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RESEARCH ARTICLE

A STUDY ON THE ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES IN THE ETHYL ACETATE EXTRACT OF *SAUSSUREA LAPPA*

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ABSTRACT

Herbal plants are known to make a wide number of bioactive secondary metabolites and several compounds have been consequent from them for potential development of novel drugs by the pharmaceutical and agriculture industries. However medicinal plants have not been adequately explored for their potential as a source of bioactive substances. In this context *Saussurea lappa* ethyl acetate extract were evaluated for their potential for bioactivity. In addition, the phytochemical analysis demonstrated the greatest amounts of total phenolic and flavonoid compositions in *Saussurea lappa.* Ethyl acetate extracts of the *Saussurea lappa* for the study were prepared using antioxidant and antibacterial activity against, and Xanthomonasoryzae pv**.** Oryzae and *Erwinia chrysanthemi*, *Pseudomonas fuscovaginae*. Results also indicated that the ethyl acetate extract of *Saussurea lappa* were more effectual against the tested bacterial strains and followed by antioxidant of same extract. Chemical analyses showed that *Saussurea lappa* ethyl acetate extract recorded the highest percentages of the polyphenol. In conclusion, results indicated that these plants could performance as a hopeful antimicrobial agent, due to their short assassination time.

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INTRODUCTION

The non-toxic, natural antioxidants are best alternative to synthetic antioxidants due to its low cost and no side effects to humans can be used in food, cosmetic and therapeutic industry and could serve as a good candidate for the development of phytomedicine. Naturally, many antioxidant compounds are present in various medicinal plants have been identified as free radical or active oxygen scavengers (Rice-Evan, 1998). Efforts are made to determine the antioxidant potential in many vegetables like potato, spinach, tomatoes, legumes and fruits. Fruits like berries, cherries, citrus, prunes and olives are rich in antioxidants. Lycopene is a strong antioxidant found in tomatoes, apricots, pink grapefruit, oranges, watermelon, guava, papaya and other foods. It has been estimated that 85% of American intake of lycopene isolated from tomatoes and tomato products (Xianquan *et al*., 2005). As green and black teas contain up to 30% of the dry weight as phenolic compounds such as flavandiols, flavonoids, flavonols, and phenolic acids, they are extensively studied for antioxidant properties. Also, tea has other phenolic acids, called gallic acids and characteristic amino acids, referred as the anine.

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Plants have the capability to synthesize an extensive verity of phytochemical constituents as secondary metabolites. Many of the plant metabolites have been used to efficiently treat the numerous illnesses for mankind. WHO (World Health Organization) has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species. Greatest of the medicinal plant parts are used as raw drugs and they retain different medicinal properties. Plants have a great budding for producing novel drugs and used in traditional medicine to treat long-lasting and straight infectious diseases. The Phytomedicine are more consequence in the handling of inflammation. In current years, there is a growing alertness about the standing of medicinal plants. Antibacterial resistance is sharp as the fight of bacteria to treatment with antibiotic drugs that was initially found to be operative for the treatment of infection triggered by that microorganism. This means that antibiotics become unsuccessful against resistant bacteria permitting infections to continue in patients, tapping them at enflamed risk of worse clinical consequences and death. In statistic, on average, the death rate for patients with infections caused by non-resistant bacteria is less than half of that of people with a resistant form of the same infection. Antibiotic resistance is present worldwide and new resistance mechanisms are continuing to emerge, strongly increasing the risk of spread of resistant strains.

Thus, antibiotic resistance represents a threat to global public health and represents an important economic issue, due to the higher health care costs of necessary therapies and the increased duration of illness, treatment, and potential hospitalisation when compared with non-resistant, common infections. It has been scientifically proven that the indiscriminate and inappropriate use of antibiotics has accelerated the emergence of drug-resistant strains. In addition, poor sanitary conditions and inadequate food-handling encourage the further spread of antimicrobial resistance. Considering that antibacterial resistance is a multifactorial problem, driven by many interconnected factors, the World Health Organization suggests a series of concerted and coordinated actions.

Rice bacterial blight**,** also called bacterial blight of rice**,** deadly bacterial disease that is among the most destructive afflictions of cultivated rice, the bacterium Xanthomonas oryzae pathovar oryzae**.** Since rice paddies are flooded throughout most of the growing season, Xoo may easily spread among crops; bacteria travel through the water from infected plants to the roots and leaves of neighboring rice plants. Wind and water may also help spread Xoo bacteria to other crops and rice paddies. Various mechanisms of disease, including quorum sensing and biofilm formation, have been observed in rice bacterial blight and Xoo. On the other hand, the study of medicinal plants as possible natural sources of obtaining active compound (secondary metabolites) against phytopathogens has gained increasing interest in recent years, due to several aspects, mainly that they are obtained from a natural source through the production or synthesis of secondary metabolites considered as nontoxic such as phenols, flavonoids, terpenes, alkaloids, etc. Another advantage is that phytopathogens still do not develop resistance to the antifungal, antimicrobial, and nematicide effect of the phytochemical compounds produced by some medicinal plants (Żaczek *et al*., 2015).

*Saussurea lappa*C.B. Clarke, syn. *Saussurea costus* (Falc.) Lipsch belonging to family Asteraceae. It is perennial herb that grows to a height of 1-2 m; stem is upright, stout and fibrous while root is a long stout of approximately 60 cm with a characteristic odour; leaves are lobate, stalked, membranous, irregularly toothed; upper leaves are small while basal leaves are large with long lobately winged stalks. Flowers are stalkless, dark purple to black in colour and are arranged in terminal and axillary heads. Pappas is approximately 1.6 cm long, fluffy, feathery giving an inquisitive appearance to the fruiting flower heads. Fruit of S. lappa is cupped, curved, compressed and hairy. Its active constituents are mainly terpenes, but it also contains anthraquinones, alkaloids and favonoids. Plant has various terpenes that mainly have antitumor properties and anti-infammatory, such as costunolide, dihydrocostunolide, 12-methoxydihydrocostunolide, dihydrocostus lactone, dehydrocostus lactone (Yang *et al*., 1998).

MATERIALS AND METHODS

Plant Materials: Root of *Saussurea lappa* were collected from Government Siddha Medical College, Herbal Garden, Chennai, Tami Nadu, India. Plants were authenticated by Dr. S. Sankaranarayanan, Head, Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, Chennai-600 106, Tamilnadu.

Phytochemical screening and extraction: The root of *Saussurea lappa* were dried in hot air oven at (40 °C) for 1 hour, after which it was ground to uniform powder with house hold mixer grinder. The aqueous extracts were prepared by soaking 100 g of the dry powdered plant materials in 500 L of aqueous at 4 °C for 24 hour. The extracts were filtered first through a Whatmann filter paper No. 42 (125 mm) and then centrifuged at 5000 rpm for 10 min (Remi-R-8C, India). The clear solution was partitioned with petroleum ether for elimination of lipid and fatty acids compound and finally aqueous extracts were partitioned with ethyl acetate. It was concentrated using a rotary evaporator with the water bath set at 40 °C. The percentage yield of extracts ranged from 7–19% w/w (Debiyi and Sofowora, 1978)

Determination of total Phenolics: The concentration of total phenolics in the ethyl acetate extract of *Saussurea lappa* were determined by using Folin-Ciocalteu reagent and calibrated externally with gallic acid. Briefly, about 0.2 ml of flavonoid extract and 0.2 ml of Folin-Ciocalteu reagent were added and mixed vigorously. After shaking for 4 min, 1 ml of 15% Na_2CO_3 was added, and finally the mixture was allowed to stand for 2 hours at room temperature. The absorbance was measured at 760 nm using Deep Vision 1371 spectrophotometer. The concentration of the total phenolicwas estimated as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve. The quantification of phenolic compounds in all the fractions was carried out in triplicate and the results were averaged (Singleton *et al*., 1999).

Determination of Flavonoid content: The amount of total flavonoids in the extracts was measured according to Quettier-Deleu *et al*. (2000). This method is based on the formation of a complex flavonoid-Aluminium, with the absorbance maximum at 430nm. Rutin was used to make a calibration curve. To 1ml of flavonoid rich fraction, added 1ml of 2% $AlCl_3$ and it was incubated at room temperature for 15 min. Then absorbance was measured at 430 nm using Deep Vision 1371 spectrophotometer.

Invitro antioxidants properties

ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical scavenging assay: ABTS radical scavenging activity of ethyl acetate extract of *Saussurea lappa* was determined according to Re *et al.,* (1999). ABTS radical was freshly prepared by adding 5 ml of 4.9 mM potassium persulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with distilled water to yield an absorbance of 0.70 at 734 nm and the same was used for the antioxidant assay. The final reaction mixture of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of Ascorbic acid. Similarly, in the test group, 1 ml reaction mixture comprised 950 µl of ABTS solution and 50 µl of different concentration of flavonoid rich fraction. The reaction mixture was vortexed for 10 s and after 6 min, absorbance was recorded at 734 nm against distilled water by using a Deep Vision (1371) UV–Vis Spectrophotometer and compared with the control ABTS solution. Ascorbic acid was used as reference antioxidant compound.

ABTS Scavenging Effect (%) = $[(A_{0-}A_1/A_0) \times 100]$

Where *A*0 is the absorbance of the control reaction and *A*1 is the absorbance of flavonoid rich fraction.

Inhibition of lipid peroxidation activity: Lipid peroxidation induced by Fe^{2+}ascarbate system in egg yolk by the method of Bishayee and Balasubramaniyam1971, was estimated as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa *et al*.(1979). The reaction mixture contained 0.1 ml of egg yolk (25% w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); $FeSO_4$ $(NH4)_2SO_4.7H_2O$ (0.06 mM); and various concentrations of ethyl acetate extract of *Saussurea lappa* in a final volume of 0.5 ml. The reaction mixture was incubated at 37°Cfor 1 hour. After the incubation period, 0.4 ml was removed and treated with 0.2 ml sodium dodecyl sulphate (SDS) (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The total volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95 to 100 °C for 1 hour. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The butanol-pyridine layer was removed and its absorbance was measured at 532 nm in Deep Vision (1371) UV–Vis Spectrophotometer) to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the optical density (OD) of test sample with control. Ascorbic acid was used as standard. Inhibition of lipid peroxidation (%) by the extract was calculated according to 1-(E/C) X 100, where C is the absorbance value of the fully oxidized control and E is absorbance of the test sample (Abs_{532}+TBA–Abs_{532}+TBA).

Superoxide radical scavenging assay: This assay was based on the capacity of the extract to inhibit the photochemical reduction of Nitroblue tetrazolium (NBT) (Beauchamp and Fridovich 1979) in the presence of the riboflavin-light-NBT system, as described earlier Tripathi and Pandey (1999) and Tripathi and Sharma (1999). In brief, each 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM Ethylene diamine tetra acetic acid (EDTA), NBT (75 µM) and different concentration of test sample solution. It was kept in front of fluorescent light and absorbance was taken after 6 min at 560 nm by using a Deep Vision (1371) UV–Vis Spectrophotometer. Identical tubes with reaction mixture were kept in the dark and served as blanks. The percentage inhibition of superoxide generation was measured by comparing the absorbance of the control and those of the reaction mixture containing test sample solution.

% Super oxide radical scavenging capacity= $[(A_0-A_1)/A_0] \times 100$

Where A_0 was the absorbance of control and A_1 was the absorbance of flavonoid rich fraction.

Nitric oxide radical scavenging activity: Nitric oxide scavenging capacity of ethyl acetate extract of *Saussurea lappa* was measured according to the method described by Olabinri *et al*.(2010*)*. 0.1 ml of sodium nitroprusside (10 mM) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration of flavonoid rich fraction and incubated at room temperature for 150 min. After incubation period, 0.2 ml of Griess reagent (1% Sulfanilamide, 2% Phosphoric acid and 0.1% N-(1-Naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the reaction mixture was read at 546 nm against blank. All readings were taken in triplicate and ascorbic acid was used as the standard. The % inhibition was calculated by following equation.

% Nitric oxide radical scavenging capacity= $[(A_0-A_1)/A_0] \times 100$

Where A_0 was the absorbance of control and A_1 was the absorbance of flavonoid rich fraction.

Metal chelating activity: Metal chelating capacity of ethyl acetate extract of *Saussurea lappa*was measured according to the method described by Iihami *et al*.(2003). 1 ml of different concentrations of flavonoid rich fraction was added to 0.05 ml of 2 mM ferric chloride solution. The reaction was initiated by the addition of 0.2 ml of 5 mM Ferrozine and the mixture was shaken vigorously. After 10 min, the absorbance of the solution was measured at 562 nm against blank. All readings were taken in triplicate and ascorbic acid was used as the standard. The % inhibition of ferrozoine-Fe2+complex was calculated by following equation.

% Inhibition of ferrozoine-Fe^{2+}complex = $[(A_0-A_1)/A_0] \times 100$

Where A_0 was the absorbance of control and A_1 was the absorbance of flavonoid rich fraction.

Antibacterial Properties

Bacterial strains

Bacteria used for the determination of antibacterial activities were *Xanthomonasoryzae pv. Oryzae* MTCC 29213 and *Erwinia chrysanthemi* MTCC 1771, *Pseudomonas fuscovaginae* MTCC 2488. The bacterial strains were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology Sector 39-A, Chandigarh-160036, India. All bacterial strains were sub cultured on nutrient agar medium, incubated at 37 °C for 24 h and stored at 4 °C in refrigerator to maintain stock culture.

Antibacterial assay: Antibacterial activity was carried out using disc diffusion method (Velickovic and Smelcerovic, 2003). Petriplates were prepared with 20 ml of sterile nutrient agar (HIMEDIA). The tested cultures were swabbed on top of the solidified media and allowed to dry for 10 min. The test was conducted in four different concentrations of the flavonoid rich fraction (5, 10, 15 & 20 µl/ml) and impregnated discs with extracts (Whatman No.1 filter paper was used to prepare discs) were prepared and air dried well. The loaded discs were placed on the surface of the medium and incubated at room temperature for 24 h. The relative susceptibility of the organisms to the flavonoid rich fraction was indicated by the clear zone of inhibition around the discs. It was then observed, measured and recorded in millimeters with three replicates.

Determination of minimum inhibitory concentration: Microdilution broth susceptibility assay for bacteria was used, as recommended by Eloff, (1998). All tests wereperformed in Mueller Hinton broth (MHB) supplemented with Tween 80detergent (final concentration of 0.5%, v/v) to enhance the oil solubility. Bacterialstrains were cultured overnight at 37 °C in MHA and the yeasts were cultured overnightat 30 °C in SDB. Test strains were suspended in MHB to give a final density of $5X10^5$ CFU/mL and these were confirmed by viable counts. Geometric dilutionsranging from 5 µmg/mL to 20 µg/mL of the ethyl acetate extract of *Saussurea lappa*were prepared in a96-well microtiter plate, including one growth control (MHB + Tween 80) and onesterility control (MHB + Tween 80 + extract). Plates were incubated under normalatmospheric conditions at 37 °C for 24 h for bacteria and at 30 °C for 48 h for theyeasts.

The bacterial growth was indicated by the presence of a white ‘‘pellet” onthe well bottom.

Statistical analysis: The influence of the ethyl acetate extract of *Saussurea lappa* on its antioxidant activity was measured by the ABTS assay, lipid peroxidation, superoxide scavenging, metal chelating and nitric oxide radical were ascertained using one-way analysis of variance (ANOVA). Furthermore, Duncan’s post hoc test was applied, so as to determine the statistically significant different values. All statistical handling was performed using SPSS software, v. 14.0 (SPSS, Chicago, Ill., U. S. A.

RESULTS AND DISCUSSION

In the present study, the **root**extract showed positive results for terpenes, steroids alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins, and phenolic acids as secondary metabolites with potential biological activities, confirming the scant data yielded by previous studies of *Saussurea lappa*.

Analysis of ethyl acetate extract of by GC–MS: Ethyl acetate extract from *Saussurea lappa* ensured highest flavonoid content and exhibited the strongest antioxidant activity.The GC-MS analysis is used to determine its chemical composition that may contribute to this activity. The GC-MS analysis showed a variety of phenolic compounds (Table-2 and Fig-1). In addition, many cinnamic acid derivatives with the phenolic hydroxyl group were considered as antioxidant and antibacterial activities were supposed to have several health benefits due to their strong free radical scavenging properties.

Free radical-scavenging ability using ABTS assay of goat urine: The radical scavenging ability was measured by ABTS assay as given in Table 3 the Percentage inhibition of the ABTS radical activity assayed on average, and higher free radical-scavenging values were found in goat urine than pure ascorbic acid. However, the agreement between this assays, in our study, probably indicate that these activities were mainly due to phenolics substance. Although these constituents were not investigated, their contributions toward antioxidant/ antiradical activities of these studied goat urine may be very minimal. Antioxidant assay is based on electron transfer reaction, whereas ABTS assays are based on electron and H atom transfer.

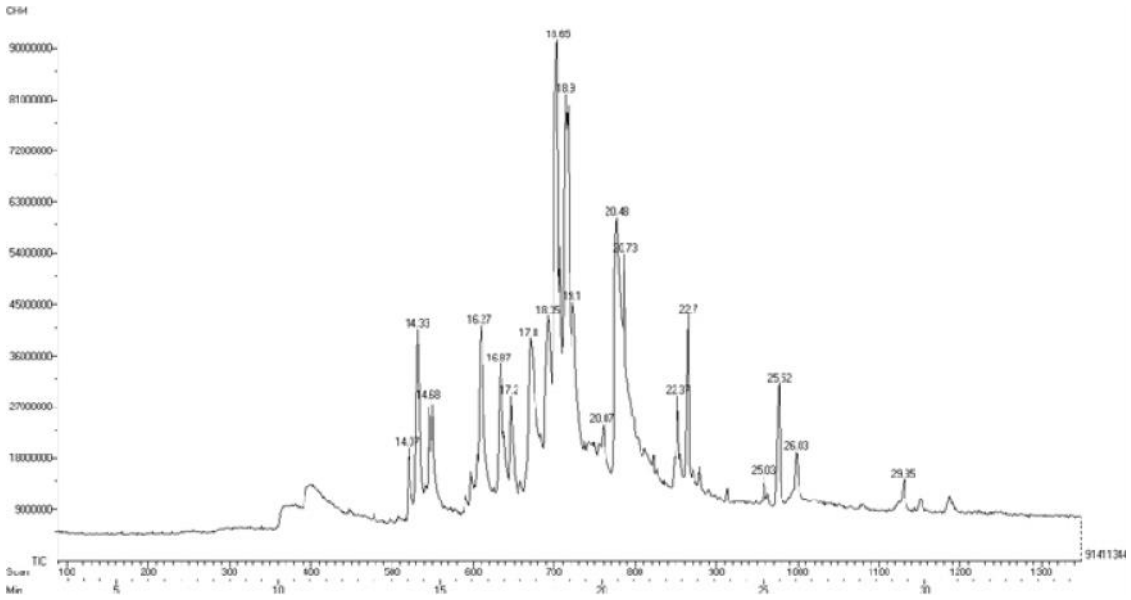

Fig. 1. GC MS analysis of Ethyl acetate extract of *Saussurea lappa*

Table 1. Anti-bacterial activity of ethyl acetate extract of *C. angustifolia*tested against plant pathogenic bacteria

Ethyl acetate extract of *C. angustifolia*	Different concentrations of extract µl/ml							
	[a]Inhibition zone of the Xanthomonasoryzae pv. Oryzae measured (mm)				[a]Inhibition zone of the*Pseudomonas fuscovaginae*measured (mm)			
	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml	5 µl/ml	10 µl/ml	15µl/ml	20 µl/ml
	9.1±0.23	12.3±1.3	14.3±2.1	17.6±1.3	8.2±1.3	11.2±1.5	13.9±0.2	16.4±0.1
	[a]Inhibition zone of the *Erwinia chrysanthemi* measured (mm)							
	7.3±1.4	9.8±1.4	12.4±1.2	15.1±0.8				

[a] Mean diameter of the zone of inhibition is in millimetres. Figures in parentheses are inhibition percentages compared to streptomycin.

Table-2. Analysis of ethyl acetate extract of by GC–MS

S.No	Compound	Retention Time(min)	Molecular weight	Major peaks
1	Pentadecanoic acid, 14-methyl-, methyl ester	17.2	270	269, 226, 184
2	3-Methylene-4-phenyltricyclo	17.8	224	251, 209, 180
3	Tricyclo[7.2.2.0(3,8)]tridec-12-en-2-one, 5,6-epoxy-4-methyl	18.35	218	251, 227, 209
4	Tricyclo dodec carboxy ethoxy	19.65	273	259, 241, 222
5	Benzamide, 2-amino-5-hydroxy	18.9	228	259, 227, 209
6	Eicosatetraenoic acid	20.48	304	253, 238, 224
7	Hexadec-9-enoic acid	22.37	254	255, 218, 194
8	Tetratriacontane	22.67	478	308, 294, 266
9	8-Octadecenal	25.03	266	251, 218, 178
10	Heptacosane	25.53	380	379, 336, 280

Table-3. Free radical-scavenging ability using ABTS assay of goat urine

Different concentration of extract	Percentage of lipid peroxidation	
	ABTS radical scavenging activityof Ethyl acetate extract of *Saussurea lappa*	Standard Vitamin-C
5 µl/ml	21.53±1.48	17.34±1.47
10 µl/ml	37.46±0.68	34.36±0.98
15 µl/ml	56.31±0.89	49.34±1.78
20 µl/ml	74.35±1.63	71.34±0.89
EC_{50} value	51.23	55.34

[a] Results are expressed as percentage of ABTS radical scavenging with respect to control. Each value represents the mean+SD of three experiments.

Table 4. Inhibition of lipid peroxidation activity of ethyl acetate extract of *Saussurea lappa*

Different concentration of extract	Percentage of lipid peroxidation	
	Lipid peroxidation activity of Ethyl acetate extract of *Saussurea lappa*	Standard Vitamin-C
5 µl/ml	20.35±2.46	16.34±1.64
10 µl/ml	37.64±1.36	32.64±1.87
15 µl/ml	60.21±1.49	48.37±0.56
20 µl/ml	76.34±2.78	68.34±2.48
EC_{50} value	47.35	58.67

[a] Results are expressed as percentage inhibit of lipid peroxidation with respect to control. Each value represents the mean+SD of three experiments.

Table 5. Superoxide scavenging assay activity of ethyl acetate extract of *Saussurea lappa*

Different concentration of extract	Percentage of Superoxide scavenging activity	
	Superoxide scavenging activity of Ethyl acetate extract of *Saussurea lappa*	Standard Vitamin-C
5 µl/ml	22.34±1.47	20.34±0.23
10 µl/ml	43.65±2.89	38.64±1.89
15 µl/ml	65.31±1.69	58.64±2.63
20 µl/ml	85.67±1.89	72.34±1.48
EC_{50} value	42.67	54.67

[a] Results are expressed as percentage of Superoxide scavenging activity with respect to control. Each value represents the mean+SD of three experiments.

Table-6. Nitric oxide radical scavenging assay of the Ethyl acetate extract of *Saussurea lappa*

Different concentration of extract	Percentage of Nitric oxide radical scavenging activity	
	Nitric oxide scavenging activity of ethyl acetate extract of *Saussurea lappa*	Nitric oxide scavenging of Standard Vitamin-C
5 µl/ml	24.56±0.39	20.34±2.48
10 µl/ml	48.67±2.61	43.64±1.68
15 µl/ml	65.31±1.78	58.38±1.89
20 µl/ml	88.67±1.47	76.98±1.78
EC_{50} value	38.97	45.68

[a] Results are expressed as percentage of Nitric oxide radical activity with respect to control. Each value represents the mean+SD of three experiments.

Inhibition of lipid peroxidation activity of ethyl acetate extract from the root *Saussurea lappa:* The ethyl acetate extract of *Saussurea lappa*also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was recorded in ethyl acetate extract of *Saussurea lappa* (76.34%) and lowest inhibition percentage of ascorbic acid was found in 68.34% (Table-4). As it is identified that lipid peroxidation is the net result of any free radical attack on membrane and other lipid components present in the system, the lipid peroxidation may be enzymatic (Fe/NADPH) or non-enzymatic (Fe/ascorbic acid). In the present study, egg yolk was used as substrate for free radical mediated lipid peroxidation, which is a non-enzymatic method. Normally, the mechanism of phenolic compounds for antioxidant activity includes neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals.

Superoxide scavenging assay activity of ethyl acetate extract of *Saussurea lappa:* The effect of the goat urineon superoxide radical was determined by photochemical reduction of nitro blue tetrazolium (NBT) in the presence of a riboflavin-light-NBT system and the results are shown in Table-5. All the tested samples significantly scavenged the superoxide radicals in a concentration-dependent manner and standard compounds (ascorbic acid) at the concentration of 5-20 µl/ml. The goat urineshowed highest radical activity in the percentage of 85.67% when compared to positive control 72.34% (Table-5). Superoxide radicals are reasonably a weak oxidant may decompose to form stronger reactive oxidative species, such as singlet oxygen and hydroxyl radicals.

Nitric oxide radical scavenging assay of ethyl acetate extract of *Saussurea lappa:* Nitric oxide radical quenching activity of goat urine was identified and compared with the standard ascorbic acid. The goat urinealso caused a moderate dose-dependent inhibition of nitric oxide with an EC_{50} (Table-6) of 38.97 µg/ml. Vitamin-C was used as a reference compound and 45.68 µg/ml Vitamin-C was needed for 50% inhibition. The EC_{50} value of the extract was less than that of the standard. At 20 µg/ml, the percentage inhibition of the ethyl acetate extract of *Saussurea lappa*were 88.67% whereas that of Vitamin-C was 76.98%.

Anti-bacterial activity of ethyl acetate extract of *Saussurea lappa*tested against plant pathogenic bacteria: Antibacterial activities of ethyl acetate extract of *Saussurea lappa*against the tested organisms are shown in Table-7. The plants differ in their activities against the micro-organisms tested. Ethyl acetate extract of *Saussurea lappa*showed maximum antibacterial activity against *Xanthomonasoryzae pv. Oryzae, Erwinia chrysanthemi* and *Pseudomonas fuscovaginae*. Highest antibacterial activity was observed with ethyl acetate extract of *Saussurea lappa*against *Xanthomonasoryzae pv. Oryzae* and *Pseudomonas fuscovaginae* (17.6and 16.4mm) respectively while lowest activity was observed against *Erwinia chrysanthemi* with the inhibition zone of 15.1mm. Results obtained in the current investigation revealed that the ethyl extract possess potential antibacterial activity against entire tested organisms. These findings are quite similar with the results of Hasson *et al*. (2013) reporting that *Saussurea lappa*aromatic oilinhibited the growth of some Gram positive and Gram negative bacteria, fungi and yeasts. As the main component, sesquiterpenoids, has proven to be particularly effective against some species of Gram positive and Gram negative bacteria.Ethanolic extract of S. lappa was assessed for antimicrobial potential using S. mutans. The study showed that the ethanolic extract caused significant. Antifungal potential of S. lappa was assessed. By using microdilution technique, nine fungal strains i.e. *Aspergillus niger, Aspergillus ochraceus, Aspergillus versicolor, Aspergillus flavus, Penicilium ochrochloron, Penicillium funiculosum, Trichoderma viride, Cladosporium cladosporioides* and *Alternaria*. KSR4 possessed the highest antifungal potential, while KSR3 showed medium activity. Compounds KSR1 and KSR2, showed good activity (Rao *et al*., 2007).

Conclusion

In this work, the antimicrobial and antioxidant activities of extracts of some plants used in Iranian folklore medicine were reported. Based on the results, ethyl acetate extract of *Saussurea lappa* showed the maximum antibacterial activity against most of the tested bacteria pathogens, attributable to higher content of phenolic and flavonoid compounds. According to the obtained results, a high resolution GC/MS method reported for the evaluation of the constituents of ethyl acetate extract of *Saussurea lappa.* Furthermore, in this study, the antibacterial and antioxidants activities of were evaluated for the first time. Furthermore, antioxidant assays including measurement of ABTS, lipid peroxidation, and nitric oxide scavenging activity were reported for the first time in this study.

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