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Original article

Deciphering chemical profiling, pharmacological responses and potential bioactive constituents of *Saussurea lappa* Decne. Extracts through in vitro approaches

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ABSTRACT

Despite advancement in modern medicines, plant derived medicines have still wide range utilities as they have less side effects and are cheap and biocompitable. *Sassurea lappa* is an extensively used plant in traditional medicinal formulations. Plant roots are used to cure various diseases including cancer, rheumatic pain, abdominal and nervous disorders. The present study was aimed for the evalution of biological potentials of methanolic and chloroform extracts of *Saussurea lappa* root, leaf, seed and flower. The methanolic and chloroform extracts were subjected to qualitative and quantitative phytochemical analyses. Identification of functional groups was performed using Fourier Transform infrared (FT-IR) spectroscopy. Antioxidant potential was determined via diphenyl-1-picrylhydrazyl (DPPH), total reducing power (TRP) and total antioxidant capacity (TAC) method, anti-hemolytic potential was conducted on human RBCs, antibacterial activity was evaluated against six American type culture collection (ATCC) and three multi drug resistance (MDR) strains, cytotoxic and phytotoxic potentials were evaluated through brine shrimp lethality assay and raddish seed assay respectively. Experiments were performed in triplicates and analysis of variance (ANOVA) was applied using statistics version-8.1. Phytochemical analysis revealed the presence of sixteen secondary metabolites. Fourteen functional groups were identified through FTIR. *S. lappa* root methanolic (SLRM) showed maximum antioxidant activity index (AAI-79.42%) whereas chloroform extract of leaves (SLLC) gave highest antibacterial activity with maximum zone of inhibition (ZOI) against *Pseudomonas aeruginosa* (21.4 mm). Maximum cytotoxicity was observed for SLRM with lethal dose concentration (LC_{50}) of 58.8 μg/mL. However, root extracts showed significant phytotoxicity (15% germination). The current study investigated that bioactive compounds present in *S.*

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lappa leaves, seed, flower and roots were responsible for enhanced biological potentials. Further studies on isolation and characterization of these bioactive compounds may help in drug development. In future, we recommend different *in-vitro* and *in-vivo* studies to further confirm it biopharmacological potencies.

1. Introduction

Despite rapid advancement in modern medicines, herbal medicines are still in use (Sreejith et al., 2016) because they have less side effects and are cheap (Iqbal et al., 2017, Iqbal et al., 2018a, Iqbal et al., 2018a,b, Iqbal et al., 2018c). They are part of various medicinal systems including Ayurvedic, Siddha and Unnani medicinal system (Sharma et al., 2008).

Medicinal plants are highly rich in bioactive phytochemicals and nutraceutical compounds such as alkaloids, flavanoids, terpenoids, tannins and saponins, vitamins, proteins etc (Abbasi et al., 2018, Abbasi et al., 2019, Iqbal et al., 2019). Phytochemicals or bio-nutrients have wide range functions based on their types and are present in different food products such as vegetables, fruits and seeds etc (Batool et al et al., 2019, Batool et al., 2020, Ali et al., 2021a, Ali et al., 2021b, Ali et al., 2021c). These active phytochemicals are useful in combating different diseases like cancer (), cardiac disease and diabetes thus help to promote basic healthcare system (Shahbaz et al., 2021, Zahra et al., 2021a, Zahra et al., 2021b).

Asteraceae is the largest and economically important family of angiosperms having 1,620 genera and about 23,000 species (Amin et al., 2013). Members of family *Asteraceae* have high medicinal values and are used by the local communities to treat wide range of ailments (Alamgeer et al., 2018). The *S. lappa* is an important medicinal plant of family *Asteraceae* (Kumar et al., 2014; Huang et al., 2017) and is commonly known as "kuth" in Pakistan (Amara et al., 2017). *S. lappa* is a herbaceous and tall perennial plant, (Sharma et al., 2010; Zahara et al., 2014) and is used as herbal medicine throughout the world to cure different ailments (Madhuri et al., 2012). The plant shows biological activities due to the presence of almost 25,000 bioactive phytochemicals (Amara et al., 2017). Various biologically active phytochemicals such as lactones, isoalantolactones, alantolactones (Kumar et al., 2014; Lee et al., 2018) sesquiterpenoid lactones, santamarin (Choi et al., 2012), dehydrocostus lactones, costunolide, cynaropicrin, (Lee et al., 2018; Lohberger et al., 2013; Lin et al., 2015; Singh et al., 2017), 1,3-cyclooctadiene, phenolics, flavonoids, tannins, polysaccharides (Bagheri et al., 2018), glycosides, saponins, steroids, alkaloids and terpenoids (Basudan, 2018) have been reported in *S. lappa* that contribute towards its disease combating ability (Rahman et al., 2015; Tabata et al., 2015). This plant is effective in curing almost 43 diseases including cancer, inflammation, ulcers, gastric and liver disorders (Zahara et al., 2014) in different medicinal systems such as Indian traditional medicine, Ayurvedic medicine system (Farooqui et al., 2018) and Persian medicine system (Bagheri et al., 2018). It is an effective and safe plant which can be used in modern medicine formulation and clinical trials (Madhuri et al, 2012). The local communities in different parts of world also use *S. lappa* to treat different aliments (Sreejith et al., 2016; Mahmood et al., 2011) due to its extreme anticancer (Kumar et al., 2014; Vadaparthi et al., 2015; Singireesu et al., 2018), anti-inflammatory, anticonvulsant (Madhuri et al., 2012; Farooqui et al., 2018), anti-allergic, anti-ulcerative, anti-microbial (Lin et al., 2015) , anti-viral (Madhuri et al., 2012), anti-proliferative (Rahman et al., 2015) and hepatoprotective properties (Madhuri et al., 2012; Singireesu et al., 2018; Alnahdi et al., 2017). It also helps to cure abdominal pain and tenesmus (Choi et al., 2013). Its essential oil (Bagheri et al., 2018) possess insect repellent properties and used in cosmetic industry in the formulation of anti-aging creams (Adnan et al., 2017). Due to high medicinal importance of roots, the previous studies on this plant mainly focused on phytochemical and biological screening of only roots of this plant, and other parts of this plant like leaves, seed and flower remained neglected. Since, there is sporadic information of phytochemical screening of leaves, flowers and seeds of *S. lappa*. The present research was planned to explore photochemistry and biological potentials of all parts of this plant for its proper utilization.

2. Materials and methods

2.1. Medicinal plant collection and sample preparation

Healthy roots, leaves, seeds and flowers of *S. lappa* were collected from Astore, Gilgit Baltistan, Pakistan. The plant specimen was identified by National Herbarium, QAU, Islamabad, Pakistan. Different plant parts were thoroughly washed, shade dried and ground into fine powder. Chloroform and methanolic extract of different parts was made by dissolving 30 g powder of each part in 300 mL of respective solvent in airtight containers and kept for 48 h with constant shaking in a shaker at 200 rpm. After filtration and evaporation through evaporator (R-200 Buchi, Switzerland) the obtained extracts were stored at 4 °C till further analysis.

2.2. Qualitative phytochemical screening

Standardized phytochemical testing procedures were followed to detect the active secondary metabolites in order to establish the chemical profile of plant for phenols, carbohydrates (Yadav and Agarwala, 2011), flavonoids, alkaloids, terpenoids, saponin, tannins, glycoside**,** cardiac glycosides, coumarins, anthocyanin and betacyanin, steroids (Vishwakarma et al., 2014), phytosterols (Yadav et al., 2017), protein, anthraquinone glycosides, fats and fixed oils (Roopalatha, 2013), emodins, phlobatanins (Rauf et al., 2013) and quinone (Ajuru et al., 2017) .

2.3. Quantitative phytochemical screening

Folin–Ciocalteu (FC) reagent method was used to determine the total phenolic content (TPC). In 20 μL of sample solution Folin–Ciocalteu (90 μL) reagent and 6 % Na_2CO_3 (90 μL) was added. The mixture was incubated for 60 min at 25 °C and OD was measured at 760 nm. Gallic acid was used as positive control. TPC was expressed as gallic acid equivalents (GAE) mg/g of plant extract (Vishwakarma et al., 2014).

Total flavonoid content was estimated using $AlCl_3$ colorimetric method. For this purpose, 10 μL $AlCl_3$ and10 μL potassium acetate solution was mixed with sample solution (20 μL), and the volume was made up to 200 μL by adding 160 μL of distilled water. After 30 min incubation at 37 °C, absorbance was recorded at 415 nm. Quercetin was used as standard and results were articulated as quercetin equivalents (QE mg/g) of plant sample (Vishwakarma et al., 2014).

β-carotene and lycopene contents were estimated by mixing 2 mg of plant extract in 10 mL of acetone and n-hexane (4:6),

and mixture was vigorously shaked for 1 min. The solution was filtered and absorbance was recorded at 453, 505, 645, and 663 nm. Calculations were made using following equation (Randome et al., 2017).

$$\beta - \text{carotene(mg/100mL)} = 0.216xA663 - 1.22xA645 - 0.304xA505 + 0.452xA453$$

$$\text{Lycopene(mg/100mL)} = -0.0458xA663 + 0.204xA645 + 0.372xA505 - 0.0806xA453$$

A = Absorbance recorded at specific wavelengths

2.4. FTIR analysis

In order to identify the potential functional groups present in the plant extracts, FTIR analysis was done by taking 0.5 mg of each plant extract. The absorbance peaks were obtained in the range of 4000–400 cm^{-1} wave number. The analysis of the peaks was done and functional groups were determined on the basis of the absorbance (Maitera and Chukkol, 2016).

2.5. Antioxidant assays

2.5.1. DPPH free radical scavenging assay

DPPH activity was determined at various concentrations of plant extracts (2000–31.25 μg/mL) by mixing sample solution (10 μL) with 0.004 % methanolic DPPH (190 μL) solution and incubated in dark (30 min) at 25 °C. Sample absorbance was measured at 517 nm using ascorbic acid as a positive control and below formula was used to calculate the scavenging activity (%) (Vishwakarma et al., 2014).

% scavenging activity = $(A_{(c)} - A_{(s)} / A_{(c)}) \times 100$

$A_{(c)}$ = Absorbance of DPPH only

$A_{(s)}$ = Absorbance of DPPH + sample

2.5.2. Total-antioxidant capacity (TAC) / phosphomolybdenum assay

In 100 μL sample solution, 900 μL of reagent solution (28 mM sodium phosphate, 0.6 M sulfuric acid and 4 mM ammonium molybdate) was added. After 90 min incubation at 95 °C, absorbance at 695 nm was recorded. Ascorbic acid was used for calibration curve. The activity was determined at different concentrations (31.25–2000 μg/mL). Consequently, TAC was expressed as ascorbic acid equivalents (AAE mg/g) (Uchôa et al., 2015).

2.5.3. Total-reducing power assay (TRP)

The sample solution (100 μL) was mixed with 250 μL of potassium ferricyanide (1 %) solution and 200 μL (0.2 M) phosphate buffer. After the incubation at 50 °C for 20 min, 200 μL of trichloroacetic acid was added and centrifugation was done at 3000 rpm for 10 min. Then 50 μL of 0.1 % $FeCl_3$ solution was added in 150 μL of supernatant and OD was measured at 700 nm for various concentrations (31.25–2000 μg/mL) of plant extract. Total reducing power was expressed as GAE (mg/g) of extract (Ravisankar et al., 2014).

2.5.4. Antioxidant activity index (AAI)

Antioxidant activity index indicates average antioxidant activity of the plant extracts (Puttaraju et al., 2006) determined by taking the average of DPPH, TAC and TRP.

2.6. Anti-hemolytic activity

Anti-hemolytic activity was performed using human blood with the approval of bioethical committee (BEC) of Quaid-i-Azam University (Protocol #BEC-FBS-QAU2019-142). A 5 mL of blood was collected from the healthy participant in EDTA (ethylene diamine triacetic acid) vails. Erythrocytes were separated by centrifugation for 5 min at 1000 rpm and washing of the pellet was done using phosphate buffer saline (PBS) (pH 7.4) until supernatant became clear. The erythrocytes were then suspended in PBS (4 % v/v). 500 μL of the extract fractions (1000, 500, 250, 125 μg/mL) were added in 1000 μL of PBS and 2000 μL of erythrocytes solution. After 20 min of incubation at 25 °C, H_2O_2 (500 μL) was added and centrifugation was done for 10 min at 1000 rpm. The supernatant was subjected to OD at 540 nm. The experiment was performed in triplicates. For positive control PBS was used and H_2O_2 as a negative control. Quercetin was utilized as standard and the results were shown as quercetin equivalents (QE mg/g) (Afsar et al., 2016).

2.7. Antibacterial activity

Antimicrobial assay was performed against six ATCC (American Type Culture Collection) bacterial strains (*Bacillus subtilis* (ATCC 6633), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 66332593), *E. coli* (ATCC 25922), *S. enterica* (ATCC 14028) and *L. monocytogenes* (ATCC 13932) and three MDR (multidrug resistant) bacterial strains (*Klebsiella pneumoniae*, *P. aeruginosa* and *S. aureus*). The bacterial strains were collected from Department of Biology, Allama Iqbal Open University, Islamabad. Nutrient agar was used for bacterial culturing and at 37 °C bacterial cultures were incubated for 24 hrs. The bactericidal activity was evaluated through agar well diffusion method followed by Moonmun et al. (2017). The Penicillin was taken as positive control while DMSO as a negative control. The plant extracts that gave zone of inhibition > 8 mm were considered active and their MIC were evaluated using broth microdilution method (Moonmun et al., 2017).

2.8. Cytotoxicity assay

Cytotoxicity potential was analyzed at nine different concentrations (10–2000 μg/mL) using microtiter plate by adding 100 μL of the stock (4 mg/mL) in 1st well, followed by 50 μL, 25 μL, 12.25 μL, 5 μL, 4 μL, 2 μL, 1 μL and 0.5 μL in the successive wells and evaporated. Brine shrimp (*Artemia salina)* eggs were hatched via incubation at 28 °C in the presence of light source for 24 hrs. After hatching, ten nauplii were transferred to each well along with 200 μL of sea water. Vincristine sulphate (20–0.0625 μg/mL) was taken as positive and distilled water as negative control. After 24 hrs incubation at 28 °C dead nauplii were counted and percentage mortality was calculated. Experiment was performed in triplicate and LC_{50} values were calculated.

2.9. Phytotoxicity assay

Radish seed assay was used to access the phytotoxic potential of the plant extracts at three different concentrations (1000, 100 and 10 μg/mL) under aseptic conditions. Autoclaved filter papers (Whatman filter paper No.1) were placed in petri plate and sample solution was poured on to filter paper and after evaporation 2 mL distilled water was added. Sterilization of radish seed (0.1 % $HgCl_2$) was carried out. In each petri plate 10 sterilized seeds were placed and incubated at 25 °C under dim light. After 5 days the root length was measured and percent seed germination was calculated. Experiment was repeated thrice and calculations were done (Ramalakshmi and Muthuchelian, 2013).

2.10. Statistical analysis

All the experiments were performed in triplicates and mean ± standard error was calculated. ANOVA was applied on the results using Statistics version 8.1 and least significant differ-

Table 1
Qualitative phytochemical analysis of *S. lappa*.

Phytochemicals	SLRM	SLLM	SLSM	SLFM	SLRC	SLLC	SLSC	SLFC
Phenols	+++	+++	–	–	+++	+++	–	–
Flavonoids	+++	+++	+++	+++	+++	+++	+++	++
Alkaloids	+++	+	+	+++	+++	+++	+++	+++
Terpenoids	+++	–	–	+++	+++	–	–	+
Saponins	++	++	++	++	++	++	++	++
Tannins	+	++	–	–	–	++	–	–
Glycoside	–	–	–	–	–	–	–	–
Cardic Glycoside	+++	+++	++	++	–	++	–	++
Steroid	–	–	–	–	+++	+++	+++	+++
Phytosterol	+++	–	–	–	+++	–	+++	+++
Coumarins	+++	+++	+	+	+++	+++	+	+++
Proteins	+++	+++	+++	+++	+++	+++	+++	+++
Carbohydrates	+++	+++	++	–	+++	+++	++	–
Anthocyanin	–	–	–	–	–	–	–	–
β-cyanin	+++	+++	+	+	+	+++	+	+++
Anthraquinone Glycosides	–	–	–	–	–	–	–	–
Fats & Fixed Oils	+++	+++	+	+++	++	+++	+++	++
Quinone	+++	+++	–	+	+++	+++	+++	+++
Emodins	–	–	–	–	–	–	–	–
Phlobatnins	–	–	–	–	–	–	–	–

+++ Highly present, **++** Moderately present, **+** Slightly present, **-** Absent.
Quantitative phytochemical analysis showed that *S. lappa* contains significant amount of total phenolic content (TPC), total flavonoids content (TFC), β-carotene and lycopene (Table 2). The results revealed that among methanol extracts, highest phenolic content (75.71 ± 1.75 mg/g) was present in SLRM, while flavonoids (71.66 ± 1.72 mg/g), β-carotene (0.22 ± 0.01 mg/g) and lycopene (0.48 ± 0.03 mg/g) were predominant in SLLM. Among the chloroform extracts, SLLC showed highest values for phenolic (66.94 ± 1.05 mg/g), flavonoids (66.24 ± 1.25 mg/g) mg/g, β-carotene (0.57 ± 0.06 μg/mL) and lycopene content (0.90 ± 0.07 μg/mL) respectively.

ence (LSD) was estimated. IC_{50} values were measured through Graph pad prism (version 5.01) while, LC_{50} value was estimated.

3. Results

3.1. Qualitative and quantitative analyses of bioactive phytochemicals

The present study showed that the bioactive phytochemicals were predominantly present in all extracts. The extracts were explored for the detection of twenty secondary metabolites (Table 1). Fourteen active compounds were detected in methanolic extract of roots (SLRM) followed by thirteen compounds in chloroform extracts of root and leaves (SLRC) and (SLLC), twelve in SLLM and SLFC, eleven in SLSC, ten in SLFM and nine in SLSM. Overall, sixteen active compounds were reported in different extracts. All plant extracts showed positive results for flavonoids, alkaloids, proteins, β-carotene, coumarins, saponins, carbohydrates, fats and quinones while, glycosides, emodins and phlobatanins were absent in all extracts.

3.2. FTIR analysis

FTIR is a technique used to identify the presence of functional groups present in a particular sample. In the present study FTIR analysis of methanolic and chloroform extracts was evaluated. The results revealed the availability of various bioactive functional groups; including O-H bend (alcohols, phenols), C = O stretch (α-β-unsaturated aldehydes, ketones), C-C stretch in ring (aromatics), -C = C- stretch (alkynes), C-H stretch (alkanes), C-Cl stretch (alkyl halides), C-H wag (-CH_2X) (alkyl halides), C-N stretch (aromatic amines) C-Br stretch (alkyl halides), O-H stretch (alcohols, phenols), N-H bend (1° 2° amines), C-Br stretch (alkyl halides), C-H bend (aromatics = C-H stretch (alkenes), C-H "oop" (aromatics) and C-O stretch (carboxylic-acids, esters, ether, alcohols) in chloroform and methanolic extracts (Fig. 1 and Fig. 2).

3.3. Antioxidant assays

Antioxidant potential refers to the plant's strength to scavenge the reactive oxygen species (ROS) that increases the therapeutic potential of plant.

3.3.1. DPPH (2,2- Diphenyl-1-picrylhydrazyl) assay

Results of DPPH assay are presented in terms of IC_{50} values. in our study it was observed that SLRM possess lowest IC_{50} value (43.97 ± 0.74 μg/mL) followed by SLLM (88.63 ± 0.69), SLSM (217.10 ± 0.55) and SLFM (277.80 ± 1.47) respectively. However, among chloroform extracts, SLRC showed lowest IC_{50} value (66.06 ± 0.69 μg/mL) followed by SLLC (90.17 ± 0.95), SLSC (224.50 ± 1.72) and SLFC (324.50 ± 1.05) respectively (Table 3). Ascorbic acid used as standard, showed IC_{50} value of 39.80 ± 0.09 μg/mL.

Table 2
Quantitative phytochemical analysis of *S. lappa*.

Plant Extract	β-Carotene (mg/100 mL)	Lycopene (mg/100 mL)	TPC (Gallic Acid Eq. mg/g)	TFC (Quercetin Eq. mg/g)
SLRM	0.11 ± 0.01^{C}	0.12 ± 0.01^{CD}	75.71 ± 1.75^{A}	70.01 ± 1.37^{AB}
SLLM	0.22 ± 0.01^{B}	0.48 ± 0.03^{B}	61.77 ± 1.24^{E}	71.66 ± 1.72^{A}
SLSM	0.08 ± 0.02^{D}	0.05 ± 0.01^{F}	69.58 ± 1.15^{B}	68.94 ± 1.14^{B}
SLFM	0.09 ± 0.02^{D}	0.10 ± 0.06^{DE}	51.76 ± 1.44^{G}	64.88 ± 1.15^{C}
SLRC	0.17 ± 0.03^{C}	0.08 ± 0.02^{EF}	64.49 ± 1.71^{D}	65.33 ± 1.39^{C}
SLLC	0.57 ± 0.06^{A}	0.90 ± 0.07^{A}	66.94 ± 1.05^{C}	66.24 ± 1.25^{C}
SLSC	0.04 ± 0.02^{D}	0.05 ± 0.01^{F}	54.66 ± 1.06^{F}	58.35 ± 1.29^{D}
SLFC	0.07 ± 0.01^{D}	0.18 ± 0.04^{C}	51.89 ± 1.09^{G}	58.33 ± 1.27^{D}

Results are expressed as mean ± SD (n = 3) each letter (A-G) represents level of significance at $P < 0.05$ (LSD).

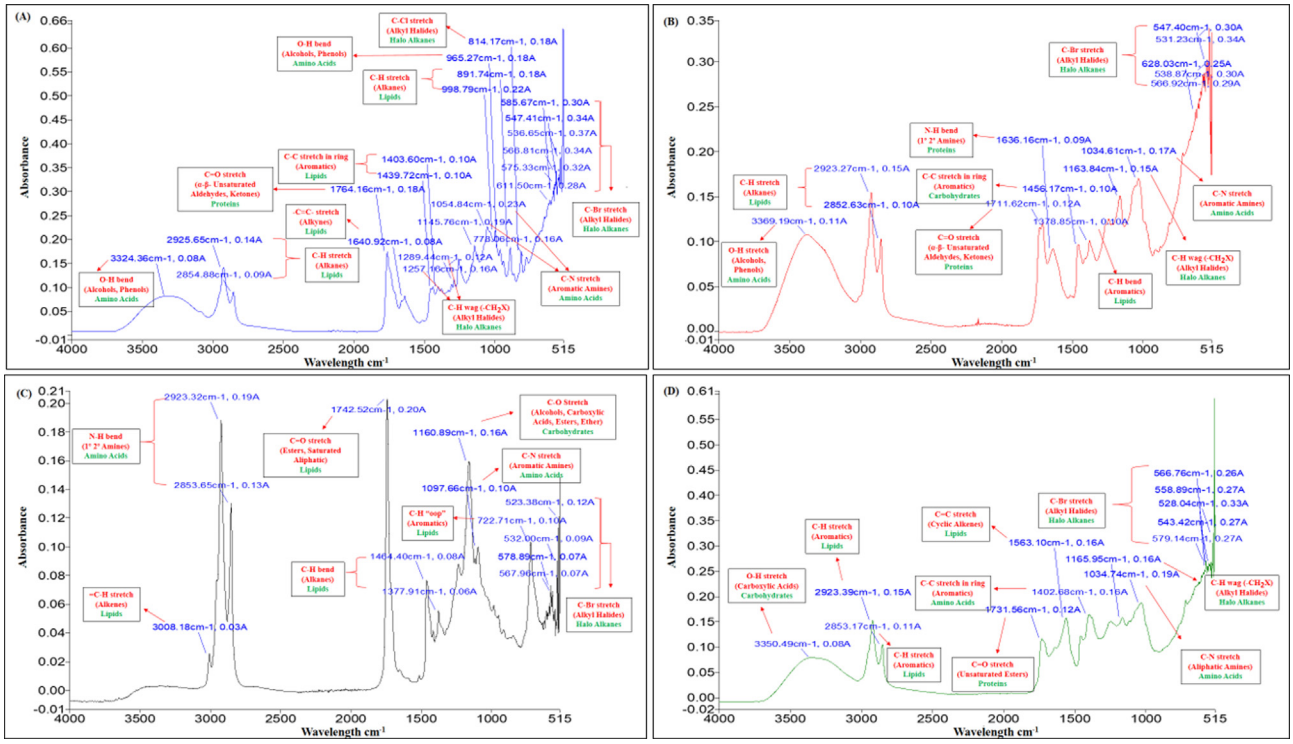

Fig. 1. FTIR analysis of methanolic extracts of *S. lappa* **(A)** SLRM **(B)** SLLM **(C)** SLSM **(D)** SLFM **(E)** SLRC **(F)** SLLC **(G)** SLSC **(H)** SLFC.

3.3.2. Total reducing power (TRP)

In this study total reducing power of extracts was determined at seven concentrations (31.25 to 2000 μg/mL) using regression equation derived from gallic acid calibration curve (y = 0.0004x + 0.22 82), and results were expressed as GAE (mg/g) (Fig. 3 A and B). According to the results the methanol extracts possess greater reducing potential as compared to chloroform extracts. Highest reducing power was observed for SLRM (75.98 ± 1.06 μg/mL). While, among chloroform extracts the highest value for TRP was 73.45 ± 1.32 μg/mL which was observed for SLRC.

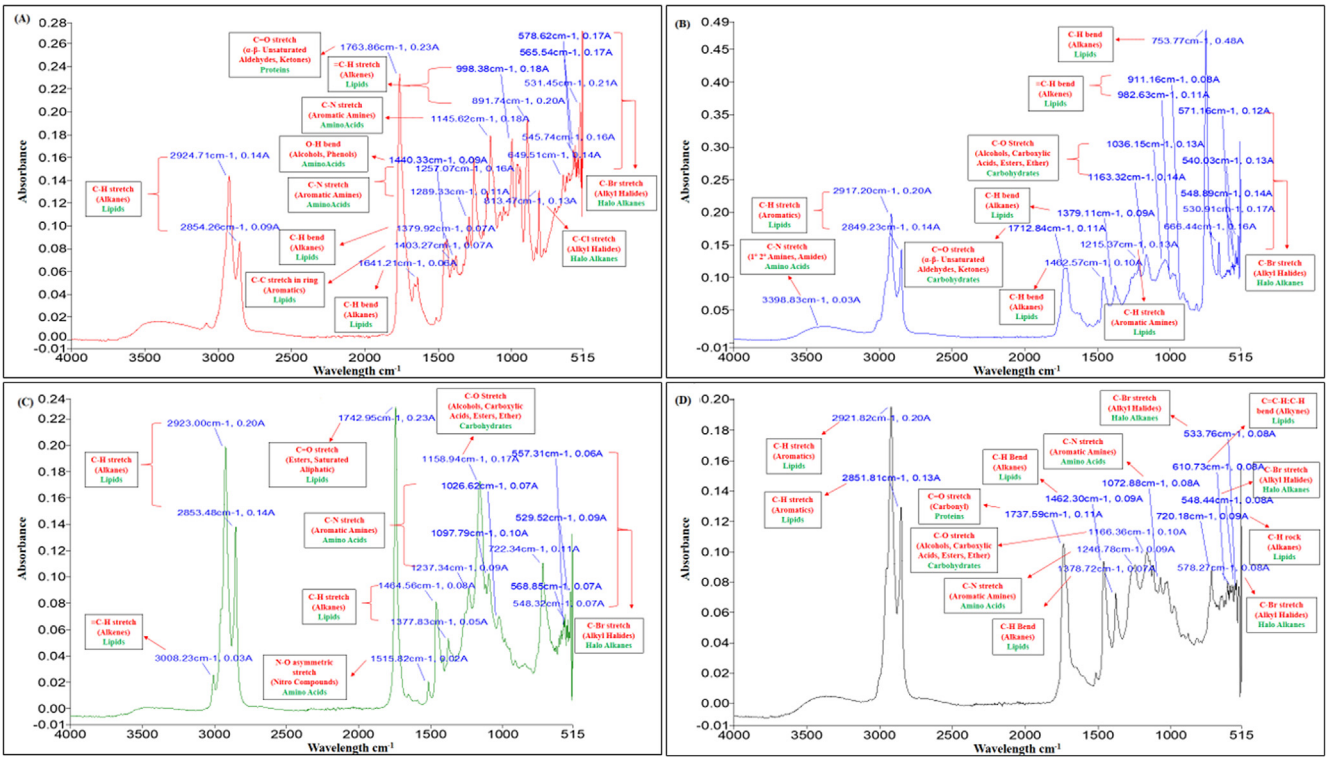

Fig. 2. FTIR analysis of chloroform extracts of *S. lappa* **(A)** SLRC **(B)** SLLC **(C)** SLSC **(D)** SLFC.

Table 3
DPPH IC_{50} (μg/mL) value of various extracts of *S. lappa*.

Plant Extract	% Scavenging at different concentrations (μg/mL)							R^2	IC_{50}	95% CI
	31.25	62.5	125	250	500	1000	2000			
SLRM	43.95	56.31	63.72	71.34	78.34	81.82	88.78	0.99	43.97 ± 0.74^{H}	36.87–52.45
SLLM	37.29	49.02	54.21	58.78	65.28	80.70	83.72	0.96	88.63 ± 0.69^{F}	60.76–129.3
SLSM	29.74	36.92	49.26	50.99	57.10	66.26	68.15	0.97	217.10 ± 0.55 ^{D}	162.3–290.2
SLFM	24.19	35.37	43.97	51.43	59.44	62.43	64.67	0.95	277.80 ± 1.47^{B}	191.7–402.8
SLRC	41.87	50.25	57.95	63.27	68.26	80.75	85.89	0.97	66.07 ± 0.69 ^{G}	48.74–89.56
SLLC	33.38	46.72	57.86	62.85	70.14	74.18	80.64	0.97	90.17 ± 0.95 ^{E}	67.45–120.5
SLSC	28.87	35.34	49.01	54.99	59.87	61.98	65.28	0.92	224.50 ± 1.72^{C}	143.0–352.4
SLFC	24.25	31.28	45.39	51.45	56.16	60.87	62.33	0.92	324.50 ± 1.05 ^{A}	205.0–513.7
Standard *	49.13	58.50	63.07	71.41	81.16	92.63	98.25	0.94	39.80 ± 0.09 ^{I}	23.51–67.40

Results for IC_{50} are expressed as mean ± SD (n = 3) each letter (A-I) represents level of significance at P < 0.05 (LSD).
IC_{50} = Inhibition concentration fifty; CI = Confidence interval
* Standard = Ascorbic Acid

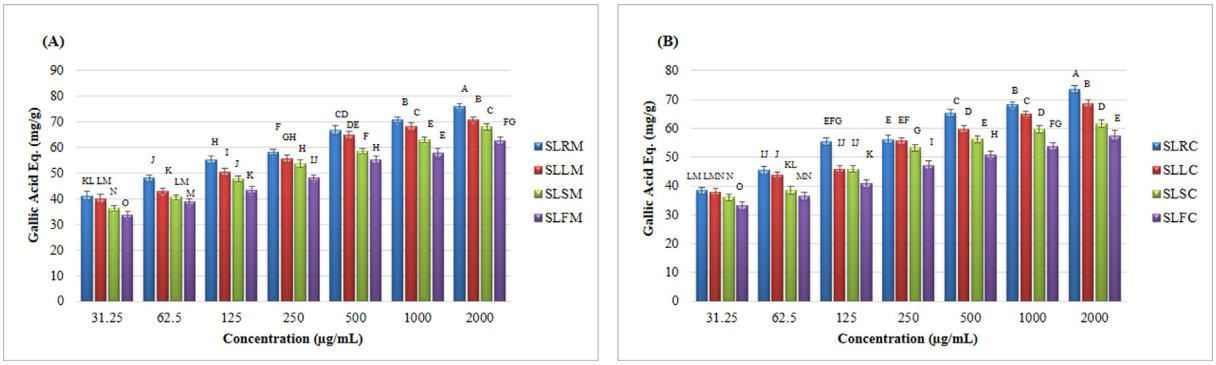

Fig. 3. Total reducing power of *S. lappa* **(A)** Methanolic extracts **(B)** Chloroform extracts Results are expressed as mean ± SD (n = 3) each letter (A-O) represents level of significance at P < 0.05 (LSD).

3.3.3. Total antioxidant capacity (TAC)/ phosphomolybdenum assay

S. lappa plant extracts were evaluated for total antioxidant capacity at seven different concentrations (3.125 to 2000 μg/mL). The results revealed that the root extracts of *S. lappa* in both solvents showed more antioxidant potential as compared to leaf, seed and flower (Fig. 4 A and B). Among the methanolic extracts, total antioxidant activity of SLRM ranged from 73.50 ± 1.00 to 48.50 ± 1 .12 μg/mL, followed by SLLM (72.83 ± 1.52 to 41.83 ± 0.58 μg/mL), SLSM (69.58 ± 1.53 to 29.50 ± 1.00 μg/mL) and SLFM (66.50 ± 1.00 to 28.50 ± 1.00 μg/mL) respectively. However, among chloroform extracts the total antioxidant activity of SLRC ranged from (69.83 ± 0.58 to 44.50 ± 1.00 μg/mL) followed by SLLC (68.83 ± 0.58 to 40.50 ± 1.00 μg/mL), SLSC (63.50 ± 1.00 to 31.63 ± 0.8029 μg/mL) and SLFC (59.50 ± 1.00 to 25.50 ± 1.00 μg/mL). The results were expressed as AAE by using ascorbic acid calibration curve (y = 0.0 001x + 0.3365).

3.4. Antioxidant activity index (AAI %)

Antioxidant activity index displays a collaborative picture of four antioxidant activities. The antioxidant activity index (AAI %) of *S. lappa* revealed that all the parts of the plant exhibit antioxidant potential. The root and leaf extracts showed the most significant results (Fig. 5). SLRM presented highest AAI (81.48 %), followed by SLLM (75.73 %), SLSM (66.37 %) and SLFM (63.10 %). Among the chloroform extracts SLRC showed maximum AAI

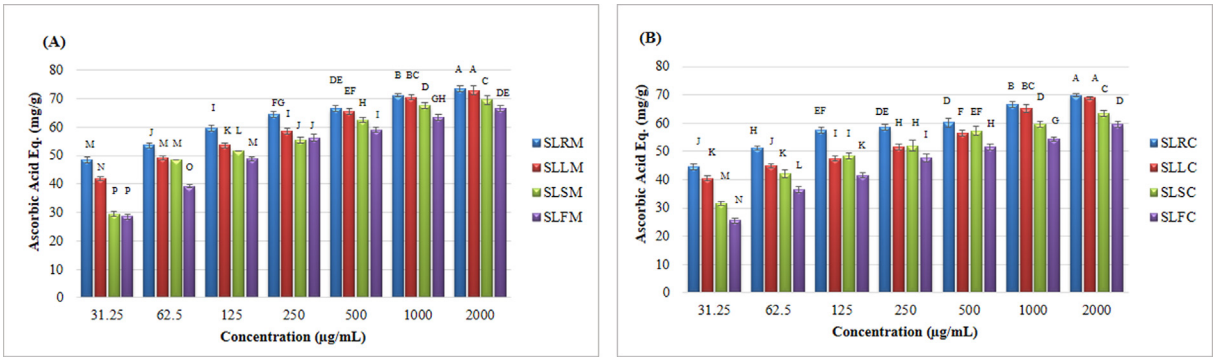

Fig. 4. Total antioxidant capacity of *S. lappa* **(A)** Methanolic extracts **(B)** Chloroform extracts. Results are expressed as mean ± SD (n = 3) each letter (A-P) represents level of significance at P < 0.05 (LSD).

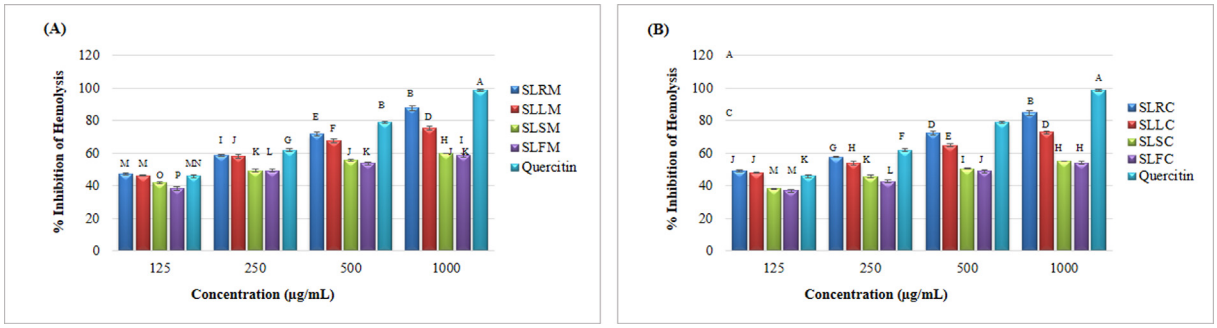

Fig. 5. Antioxidant activity index (AAI %) of *S. lappa* root, leaf, seed and flower.

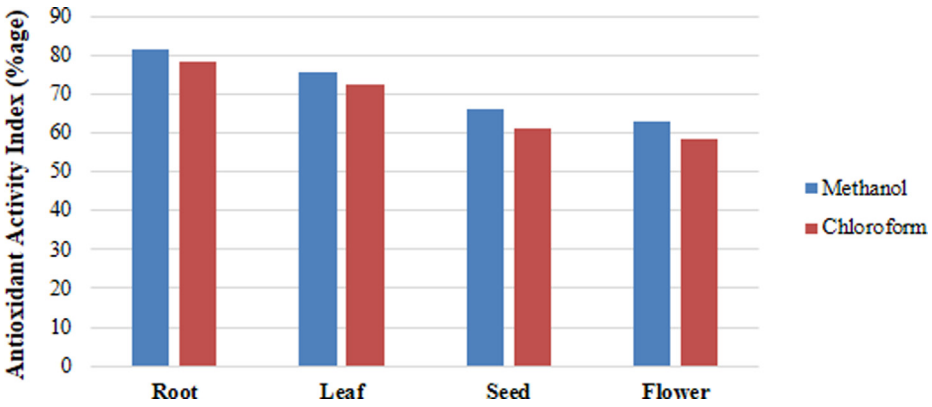

Fig. 6. Anti-hemolytic activity of *S. lappa* (A) Methanolic extracts. **(B)** Chloroform extracts. Results are expressed as mean ± SD (n = 3) each letter (A-P) represents level of significance at P < 0.05 (LSD).

(78.46 %) followed by SLLC (72.67 %), SLSC (61.34 %) and SLFC (58.32 %).

3.5. Anti-hemolytic activity

In current study, plant extracts were evaluated for anti-hemolytic potential at four different concentrations on human blood O^+ group (Fig. 6 A and B). The following trend was found for the anti-hemolytic potential of methanolic extracts: SLRM (87.67 ± 0.26 µg/mL) > SLLM (75.37 ± 0.14 µg/mL) > SLSM (59.60 ± 0.99 µg/mL) > SLFM (58.56 ± 0.47 µg/mL). The anti-hemolytic activity for chloroform extract showed the following trend: SLRC (84.68 ± 0.51 µg/mL) > SLLC (72.65 ± 0.25 µg/mL) > SLSC (54.84 ± 0.88 µg/mL) > SLFC (54.05 ± 0.60 µg/mL).

3.6. Antibacterial activity

In present investigation the antibacterial potential of *S. lappa* extracts against six ATCC and three multi drug resistant (MDR) strains were evaluated by agar well method. Results of our study revealed that all plant extracts possess remarkable antimicrobial activity against bacterial strains with varying degree of zones of

Table 4
MIC values (MIC µg/mL) and ZOI (mm ± SD) of *S. lappa* plant extracts against various bacterial strains.

Bacterial Strains		SLRM	SLLM	SLSM	SLFM	SLRC	SLLC	SLSC	SLFC	Control
B. subtilis (ATCC)	ZOI	13 ± 0.1	15 ± 0.1	NI	NI	13 ± 0.1	15 ± 0.1	NI	NI	20.16
	MIC	50	50	–	–	50	50	–	–	12.5
P. aeruginosa(ATCC)	ZOI	15 ± 0.1	19 ± 0.1	12 ± 0.1	NI	15 ± 0.1	21 ± 0.1	12 ± 0.1	11 ± 0.1	23.29
	MIC	50	25	50	–	50	12.5	50	100	12.5
S. aureus (ATCC)	ZOI	12 ± 0.1	15 ± 0.1	9 ± 0.1	NI	12 ± 0.1	17 ± 0.1	13 ± 0.1	NI	22.47
	MIC	50	50	100	–	50	50	50	–	12.5
E. coli (ATCC)	ZOI	10 ± 0.1	10 ± 0.1	NI	NI	17 ± 0.1	18 ± 0.1	NI	8 ± 0.1	21.39
	MIC	100	100	–	–	25	50	–	100	12.5
S. enterica (ATCC)	ZOI	10 ± 0.1	13 ± 0.1	NI	NI	NI	13 ± 0.1	NI	NI	19.42
	MIC	100	50	–	–	–	50	–	–	25
L. monocytogenes (ATCC)	ZOI	19 ± 0.1	16 ± 0.1	9 ± 0.1	NI	12 ± 0.1	12 ± 0.1	NI	NI	20.52
	MIC	25	25	100	–	50	100	–	–	12.5
K. pneumoniae (MDR)	ZOI	13 ± 0.1	10 ± 0.1	10 ± 0.1	9 ± 0.1	16 ± 0.1	11 ± 0.1	12 ± 0.1	12 ± 0.1	24.28
	MIC	50	100	100	100	25	100	50	50	12.5
P. aeruginosa (MDR)	ZOI	14 ± 0.1	14 ± 0.1	NI	13 ± 0.1	17 ± 0.1	21 ± 0.1	11 ± 0.1	14 ± 0.1	19.60
	MIC	50	50	–	100	25	12.5	100	50	25
S. aureus (MDR)	ZOI	NI	NI	NI	NI	12 ± 0.1	13 ± 0.1	NI	9 ± 0.1	20.67
	MIC	–	–	–	–	100	50	–	100	12.5

ZOI: Zone of inhibition (mm ± SD); MIC: (µg/mL); NI: No inhibition; Control: Penicillin.

Table 5
LC_{50} (μg/mL) of *S. lappa* plant extracts.

Plant Extract	Mortality (%) probits at different doses (μg/mL)									R^2	LC_{50}	95% CI
	10	20	40	80	100	250	500	1000	2000			
SLRM	1	3	5	6	7	7	10	10	10	0.889	58.501 ± 0.62 ^{D}	34.181–100.126
SLLM	1	2	4	6	6	8	10	10	10	0.991	64.838 ± 1.19^{C}	40.233–104.489
SLSM	0	0	0	2	2	4	8	10	10	0.925	241.554 ± 1.15 ^{A}	161.907–360.383
SLFM	0	2	4	5	5	7	8	10	10	0.979	87.745 ± 0.63^{B}	48.711–158.061
SLRC	1	2	3	5	7	10	10	10	10	0.949	66.001 ± 0.80 ^{D}	41.854–104.079
SLLC	1	1	3	5	6	8	10	10	10	0.963	78.807 ± 1.93^{C}	50.210–123.693
SLSC	0	1	1	2	4	4	6	7	10	0.929	328.698 ± 0.32 ^{A}	179.951–600.400
SLFC	0	0	1	2	4	6	9	10	10	0.966	156.583 ± 1.89^{B}	107.675–227.706

Results for LC_{50} are expressed as mean ± SD (n = 3) each letter (A-E) represents level of significance at P < 0.05 (LSD). LC_{50} = Lethal concentration fifty; CI = Confidence interval

inhibitions and MIC values (Table 4). Highest zone of inhibition of 21 ± 0.1 mm was observed for SLLC against ATCC and MDR *P. aeruginosa*, followed by SLRM (ZOI 17 ± 0.1 mm) against *E. coli* (ATCC), SLRC (16 ± 0.1) mm against *K. pneumoniae* (MDR) and SLLM (ZOI 16 ± 0.1 mm) against *L. monocytogenes* (ATCC).

3.7. Cytotoxicity analysis

In this study the cytotoxic ability of *S. lappa* by brine shrimp lethality assay revealed that plant possess significant cytotoxic potential (Table 5). Among methanolic extracts, SLRM showed most significant results with lowest LC_{50} value (58.86 ± 0.63 μg/mL) followed by SLLM (LC_{50} 65.53 ± 1.19 μg/mL), SLFM (LC_{50} 87.3 22 ± 0.63 μg/mL) and SLSM (LC_{50} 240.88 ± 1.15 μg/mL) respectively. It was observed that the methanolic extracts have more cytotoxic potential as compared to the chloroform extracts. The chloroform extracts also showed significant results with best LC_{50} value of SLRC (66.46 ± 0.800 μg/mL) followed by SLLC (LC_{50} 77.69 ± 1.93 μg/mL), SLFM (LC_{50} 157.17 ± 1.89 μg/mL) and SLSC (LC_{50} 328.88 ± 0. 32 μg/mL).

3.8. Phytotoxicity analysis

In current study, the phytotoxicity of *S. lappa* extracts was evaluated using radish seeds germination assay at three concentrations. Among methanolic extracts, SLRM and SLSM were more active in terms of inhibiting seed germination and root length as compared to SLLM and SLFM. Similarly, among chloroform extracts SLRC and SLSC were more phytotoxic as compared to SLLC and SLFC (Fig. 7).

4. Discussion

Sassurea lappa is an important medicinal plant of family Asteraceae (Kumar et al., 2014). The current phytochemical investigation of this plant showed that all parts of this plant are enriched with important phytochemicals such as flavonoids, phenols, alkaloids, proteins, β-carotene, coumarins, saponins, carbohydrates, fats and quinones. These phytochemicals have multi-functional properties such as carbohydrates have water holding capacity, binding of toxins and bile acids. Alkaloids, phenols, polyphenols, flavonoids and carotenoids are antimicrobial, anti-inflammatory, anti-tumor and antioxidant in nature (Abbasi et al., 2020, Iqbal et al., 2020, Iqbal et al., 2021, Abbasi et al., 2021, Uddin et al., 2021). Aromatic compounds and coumarins inhibit pro-carcinogen activation which may help in the treatment of chronic diseases like AIDS and cancer (Saxena et al., 2013). Tannins are known to be an effective wound healing agent (Basudan, 2018). Earlier researches also reported the significant presence of these

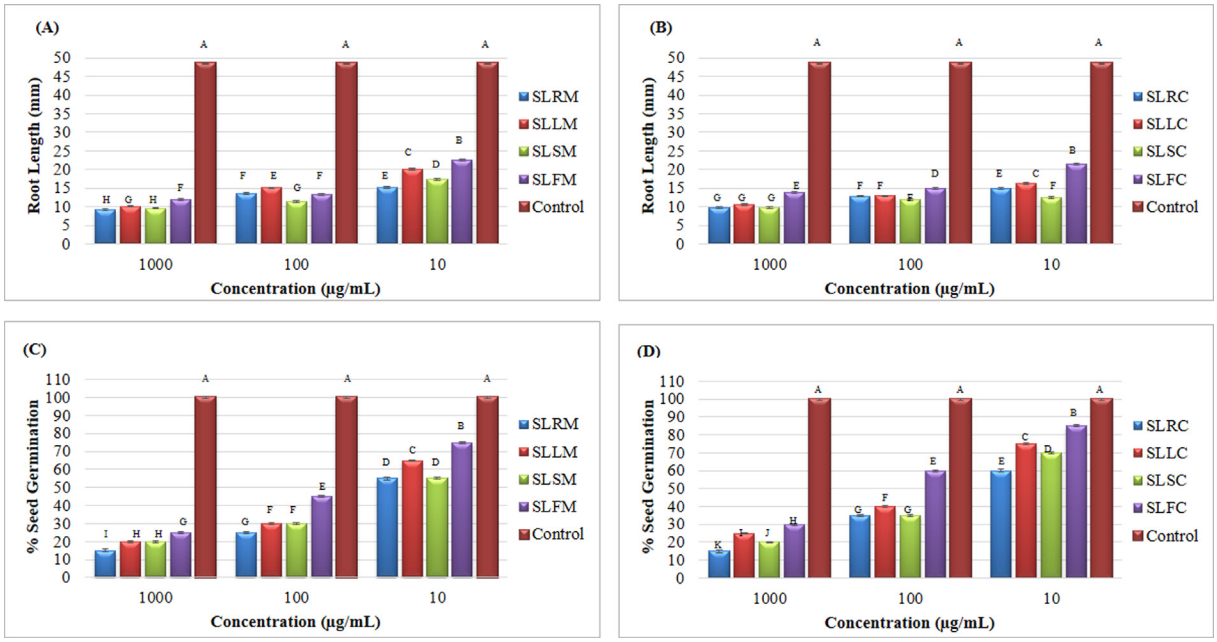

Fig. 7. Phytotoxic activity of *S. lappa* **(A)** Phytotoxic effect of methanolic extracts on radish root length. **(B)** Phytotoxic effect of chloroform extracts on radish root length. **(C)** Percentage germination of radish seeds in methanolic extracts. **(D)** Percentage germination of radish seeds in chloroform extracts. Results are expressed as mean ± SD (n = 3) each letter (A-K) represents level of significance at P < 0.05 (LSD).

phytochemicals in roots of this plant such as Mishra et al. (2011) found alkaloids, carbohydrates, glycosides, phenolics, flavonoids and saponins in root extracts of *S. lappa.* Similarly, Basudan et al. (2018) also reported the presence of eight phytochemicals including glycosides, saponin, tannins, phenols, terpenoids, alkaloid, steroids, and flavonoids in its root extracts. However, our study is preliminary to report photochemistry of seeds, flower and leaves of *S. lappa*, as no published data is available for photochemistry of these parts. The presence of important phytochemicals in seeds, flowers and leaves suggest that these parts might also be used for medicinal purposes and drug development.

The results for the quantitative phytochemical analysis revealed that on average methanolic and chloroform extracts of plant parts had high phenolic, flavonoid, β-carotene and lycopene content. The high phenolic compounds contribute to enhanced ability of plant to quench reactive oxygen species (ROS) due to their redox potential (Singh and Chahal, 2018). Phenolic compounds also have anti-inflammatory, antiallergic, antimicrobial and cardio-protective ability. (Vishwakarma et al., 2014) Similarly, flavonoids have chelating as well as scavenging ability that enhance plant's antioxidant potential (Singh and Chahal, 2018). Like phenols, flavonoids also have antifungal, antibacterial and anticancer properties (Farkas et al., 2004). In current study the highest phenolic content (75.71 ± 1.75 mg/g) was present in SLRM, while flavonoids (71.6 6 ± 1.72 mg/g) were significantly present in SLLM. Previously, the presence of 80 mg/g of total phenolic content has been reported by Alnahdi et al. (2017) in aqueous root extracts of *S. lappa.* Similarly, Chang et al. (2012) reported 44.43 μg/g of total phenolic and 92.15 μg/g of total flavonoid content in n-butanol extracts of *S. lappa* roots.

The biological potential of the plant gets enhanced with the presence of various classes of organic molecules. In current study, FTIR analysis revealed the presence of fourteen functional groups including alkanes, alkenes, alkynes, aromatic amines, alcohols, phenols, alkyl halides, aldehydes, ketones, carboxylic acid, ether, esters, hydroxyl, aliphatic amines and nitro compounds in both methanolic and chloroform extracts of this plant. The presence of these functional groups indicates the presence various classes of macro-molecules such as lipids, proteins, carbohydrates and amino acids in various parts of *S. lappa.* Our outcomes agree with the results of Al Otibi et al. (2019). They reported that the terpenes, ketones, flavonoids, phenols and aromatic compounds were significantly present in methanolic extracts of *S. lappa* roots.

Various phytochemicals in plants contribute towards antioxidant potential of plants. The antioxidants have ability to stabilize the reactive oxygen species (Sharma and Bhat, 2009). In our study the antioxidant potential of methanolic and chloroform extracts of *S. lappa* (root, leaves, flower and seeds) were evaluated by DPPH free radical scavenging activity, TAC, and TPC. DDPH analysis is extensively used to estimate the antioxidant potential of plant extracts. DPPH have ability become a stable diamagnetic molecule by accepting an electron or hydrogen radical (Kedare and Singh, 2011). A strong absorption band can be seen at 517 nm. As the absorbance decreases it shows the reduction of the DPPH radicals and the solution loses color stoichiometrically with the number of electrons taken up (Pandey et al., 2005). The results showed that SLRM possess lowest IC_{50} value (43.97 ± 0.74 μg/mL). The lowest IC_{50} value of SLRM correspond to its increased potential to scavenge free radicals (Kedare and Singh, 2011). The current results agree with the study of Chang et al. (2012). They reported that n-butanol extracts of *S. lappa* roots showed 95% DPPH free radical scavenging activity. High antioxidant potential of the plant indicates the presence of biologically active metabolites in *S. lappa*. Thus, *S. lappa* can be used as a strong antioxidant agents against ROS (Singh and Chahal, 2018). The ROS are highly reactive and are responsible for initiating the free radical chain reaction which may result in cancer, atherosclerosis and aging (Pandey et al., 2005). Therefore, plant-based antioxidants are safer and have less side effects as compared to synthetic antioxidants (Singh and Chahal, 2018). In our study the results showed that all the plant extracts exhibited significant total reducing power. SLRM exhibited highest TRP (75.98 ± 1.06 μg/mL) followed by SLLM (71.01 ± 1.07 μg/mL). The significant level of TRP of plant extracts indicates the high level of reductones present in the plant which might be responsible for the reducing power of the plant. The reductones have ability to scavenge ROS, as they can donate the hydrogen to free radical chain and thus break the chain (Jayakumar et al., 2016). TRP measures the plant ability to reduce the Fe^{3+}/ferricyanide complex to Fe^{2+}. High absorbance values correspond to more reducing power (Afsar et al., 2016). The results for the total antioxidant activity showed that SLRM (73.50 ± 1.00 μg/mL) and SLLM (72.83 ± μg/mL), showed significant results. TAC / phosphomolybdenum assay involves Mo (VI) reduction to Mo (V). Secondary metabolites in *S. lappa* extracts might be responsible for this reduction (Afsar et al., 2016) and thus helps to minimize the effect of the oxidative species (Pandey et al., 2005). Moreover, the AAI exhibited by root and leaf extracts can be correlated with the presence of significant amount of phytochemicals (phenols and flavonoids). It was reported that the antioxidant potential of plant correlates with the amount of phenolics (Sadik et al., 2017), flavonoids (Singh and Chahal, 2018) and other phytochemicals present in it. These are responsible for enhancing therapeutic potential of plant. The increased antioxidant potential enables the plant to combat diseases such as cancer, hemolysis and stimulate antiaging (Saxena et al., 2013).

The antioxidant agents (phenols and flavonoids) present in the plant have multiple functions. In our study significant antihemolytic activity of SLRM (87.67 ± 0.26 μg/mL), SLLM (75.37 ± 0.1 4 μg/mL), SLRC (84.68 ± 0.51 μg/mL) and SLLC (72.65 ± 0.25 μg/m L) can be correlated with the presence of these antioxidant agents (phenols and flavonoids) also known as bioactive compounds. Erythrocytes are present in abundance in human body which may become targeted by oxidative drugs, transition metals and radiations which may cause oxidative damage to erythrocytes membrane lipids and proteins, thus result in hemolysis (Afsar et al., 2016). Anti-hemolytic activity reported by the plant extract refers to their ability to inhibit blood hemolysis. The phytochemicals reported in the current study (flavonoids, alkaloids, phenols, coumarins, saponins, carbohydrates, fats and quinones) might be responsible for the antioxidant potential and thus help to protect the erythrocytes from oxidative damage (Armstrong et al., 2020).

Microbes are ubiquitously present in nature. Some of them are pathogenic in nature and cause serious health issues. The greater use of antibiotics is resulting in the increased resistance of bacteria (Alnahdi et al., 2017). Plant based antimicrobial agents can be alternative to synthetic antibiotics and can be used against bacterial infections caused by MDRs (Chandra et al., 2017). The results showed that the leaf and root extracts of *S. lappa* were quite effective against wide range of bacteria specially against multi drug resistant bacteria. SLLC showed maximum antibacterial activity against both ATCC and MDR *P. aeruginosa* (21 ± 0.1 mm). The antibacterial activity this plant may be due to the significant amount of lactones, terpenoids, flavonoids, alkaloids, phenols and coumarins in the plant (Khalid et al., 2011). These phytochemicals interfere with growth of pathogenic bacteria by either interfering the synthesis of cell wall and cell membrane components or by limiting the metabolism of proteins and nucleic acids of bacteria (Maitera and Chukkol, 2016). Our findings are in concordance with Alnahdi et al. (2017) who reported the antibacterial activity of *S. lappa* against four bacterial strains (*E. coli*, *B. subtilus, S. aureus and* -

Sarcina lutea). Similar study was conducted on clinical isolates of human pathogens by Hasson et al. (2013). They also used agar well diffusion method. According to the findings of Khalid et al. (2011) root extracts of *S. lappa* can be used as an alternative of gentamycine, cefuroxime, metronidazole and tranexamic acid, as the extracts gave more antimicrobial potential than antibiotics used their study.

The cytotoxic potential of the plant refers to its ability to produce phytochemicals that act as toxins to other organisms (Basudan, 2018). In current study cytotoxicity analysis showed most significant results for SLRM lowest LC_{50} value (58.86 ± 0.63 μg/mL) followed by SLLM (LC_{50} 65.53 ± 1.19 μg/mL). The cytotoxic potential of *S. lappa* extracts correlates with the presence of dehydrocostus lactone, costunolide and α-methylene-γ-lactone moiety in the plant (Wu et al., 1997). Similar, results were also reported by Saha et al. (2013) who reported that *S. lappa* root extracts were highly toxic with LC_{50} value of 50.11 μg/mL in their study. The excessive presence of hydroxyl group is responsible for reducing the cytotoxic effect of the plant (Sun et al., 2003).

In the current study root and seed extracts showed significant phytotoxic potential as compared to leave and flower extracts of both solvents. SLRM and SLSM showed 55% seed germination at 10 μg/mL. The significant phytotoxic potential indicates that the plant has ability to show allelopathic affect against the weeds (Araniti et al., 2015) which can be regarded as the presence of sesquiterpene lactones (Cárdenas et al., 2017). Another class of secondary metabolites i.e., coumarins which have benzopyrone moiety which is highly responsible for the phytotoxic ability of the plant extracts (Araniti et al., 2015).

5. Conclusion

S. lappa is a miracle plant which is known from ancient times due to its remarkable ability to cure various ailments. Till date, the phytochemical and biological evaluation of the roots were carried out. However, the leaf, seed and flower of the plant were not explored for the useful phytochemicals. The present study documents phytochemical and biological potential of methanolic and chloroform extracts of leaf, seed, flower and root of *S. lappa.* The phytochemical screening of these parts revealed that these parts are also enriched with variety of phytochemicals such as flavonoids, alkaloids, proteins, β-carotene, coumarins, saponins, carbohydrates, fats and quinones. Furthermore, FTIR analysis showed the presence of various functional groups (O-H, C = O, C-C, -C = C-, C-H, C-Cl, -CH_2X, C-N, C-Br, N-H and = C-H) in these parts. These metabolites and functional groups play key role in the antioxidant, cytotoxic, antihemolytic, and antibacterial activities of this plant. The bioactive compounds present in the plant enhances the allelopathic potential as well. Current study shows that the leaf, seed and flower of *S. lappa* also have potential to be used as medicine. However, future studies on isolation and characterization of active compounds are needed, for their application in drug development against various infections and diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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