oxidizing substances included enzymatic (peroxidase such as H_2O_2) and non-enzymatic (ascorbic acid) antioxidants are used generally. The antioxidant compound can protect our body itself from oxidative damages. These oxidative damages offer various health problems and diseases such as cancers, inflammation, Alzheimer's, Neuro, and cardiovascular disease. These health problems can be solved by using natural antioxidant agents[6]. Many types of antibiotics are used for antimicrobial activity but their resistance is a serious health problem worldwide. Natural antimicrobial agents are widely used in clinical and veterinary medicine. Many research studies confirmed that extract and combination with an antibiotic may give synergetic effect with lower side effect with affordable treatment options [7]. During this study, research aimed to

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reveal the phytochemical composition, anthelmintic activity, antioxidant activity, and antimicrobial potential of extract of Indian origin Salvadorapersica leaves.

MATERIALS AND METHODS

Material: Leaves of *Salvadora persica* were collected from the field area of Shiddbaba Markhandi Ashram Ghanghauli Aligarh, India. Leaves were powdered by using the electric granulating machine.

Chemicals: Chloroform, ethyl acetate, methanol, ethanol were used from the research lab of Galgotias University.

Method of extraction of plant material: A modified fractional maceration method was used to extract the plant material. Dried coarse powder of leaves was placed in a closed vessel. A sufficient amount of selected solvent was added. Allow standing for two days with occasional shaking. Strained off the liquid part and repeated it three times. Filtered and concentrate at below 40°C. The remaining powder was treated with different solvents[8].

Determination of % yield of extract: Percentage yield of extract was calculated using the following formula:

% Yield = Practical Yield × 100 / Theoretical Yield

Phytochemical screening of extract: Phytochemical studies were done to check the presence of different phytochemicals like alkaloids, glycosides, tannins, saponins, Flavonoids, protein, carbohydrates, and steroids were calculated from the extract of the different solvent system[9].

Solubility studies of extract: Different dry extracts were shaken in selected solvent and solubility was observed[10].

Determination of pH of extract: One percent of solutions of the different dry extract was prepared and determines the pH using a digital pH meter [10].

Determination of microbial load in the extract: A modified spread plate technique was used to determine the microbial load of extracts[11]. 1000µg/ml solutions were prepared and spread aseptically on a nutrient agar plate for enumeration of the total viable microbe. Prepared plates were incubated for two days at 37°C. Microbial colonies were counted using colony counter and the average number of colonies were recorded in CFU (colony forming unit).

Limit test for heavy metals of extract: Limit tests for heavy metals (lead, arsenic) were performed as 'Indian Pharmacopoeial' procedure [12].

Determination and validation of λ max of extract: Dilutions of extract were prepared using buffer solution of 0.1N HCL, phosphate buffer solution (PBS) pH6.8, phosphate buffer solution pH 7.4. Wavelengths of extract were recorded at the range of 200-800nm for six days in the morning, afternoon, and evening [13].

EVALUATION OF ANTIOXIDANT ACTIVITY

Antioxidant activity of extract using DPPH radicals scavenging activity model

The antioxidant activity of the plant extracts and the standard drug was assessed based on the radical scavenging effect of the stable DPPH free radical by the method previously described[14]. The diluted working solutions of the test extracts (20-100µg/ml) were prepared in methanol. Ascorbic acid was used as the standard drug in solutions (20-100µg/ml). 0.1mM DPPH solution (1.9mg in 100ml methanol) was prepared. Then 5ml of this solution was mixed with 5ml of working sample solutions and the standard solution to be tested separately. These solution mixtures were kept in the dark for 30 min and optical density was measured at 517nm using a Shimadzu spectrophotometer against methanol. Methanol was used as a control solution. The optical density was recorded and the percentage of inhibition was calculated using the following equation.

DPPH scavenged (%) = [(Abs_{con} - Abs_{test})/Abs_{con}] x 100

Abs_{con}: is the absorbance of the control reaction
Abs_{test}: is the absorbance in the presence of the sample of the extracts

A. Antioxidant activity of extract using H_2O_2 scavenging activity model

Antioxidant activity of *Salvadorapersica* leaves extract was performed using H_2O_2 scavenging method previously described[15]. 40mM solution of H_2O_2 was prepared in phosphate buffer solution pH 7.4. Dilutions (20, 40, 60, 80, 100µg/ml) of different extracts were prepared. 5ml of each dilution (20, 40, 60, 80, 100 µg/ml) were transferred into 10 ml volumetric flask and add 5ml of H_2O_2 solution prepared in phosphate buffer pH 7.4. The absorbances were recorded at 230nm. Phosphate buffer solution pH 7.4 was used as a control solution. H_2O_2 scavenging activity was calculated using the following formula.

H_2O_2 scavenging ability of extract (%)= [(Abs_{con} - Abs_{test})/Abs_{con}] x 100

Abs_{con}: is the absorbance of the blank solution
Abs_{test}: is the absorbance in the presence of the sample of the extracts.

Antimicrobial activity of extract

The antimicrobial activity of *Salvadorapersica* leaves extract (SPLE) and S1 (Standard drug) was evaluated and compared using the disc diffusion method. Test organisms i.e. *Escherichia coli* (gram-negative) and *Bacillus subtilis* (gram-positive) was obtained from the Department of Medical Lab Technology, School of Medical and Allied Science, Galgotias University, India. According to Indian pharmacopoeia, the disc diffusion method is one of the standards and reliable methods to determine the antimicrobial activity of a drug

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sample. In this method, MH agar plates were prepared using a sterile Petri dish. Solutions of both types of microbes were prepared in a separate test tube. Another solution of different concentrations of extracts and ciprofloxacin (standard) were prepared. The microbial solution was spread on the MH agar plate surface then extract and the standard solution was applied on the surface using the disc. So the presence of antimicrobial content in test extract solution restricted the growth of bacteria in a specific area. These specific areas referred to as growth inhibition zone diameter was measured in millimeters[16].

Anthelmintic activity of *Salvadorapersica* leaves extract

Anthelmintic activity was done as a modified procedure by Goswami*et al* (2011). Indian earthworms (*Eiseniafetida*) were used as the animal. SPLE and albendazole were used as test and standard drugs respectively. Normal saline (0.9% NaCl) was used as the negative control. All equal earthworms in size and weights were used for the experiment. Washed earthworms were placed in a petri dish. Dissolve the different concentrations of drug (test and standard) in normal saline solution and poured into Petri disc. All Petri discs observe at room temperature and recorded the paralysis and death time in minutes. The death of earthworms was confirmed with no movement in hot water (50°C)[17].

RESULTS AND DISCUSSION

Determination of % yield of extract

Before obtaining the phytochemicals from plant material, there are many steps to take place i.e. drying; size reduction, and extraction, etc. extraction is the main step among them. The extraction efficiency is affected by particle size, crude drug, the solvent used, the method applied, and the nature of phytochemicals present in the extract. While the yield of extraction affects by the polarity of the solvent, temperature during extraction, pH, extraction time, and composition of the crude drug. In this study, *Salvadorapersica* leaves extract was obtained by using chloroform, ethyl acetate, methanol, ethanol, and water. Extraction yield (%) ranged from 2.14% for ethyl acetate and 7.6% for water. The comparative percentage yield of the extract is listed in table 1.

Table 1: % yield of extract

Extract	% yield
SPLEC	3.08
SPLEEA	2.14
SPLEM	4.17
SPLEE	5.50
SPLEW	7.60

Where,
SPLEC: Salvadorapersica leaves extract in chloroform
SPLEEA: Salvadorapersica leaves extract in ethyl acetate
SPLEM: Salvadorapersica leaves extract in methanol
SPLEE: Salvadorapersica leaves extract in ethanol
SPLEW: Salvadorapersica leaves extract in water

Solubility studies of extract

Solubility profiles of different extracts were observed in different solvents. SPLEC and SPLEEA were found insoluble in cool water, while partial soluble in hot water, ethanol, and methanol and soluble in 0.1 N HCL, phosphate buffer solution pH 6.8, and phosphate buffer solution pH 7.4. SPLEM, SPLEE, and SPLEW were soluble in cool water, hot water, ethanol, methanol, 0.1 N HCL, phosphate buffer solution pH 6.8, and phosphate buffer solution pH 7.4, while partial soluble in ethyl acetate and chloroform listed in table 2.

Table 2: Solubility study of *Salvadorapersica* leaves extract

	SPLEC	SPLEEA	SPLEM	SPLEE	SPLEW
Cool water (25°C)	IN	IN	S	S	S
Hot water (40°C)	PS	PS	S	S	S
Ethanol	PS	PS	S	S	S
Methanol	PS	PS	S	S	S
Ethyl acetate	PS	S	PS	PS	PS
Chloroform	S	PS	PS	PS	PS
0.1N HCL	S	S	S	S	S
Phosphate buffer pH 6.8	S	S	S	S	S
Phosphate buffer pH 7.4	S	S	S	S	S

S: Soluble, PS: Partial soluble, IN: insoluble

Determination of pH of extract

Different solutions of (1% extract) were used to determine the pH of extracted and found to be near neutral listed in table 3. Present studies claimed that if plant extract will use in formulation offer no irritation effect on the target site.

Table 3:pH of the extract

1% solution of the extract	pH
SPLEC	6.6±0.2
SPLEEA	6.6±0.1
SPLEM	6.8±0.3
SPLEE	6.8±0.2
SPLEW	6.8±0.2

Phytochemical screening of extract

Phytochemical studies have been performed to confirm the bioactive compound in extract. The result of these studies listed in Table 4.

Table 4: Phytochemical Screening of *Salvadorapersica* leaves extract

	Test performed	SPLEC	SPLEEA	SPLEM	SPLEE	SPLEW
Alkaloids	Dragen-dorff test, Wagner test, Hager's test	-	-	+	+	+
Glycosides	Legal test	-	-	+	+	+
Tannins	Ferric chloride test	-	-	+	+	+
Saponins	Froth formation test	-	-	+	+	+
Flavonoids	Zinc Hydro-chloride reduction	+	-	+	+	+

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	test					
Proteins	Biuret test	-	-	+	+	+
Carbohydrates	Molish's test,	-	-	+	+	+
Sterols and Terpenoids	Libermann-burchardtest	+	-	+	+	+

Determination of microbial load in the extract

The results of studies were showed zero microbial loads in all extracts. The absence of microbes in extract might be due to the use of chloroform, ethyl acetate, methanol, and ethanol during the fractional maceration process.

Limit test for heavy metals of extract

A limit test for heavy metal (lead, arsenic) was performed and extracts were found to be free from heavy metal (lead, arsenic).

Determination and validation of λ max of extract

The procedure discussed in the present manuscript provides an accurate and convenient method to determine and validate the λ max of the unknown sample. The different concentrations of extract in different buffer solutions were recorded and listed in table 5.

EVALUATION OF ANTIOXIDANT ACTIVITY

Antioxidant activity of extract using DPPH Free radical scavenging activity model

In-vitro antioxidant study of *Salvadorapersica* leaf extract was performed using different extracts viz. SPLEC, SPLEEA, SPLEM, SPLEE, and SPLEW. Inhibition (%) was listed in table 6 and the comparative assessment presented in figure 1. In this study, SPLEC showed the least IC50 value of 82.34µg/ml and Standard ascorbic acid showed IC50 value of 861.42µg/ml. IC50 values obtained from the study were presented in table 7. Gupta et al (2015) investigated the antioxidant activity of Salvadorapersica twig and stem extract using DPPH scavenging assay. Gupta et al reported 181.33µg/ml, and 187.33µg/ml, IC50 value of chloroform extract Salvadorapersica twig and stem respectively[18].

Table 5: Determination and validation of λ max of extract

Extracted part of the plant	The solvent used in the extraction	Buffer solution used for dilution	Table 5: Determination and validation of λ max of extract																
			Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
			9:30 am	12:30 pm	4:30 pm	9:30 am	12:30pm	4:30 pm	9:30 am	12:30 pm	4:30 pm	9:30 am	12:30 pm	4:30 pm	9:30 am	12:30 pm	4:30 pm	9:30 am	12:30 pm	4:30 pm
Leaf	Chloroform	0.1N HCL	274.5±2.5	274.0±3.0	274.0±2.0	273.5±2.5	274.0±2.0	273.0±2.5	274.0±1.5	274.0±2.0	274.0±4.0	273.0±2.0	273.5±2.0	273.5±3.5	272.0±2.0	274.0±2.0	272.0±3.5	271.5±2.0	273.0±1.5	273.0±2.0
		PBS PH 6.8	674.0±2.0	674.0±1.5	674.0±2.0	674.0±3.0	674.5±1.5	674.5±2.0	674.5±3.5	674.0±4.0	674.5±3.0	675.0±2.5	675.0±2.0	675.0±1.5	676.0±2.0	676.0±1.5	676.0±3.0	672.5±2.0	672.5±1.5	673.0±2.0
		PBS pH 7.4	674.5±1.5	674.0±2.0	674.5±2.5	673.0±3.5	673.0±3.0	674.0±2.0	674.0±3.5	674.5±4.0	674.0±2.5	673.5±3.0	673.5±2.0	673.0±4.0	675.5±3.5	675.0±3.0	675.5±2.0	675.0±2.5	675.0±3.0	675.0±2.0
	Ethyl acetate	0.1N HCL	223.0±5.0	234.0±7.5	230.0±5.5	267.0±3.00	267.00±2.0	267.0±2.5	265.5±4.5	265.5±6.5	267.0±2.0	267.5±2.5	267.0±3.0	267.5±2.5	267.0±3.0	267.0±2.5	267.0±3.0	265.5±2.5	265.5±3.5	267.0±3.0
		PBS pH 6.8	675.0±2.0	675.0±3.0	675.0±1.5	676.0±2.0	676.0±2.5	676.0±3.5	675.0±1.5	675.5±2.5	675.0±2.0	675.0±2.0	675.0±3.0	675.0±2.5	678.5±4.5	675.0±2.5	675.0±3.0	674.0±2.0	674.0±1.5	674.0±2.0
		PBS pH 7.4	674.5±2.5	674.0±3.0	674.0±1.5	616.0±9.0	620.0±8.5	620.0±6.5	674.0±3.0	674.0±3.0	674.0±2.5	674.5±2.5	674.5±3.5	674.0±4.0	676.0±4.0	676.0±3.0	676.0±2.5	672.0±4.0	672.0±3.0	672.0±3.5
	Methanol	0.1N HCL	675.0±2.5	675.5±3.5	675.5±2.5	676.5±3.5	676.0±0.5	676.0±3.0	674.0±2.5	674.5±2.5	674.0±2.0	676.5±2.5	676.0±2.0	676.5±2.5	674.0±2.0	674.0±3.0	674.0±2.0	635.5±2.5	640.0±3.0	640.0±2.0
		PBS pH 6.8	669.5±2.5	668.0±3.0	668.0±2.5	667.0±3.0	667.0±2.0	667.0±4.5	667.5±2.5	667.0±1.5	667.0±3.0	666.0±4.5	666.5±3.5	666.5±2.5	666.0±4.0	666.0±4.0	666.0±3.5	671.0±4.0	672.0±2.5	672.0±3.5
		PBS pH 7.4	270.0±2.5	270.0±3.0	270.0±2.5	270.5±3.0	270.5±2.5	270.0±5.0	270.5±2.5	270.0±3.0	270.0±3.5	270.5±2.5	270.0±5.5	270.5±2.5	272.5±3.5	272.5±1.5	272.0±3.5	272.0±3.0	272.5±2.5	272.0±3.0
	E t	0.1N	272.	271.	271.	271.	271.	271.	271.	271.	271.	271.	271.	271.	271.	271.	271.	271.	271.	271.

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		HCL	0±3.0	5±2.5	5±2.5	0±4.0	5±3.5	5±2.5	0±3.0	0±0.5	0±2.0	5±2.5	5±2.5	0±3.0	0±0.0	0±1.0	0±0.0	0±1.0	5±0.5	5±1.0
		PBS pH 6.8	778.0±2.0	778.0±1.5	778.0±2.0	774.0±5.0	774.5±5.5	774.5±2.5	780.5±4.0	780.0±3.5	780.0±2.5	780.0±1.0	773.0±2.5	773.0±2.0	780.5±2.5	780.5±2.5	780.5±2.0	776.0±2.0	776.0±3.5	776.0±2.0
		PBS pH 7.4	268.5±2.5	268.0±2.0	268.0±0.0	270.0±0.0	270.5±0.5	270.5±1.5	268.5±2.5	268.0±2.0	268.0±1.0	267.0±2.0	267.5±2.0	267.5±2.5	276.5±1.5	276.0±2.0	276.0±2.0	276.0±2.0	278.0±1.5	278.0±2.0
	Water	0.1N HCL	275.0±2.0	275.0±1.5	275.0±1.0	275.5±0.5	275.5±2.0	275.0±1.0	274.5±0.5	274.0±2.5	274.0±1.5	277.0±0.5	276.0±1.0	276.0±2.0	275.5±3.0	276.0±1.5	276.0±2.0	270.5±1.5	270.0±2.0	270.0±1.5
		PBS pH 6.8	778.0±1.0	775.0±2.0	767.0±3.0	780.0±4.0	778 6.0±2.0	785.0±2.0	776.0±3.0	672.0±2.0	795.5±2.0	788.0±2.5	778.0±2.0	776.0±5.0	277.5±2.5	276.0±2.0	276.0±2.0	796.0±1.5	796.5±0.0	795.5±1.5
		PBS pH 7.4	268.5±2.0	268.0±1.0	268.0±0.5	255.0±4.5	260.0±2.5	260.0±3.0	202.5±9.5	220.5±1.5	220.0±4.0	272.0±2.0	272.5±2.5	272.5±1.5	658.0±2.0	658.5±2.5	658.5±1.5	269.5±1.5	270.0±0.0	270.0±0.0

Table 6: DPPH inhibition (%) of *Salvadorapersica* leaves extract

Extract	Concentration (µg/ml)	Inhibition (%)
SPLEC	20	67.20
	40	70.20
	60	72.05
	80	75.05
	100	81.06
SPLEEA	20	78.75
	40	80.13
	60	80.83
	80	80.83
	100	81.98
SPLEM	20	84.52
	40	94.45
	60	96.30
	80	97.92
	100	99.07
SPLEE	20	86.35
	40	87.52
	60	93.53
	80	95.38
	100	97.22
SPLEW	20	78.06
	40	80.36
	60	83.14
	80	83.14
	100	84.52
Ascorbic acid	20	93.30
	40	94.22
	60	95.15
	80	95.84
	100	97.45

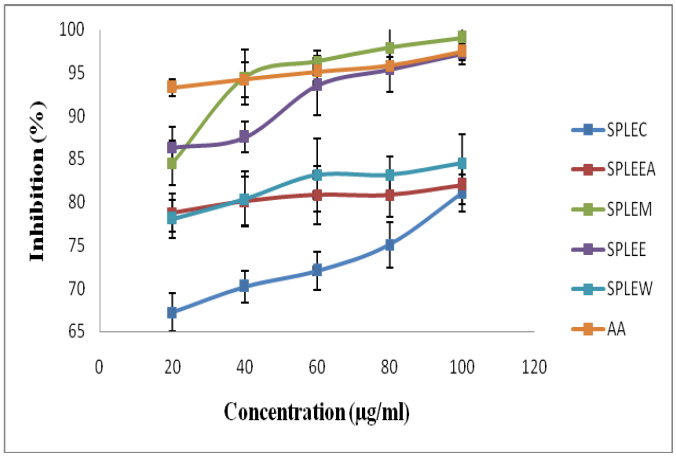

Fig. 1: Antioxidant potential (using DPPH scavenging model) of SPLEC: *Salvadorapersica* leaves extract in chloroform, SPLEEA: *Salvadorapersica* leaves extract in ethyl acetate, SPLEM: *Salvadorapersica* leaves extract in methanol, SPLEE: *Salvadorapersica* leaves extract in ethanol, SPLEW: *Salvadorapersica* leaves extract in water, AA: Ascorbic acid

Table 7: Determination of IC-50 of *S. persica* leaves extract

Sample	DPPH assay IC-50 (µg/ml)
AA	861.42
SPLEC	82.34
SPLEEA	809.71
SPLEM	214.07
SPLEE	225.37
SPLEW	347.82

Antioxidant activity of extract using H_2O_2 scavenging activity model

Antioxidant activities of *Salvadorapersica* leaves extract (SPLEC, SPLEEA, SPLEM, SPLEE, and SPLEW) were performed using H_2O_2 scavenging model. The present study showed thatIC50 values of SPLEW and SPLEC were found to be 31.17µg/ml (Min.) and 66.53µg/ml (Max.) respectively.

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Result obtained from the study was presented in table 8, figure 2 and table 9.

Table 8: H2O2 inhibition (%) of *Salvadorapersica* leaves extract

Extract	Concentration (µg/ml)	Inhibition (%)
SPLEC	20	28
	40	38
	60	44
	80	56
	100	68
SPLEEA	20	31
	40	39
	60	46
	80	59
	100	68
SPLEM	20	40
	40	54
	60	62
	80	72
	100	82
SPLEE	20	42
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	60	66
	80	78
	100	88
SPLEW	20	38
	40	58
	60	72
	80	84
	100	94

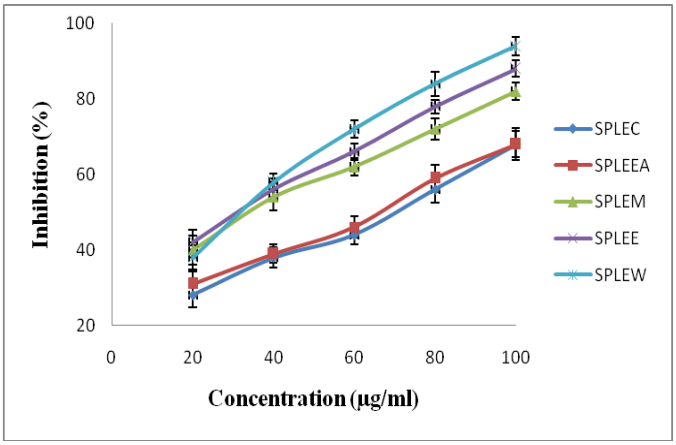

Fig. 2: Antioxidant potential of extract using H_2O_2 scavenging model

Table 9: Determination of IC-50 of *S. persica* leaves extract

Sample	H_2O_2 assay IC-50 (µg/ml)
SPLEC	66.53
SPLEEA	62.08
SPLEM	34.11
SPLEE	31.92
SPLEW	32.17

Antimicrobial activity of extract

Extract of *Salvadorapersica* leaves (SPLEC, SPLEEA, SPLEM, SPLEE, and SPLEW) was investigated to find out their growth inhibit antimicrobial activity against gram-positive bacteria (*E. coli*) and gram-positive bacteria (*B. subtilis*) using the disc diffusion method. As per our results, SPLEE have good antimicrobial activity i.e. the zone of inhibition was 4mm (dose: 100 µg/ml) and 6mm (dose: 200µg/ml) against *E. coli* while a previous research group i.e. Balto*et al* reported that ethanol extract (dose: 100mg/ml), inhibited 16mm,18mm and 20mm against *S. mutans*,*S. salivarius*, and *S. sanguis* respectively[19]. Zayed Al-Ayed*et al* was recorded the highest inhibition growth of methanol extract (dose: 400mg/ml) i.e. 4.8-13.6mm, 4.6-12.7mm, and 4.5-12.5mm against *E. coli*, *K. pneumonia* and *Serratiamarcescens* respectively[20]. Abhary*et al* reported the antibacterial activity of *Salvadorapersica* sticks extract. They were recorded inhibition zone against *E.coli* (dose: 100 µg/ml) i.e. 6mm, 7mm and 6 mm while (250 µg/ml) i.e. 7mm, 7.5 mm, and 6.5mm of water extract, ethanol extract, and hexane extract respectively[21]. Khalil et al reported the antibacterial activity of extract of aerial part of Salvadorapersica. They were found that methanol extract exhibited the highest zone of inhibition (100mg/ml) i.e. 34 mm and (250mg/ml) 36 mm against Streptococcus spp.[22].

Table 10: Antimicrobial activity of the extract

Bacteria type	Zone of inhibition (mm)											
	SPLEC (µg/ml)		SPLEEA (µg/ml)		SPLEM (µg/ml)		SPLEE (µg/ml)		SPLEW (µg/ml)		Cipro-floxacin (µg/ml)	
	100	200	100	200	100	200	100	200	100	200	100	200
E. Coli	2	3	2	2	3	5	4	6	2	2	8	14
B. Subtilis	1	2	1	1	2	4	3	5	2	2	7	12

Anthelmintic activity of extract

Albendazole (standard drug) showed the highest anthelmintic activity against the Indian worm *Eiseniafetida.Salvadorapersica* extract exhibited its anthelmintic activity but less compare to standard drug albendazole.

Our studies found the highest anthelmintic activity (dose: 150mg/ml) i.e. 13.8 minutes and 14.5 minutes of SPLEE and SPLEC respectively. Majeed Abdul reported anthelmintic activity of Salvadorapersica root extract. Majeet A. recorded the highest anthelmintic activity (dose: 80mg/ml) i.e. 15.5 minutes followed by 31.1 minutes of the aqueous extract against *Pheretimaposthuma*[23].

Table 11: *In vitro*, Anthelmintic activity of *Salvadorapersica* leaves extract

Treatment	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Albendazole	50	12.8±0.40	22.4±0.80
	100	7.5±0.20	16.5±0.70
	150	3.8±0.40	8.4±0.20
SPLEC	50	26.5±0.60	35.2±0.40
	100	18.4±0.40	28.5±0.60

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	150	14.5±0.20	22.4±0.80
SPLEEA	50	24.5±0.2	32.5±0.2
	100	20.2±0.2	28.5±0.4
	150	17.2±0.4	24.2±0.6
SPSEM	50	20.2±0.4	32.2±0.6
	100	16.4±0.6	26.8±0.2
	150	15.2±0.2	22.4±0.2
SPLEE	50	19.6±0.2	30.2±0.4
	100	15.4±0.2	22.4±0.2
	150	13.8±0.2	19.2±0.2
SPLEW	50	25.4±0.4	38.2±0.4
	100	20.4±0.2	35.4±0.2
	150	17.2±0.2	30.2±0.4
Saline solution	000	No paralysis seen till 60 minutes	No death observed till 60 minutes

CONCLUSION

The used modified maceration technique can be considered as low-cost extraction method because required fewer amount of solvents. Phytochemical studies of the extract revealed the presence of alkaloids, glycosides, tannins, saponins, flavonoids, proteins, carbohydrates, sterol, and terpenoids. These may be responsible for the pharmacological activity of the extract. Extracts were found to be soluble in 0.1 N HCL, Phosphate buffer solution pH 6.8, and Phosphate buffer solution pH 7.4, so suitable for maximum possible drug delivery systems. the pH of the extract was found to be near neutral. The study was claimed that extracts were free from heavy metals (lead, arsenic) and microbial load. The extract showed high antioxidant activity in both models DPPH scavenging and H_2O_2 scavenging model. So extract can be considered a natural source of the antioxidant agent. The outcomes of antimicrobial studies exhibited that plant extract was rich in antimicrobial capacity to both gram-negative and gram-positive bacteria. All extracts of *Salvadorapersica* showed significant Anthelmintic activity but activity was less than standard drug albendazole.

COMPETING INTERESTS DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

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