Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i19.3478

PHYTOCHEMICAL POTENTIAL OF SAUSSUREA LAPPA OOTS EXTRACT AS AN ANTIOXIDANT AND ANTIMICROBIAL THROUGH IN VITRO APPROACH.

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Abstract:
Medicinal plants contain collections of bioactive compounds like alkaloids, flavonoids, tannins, etc. which are prescribed for the management of acute and chronic diseases. The medicinal plants having bioactive compounds used as therapeutic agents has an important area in biomedical and natural research products. Saussurea lappa is an effective medicinal plant used in traditional medicines to cure the sore throat, tonsilitis, pleurisy, rheumatoid arthritis, typhoid fever and many of bacterial and fungal infections. The extracts and bioactive compounds from Saussurea lappa roots have been evaluated and found to be medicinal importance as an antioxidant, antibacterial and antifungal activities. In this study, the hydro-ethanolic extract of Saussurea lappa was prepared by microwave-assisted extraction (MAE) process. Saussurea lappa HPLC analysis of the chemical compound contained the highest concentration of chlorogenic acid, cinnamic acid, P-coumeric acid, fenalic acid, syringic acid, vanillic acid, sinapic acid, quercetin, gallic acid, caffeic acid, M-coumeric acid concentration ppm. The extract was qualitatively tested for different phytochemicals and the total phenolic content (TPC) of the extract was calculated in terms of gallic acid equivalent (GAE) per gram of dry extract while the contents of flavonoids were determined as quercetin equivalent.
The highest value was 76.14 ± 0.32 and 21.18 ± 0.38 in DPPH and FRAP assay respectively. Antioxidant enzymes SOD, CAT, and POD were also measured as 19.48 ± 0.04, 39.90 ± 0.09, and 4.85 ± 0.04 units/mg respectively. Antimicrobial activity of Saussurea lappa against salmonella typhi strains of bacteria and fungus was also detected against standards. The study results have created an opportunity for researchers and medical practitioners for the treatment of typhoid fever and oxidative stress-related diseases by natural medicines.
Keyword: Antimicrobial, antifungal, Antioxidants, HPLC, Phytochemical, Reactive oxygen species, Salmonella typhi, Typhoid fever,

Introduction.

According to the WHO updated survey reported that nearly above 60-80% people of in developing countries directly or indirectly depend on plants and plant-based medicines for the treatments of their health issues (Franzotti et al.,2000). From beginning of the world, these medicines are used against fever, antimicrobial, hepatoprotective, antioxidants and chronic disorders like diabetes or cancers etc.

In many developing countries natural medicines has gained popularity and used as prime component of primary healthcare system. Medicinal plants contain clusters of bioactive compounds like alkaloids, flavonoids, tannins etc. which are used against different degenerative ailments (Karagoz et al.,2015). Plants curative potential extensively exploited in herbal medicine and approximately 25% of prescribed drugs around the globe are plant origin (Sevindik, M.,2018).

Saussurea lappa (Family: Costaceae) is representative perennial herb growing wildly in Himalaya regions 2500-3500 m altitude and favorably grow in wet places along the rivers. (Nageswara et al., 2013). The parts of plants are commonly used barks, leaves, and roots as medicine in the Pakistan, China, India and Japan. It is indigenous to India, Pakistan and China. its roots are harvested in autumn and spring (Rao SA et al.,1959). S.lappa contains many compounds like alkaloids, saponins, phenols and flavonoides with multiple pharmacologic activities. Traditionally, without obvious adverse effects it has been used extensively in medicines as anti-inflammatory, analgesic, antioxidants, anti-anthelmintics and to treat pleurisy and multiple of systemic infections (Choi JY, et al., 2009). Saussurea lappa also prescribed for the treatment of enteric fever in the local communities (Alaq et al., 2021).

Typhoid fever is an acute and chronic life-threatening febrile illness caused by the bacterium *Salmonella enterica* serotype *salmonella typhi*. *Salmonella typhi* is a gram-negative bacterium that is responsible for typhoid fever. The 1st one who named the term typhoid fever was pierre Louis, in 1829 after perceiving lesions in the abdominal lymph nodes of patients who had died from gastric fever. (John et al., 2022). The contributing agent of typhoid fever is "*Salmonella typhi"* is approximate 50,000 years old (Claire et al., 2002). *S. typhi* is a gram-negative, rod shaped, flagellated bacteria only are the strict human host. (Crump et al., 2015).

Salmonella typhi is usually transmitted through contaminated food and water. The most clinical signs are slowly developing persistent fever with chills, hepatomegaly and pain in abdomen. Patients may have rashes, nausea and vomiting, constipation or diarrhea, headache, anorexia, coughing, relative bradycardia, and a focused state of consciousness in certain instances. (Getahun et al., 2019) Proportionality 40% to 80% of the individuals shows that they have positive blood cultures and 30% to 40% patients have positive stool cultures. The most sensitive diagnostic test available for medical professionals is a bone marrow aspirate. Even though Salmonella enterica serotype typhi is invasive, more than 90% of cultures from infected individuals are positive, and these results can persist for days after antimicrobial therapy is started. (Gilman R.H et al., 1975).

Multiple drug resistance (MDR) types of S. Typhi revealing resistance to both fluoroquinolones and third-generation cephalosporins, as well as to the typical first-line antibiotics ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol. The sole drugs that can treat these infections are tigecycline, carbapenems (meropenem), and azithromycin. (Zoe A et ai., 2019).

Antibiotics that are bactericidal have the ability to trigger a common oxidative damage pathway, which results in the generation of reactive oxygen species and cell death. Additionally, antibiotics can harm many kinds of mammalian cells and tissues. Antibiotics cause oxidative stress and target the liver in humans, and the major effect is their toxicity. (Foti, J et al., 2012).

Plants and plants-based medicines can play a key role to cope with this challenge of Various damaging effects caused by ROS are membrane lipid peroxidation, moreover, peroxidation products and their metabolites are highly reactive (Angelov et al., 2021). They react with biological substances, DNA, amines, and proteins. Recent inquiries show that oxidant scavenging activities of different plants may be linked with defense against stress because of oxidants and different human disorders produced by ROS (Mattioli et al., 2022). Antioxidants may interfere with the process of oxidation through reaction with free radicals, chelation of free catalytic metal and scavenging oxygen (Kunwar et al.,2011).

The natural antioxidants present in plants like alkaloids, flavonoids, and phenols play a vital role in the healthcare system (Shakya et al.,2016). Flavonoids, phenolic acids, bioflavonoids, anthocyanins, and isoflavonoids are subclasses of phenols having properties of antioxidants and work against several infectious diseases, allergies, ulcers, tumors, platelet aggregation, reproductive issues, cardiovascular diseases and can reduce cancer risk (Minich et al., 2019). Currently, research on plants has been used

worldwide and is important in traditional systems (Yildiz et al., 2021). Antibiotics are the known magical drugs that are playing a vital role against bacterial infections (Levy et al., 2013). But due to extensive use of these antibiotics leads to less effectiveness and ultimately globally health benefits are under threat in last few decades. Emergence of antibiotics resistance is a worldwide threat, it is much important to search for innovative alternative medicine (Tacconelli et al., 2018). Plants or plants extracted medicines due to their temperamental based action and less resistance may be a suitable alternate of these synthetic drugs in future and play pivotal role in treating infectious diseases (Gurib-Fakim et al 2013).

The virulent and multiple drug resistant bacteria (MDR) are intimidating the global health care system in the present epoch. Currently, physicians are using multiple of antibiotics to treat the bacterial infections that are harmful to multiple of tissues and causing oxidative stress at tissues levels. The reason behind the selection of this study was to investigate the phytochemicals analysis of selected medicinal plant Saussurea lappa roots extracts their antioxidant potential, antimicrobial impact against typhoid fever and to explore the antibacterial potential of plant extracts to treat the drug resistance *Salmonella typhi*.

Material and Methodology.

Collection, Authenticity, and Processing of Plant Material

From the local market of Faisalabad, the roots of *Saussurea lappa* were purchased and legitimate by the Department of Botany, Govt. College University, Faisalabad, under voucher No. GBM-259/22.

Extraction of Plant Material

The roots were thoroughly examined for contamination and adulteration. Hydro ethanol extracts: ethanol and water were used in the extraction process just as given by Sidgi et al., (2013). A total of 750 grams of ground samples were soaked in 1500 mL of 70% +30% ethanol and water, correspondingly. After homogenizing the samples for about 5 minutes in an electric blender, the samples were allowed to incubate for 4 days at room temperature. After twice filtering the mixture under vacuum, the solvent was allowed to evaporate at normal temperature. It was then extracted again using 750 mL of hydro ethanol in a shaking water bath at 70 °C temperature for six hours. Following filtering, the solution was placed to evaporate at 25°C temperature to release the solvent. After being collected, the residue (extract) was stored at 4 °C.

Inclusion and Exclusion Criteria.

The fresh roots of the latest harvest were included in the study which was confirmed by purchase invoice of vendor and source documents. The old roots, contaminated, dusty, decomposed and unknown sourced material was excluded from the study. The clinical isolate of bacteria salmonella typhi strains were included in the study which were properly identified, isolated and authenticated with documents. The unknown isolates were excluded from the study.

Phyto-chemical Estimation.

First, the stock solution was prepared by dissolving half gram of ethanolic *Saussurea lappa* extract in 20 ml of methyl alcohol and used for the initial detection and determination of phytochemicals.

Qualitative Evaluation of Phytochemicals.

The extract of Saussurea lappa was detected for secondary active metabolites by using qualitatively systemized phytochemical techniques in order to prepare the chemical profile for carbohydrates, phenols (Yadav and Agarwala, 2011), alkaloids, flavonoids, saponins, terpenoids, glycosides, tannins, coumarins, cardiac glycosides, steroids, betacyanin and anthocyanin (Vishwakarma et al., 2014), quinone (Ajuru et al., 2017), protein, fixed oils, fats and anthraquinone glycosides (Roopa latha, 2013), phytosterols (Yadav et al., 2017) and phlobatanins, emodins (Rauf et al., 2013).

Quantitative Evaluation of Phytochemicals.

HPLC Fractionation Reversed Phase Chromatography:

High performance liquid chromatography (HPLC) was performed for the detection and quantification of phenolic acids. Sample was prepared through proper method for HPLC study. It was treated with HCl and then dissolved in water and Ethanol. Micro syringe filter was used for sample filtration.

Evaluation of Total Phenolic Contents (TPC).

To evaluate the TPC, a hundred microliter extract sample was mixed with 0.5 milliliters of folin-Ciocalteu reagent, which had first been diluted with 7 milliliters of deionized H_2O. The solution was allowed to stand for 3min at 25ºC, and 0.2ml of a saturated sodium carbonate solution was added. The mixture was allowed to stand for another 120 minutes in a dark place for the completion of the reaction and formation of complexes. The absorbance of all aliquots was measured at 725 nm. The total phenolic content TPC of the extract was determined using the gallic acid equivalent (GAE) per gram of dry extract using the following equation for calculation.

$y = mx + b,\ x = (y-b)\ m,\ c = x$ (v m)

Were, v = Volume of sample, m = Concentration of sample in g, y = Absorbance of the sample at 725nm, M = Slope, b = Intercept,x = Concentration of gallic acid in µg/mL, c = Total phenolic contents The total amount of phenolic content in plant samples is represented as milligrams of equivalent amount of gallic acid per gm of dry extract.

Evaluation of total flavonoid contents (TFC).

The TFC was estimated by the aluminum chloride colorimetric method. The number of flavonoids was determined to be equivalent to quercetin. One milliliter extract of plant and AlCl3(2% w/v) was blended with each other in the appropriate solvent (stock solution SS), and the solution was prepared up to 25 milliliters with a methanolic solution of acetic acid (0.5% v/v) (Probe solution PS). A methylated acetic acid solution (contrast solution CS) was used to make 25 ml of SS from 1 ml. After 30 minutes, the absorbances of CS and PS were calculated at 420 nm. The end result was expressed as mgs of quercetin equivalent per gram of dry extract.

Evaluation of Total Soluble Proteins (TSP).

Following Bradford's (Hameed et al.,2021) instructions, the total protein content in 0.1 g of fresh *Saussurea lappa* was determined by homogenizing the sample in 2mL of phosphate buffer saline (pH 7.2) and centrifuging it for 10 minutes at 16128 g (MIKRO-200 R; Hettich GmbH and Co. KG). For thirty minutes, the sample was kept at room temperature for incubation. A UV-VIS Spectro-photometer (Hitachi u-2910, Tokyo, Japan) was used to estimate the mixture's optical density (OD) at 595 nm, and BSA served as the standard.

Estimation of Anti-Oxidant Activity.

DPPH radical scavenging activity

Analysis of DPPH will be used for the detection of antioxidants in plant sample extracts. Utilizing the free radical producer DPPH (2, 2-diphenyl-1-picrylhydrazyl) in a manner that was comparable to that of Brand-Williams, Cuvelier, & Berset, 1956, the free radical scavenging effect was ascertained. The test extract was then diluted in a 1:3 ratio with the standard DPPH solution. For 90 minutes, the mixes were retained at approx. 25 ºC temperature under dark conditions. Using a UV-VIS spectrophotometer, the optical density (OD) was evaluated at 517 nm. The reference was taken from ascorbic acid. The ability to scavenge the DPPH radical was calculated by using method:

DPPH $\%age = (A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}} \times 100$

Shown as,

Ac = The absorbance of the control reaction

As = The absorbance of all extract samples

Antioxidant activity of radical cations (ABTS).

The ABTS assay was performed using a modified version of the method that Huang described (Huang et al., 2021). When 7.45 mM potassium persulfate was mixed with the ABTS solution, ABTS+ cations were formed. Before using the mixture was dilute with ethanol until it shows an absorbance of 0.70+0.02 at 734 nm. after being in the absence of light for 11 to 15 hours at 20-25 ºC temperature. The Genesys 10S UV-VIS (Thermo Scientific) was used to quantify scavenging activity at 734nm absorbance precisely six minutes after adding 100 ml of the sample or the Trolox standard to 3.9 mL of diluted ABTS + solution and the results were stated as (TEAC) Trolox equivalent antioxidant capacity by using the formula,

$RSA\ \%age = (Ac-As)/\ Ac\times 100$

Shown as,

Ac = The absorbance of the standard.

As = The absorbance of the sample extract, and Results were averaged after taking in triplicate.

Ferric reducing activity power of plant extract (FRAP).

The Ferric reducing activity power of plant extract (FRAP) was determined using the technique developed by (Müller et al., 2011). One mL of a solution containing 10, 20, 30 & 40 µg/mL of extract was mixed with potassium ferricyanide (2.5ml, 1%) and phosphate buffer (2.5ml, 2M, pH 6.6). The mixed solution was incubated for twenty minutes at 50ºC. Additionally, the mixture was supplemented with 2.5 ml of 10% trichloroacetic acid and centrifuged for 10 minutes at 1500 rpm. The absorbance was calculated to be 700 nm after the upper layer of the solution (2.5 ml) was combined with 0.5 ml of FeCl3 (0.1 percent) and 2.5 ml of distilled water. A higher absorbance indicates that the reaction mixture has a higher reducing power. The outcome was shown as RSA% age, by using the formula

$RSA\ \%age = (Ac-As)\ Ac\times 100$

Where,

As = the absorbance of the extract samples & reference

Ac = is the absorbance of the control reaction, and Results were averaged after taking in triplicate.

Antioxidant enzymes assay.

Fresh roots of Saussurea lappa were grinded in a mortar and pestle in the presence of cooled phosphate buffer (50 mM;) for antioxidant enzyme extraction. dithiothreitol (1 mM) and pH7.0. The supernatant from the centrifugation of this solution at 25200 rpm for approximately 20 minutes at 4 O C was used to measure the antioxidant and enzyme activities following method quoted by (El-Esawi et al., 2020).

Superoxidase dismutase (SOD) contents.

With minor modifications, SOD activity of *Saussurea lappa* roots was estimated by the method developed by Gong et al. as coated by (Chang et al.,2022). For 15 minutes at 78 µmol m/2/s, the glass vials containing the reaction mixture were illuminated with 15 watts of fluorescent light and at 560 nm, the absorbance was measured.

Catalase (CAT), peroxidase (POD) contents.

By Cakmakk et al.'s method (Abbasvand et al.,2020) minor modifications of the CAT and POD activities were performed. Every 20 seconds, the absorbance of the reaction blend was estimated at 240 nm. Following the breakdown of H2O2, the absorbances of the CAT and POD reaction solutions decreased at 420 and 470 nm, respectively. Units of enzyme activity were expressed as mg-1 of protein (U = 1 mM of H2O2 reduction min-1 mg-1 protein).

Antimicrobial Studies.

Test Organisms

The samples were individually tested against bacterial strain *Salmonella typhi*. Purity and identity were verified by the Department of Microbiology, Faculty of Life Sciences, Government College University Faisalabad. The microorganisms were clinical isolate of salmonella typhi, and a fungal strain Candida albicans.

The bacterial strains were cultured in Nutrient agar (Oxoid, UK) for an entire night at 37 ºC. by using the well diffusion method, the compounds' antibacterial activity was determined. To test the samples' antibacterial activity, the well diffusion method was employed. The sensitivity of each strain and isolate in the examined microbial species was compared using ciprofloxacin as a drug of reference for bacteria. After two hours at 4 ºC, plates were incubated for eighteen hours at 37 ºC to cultivate bacterial strains. By measuring the organisms' growth inhibition zone diameter (zone reader) in millimeters and comparing with the control. (Shahid et al., 2021).

Statistical Analysis

Entire procedures were carried out in triplicate. Shapiro Wilk test was applied to determine the normality of data. The normality p value was insignificant, so the null hypothesis was rejected, the relationship was confirmed, and results were calculated by using independent sample t-test.

Results:

Percentage Yield and Qualitative Phytochemical Screening.

The percentage yield of hydro-ethanol extract is 18.21% for Saussurea lappa. The results of the extraction revealed that Saussurea lappa roots consist of nonpolar and polar compounds. Alkaloids, flavonoids, phenols, terpenoids, tannins, saponins, cardiac glycosides, steroids, phytosterols, coumarins, proteins, carbohydrates, fats and quinones were found in the hydro-ethanol extract during the phytochemical analysis as shown in (Table-1). The phytochemical compound's positive intensity was determined by an arbitrary scoring system of 3 to 1, with 1 representing the lowest possible concentration.

Table 01: Qualitative Phytochemical Screening of *Saussurea lappa* roots Extract

Sr #	Phytochemicals	*Saussurea lappa*	Test Name
1	Alkaloids	+++	Dragendorff's test
2	Flavonoids	+++	Shinoda's test
3	Phenols	+++	Liebermann's test
4	Terpenoids	+++	Salkowski test
5	Tannins	+	FeCl3 test
6	Saponins	++	Frothing test
7	cardiac glycosides	+++	Kedde test
8	Steroids	+	Liebermann-burchardt test
9	Phytosterols	+++	TLC
10	Coumarins	+++	NAOH test
11	Proteins	+++	Hopkin's,Millon test
12	Carbohydrates	+++	Benedict's test
13	Fats & Fixed oils	+++	Oil content analyzer
14	Quinones	+	Borntrager's test

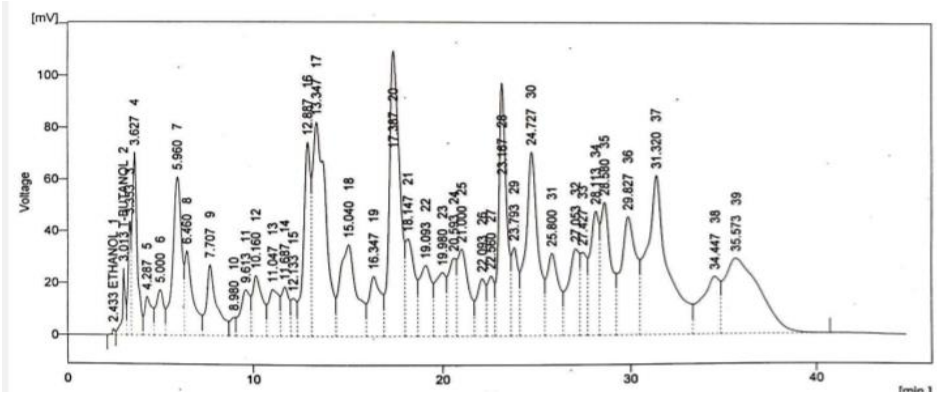
HPLC High Performance Liquid Chromatography

The HPLC analysis for the hydro – ethanolic extracts (Fig. A), showed the identification of 11 compounds. The hydro ethanolic extract contained the highest concentration of chlorogenic acid,

cinnamic acid, P-coumeric acid, fenalic acid, syringic acid, vanillic acid, sinapic acid, quercetin, gallic acid, caffeic acid, M-coumeric acid. (Table 2).

Table 02: HPLC analysis of the chemical compounds of the extracts

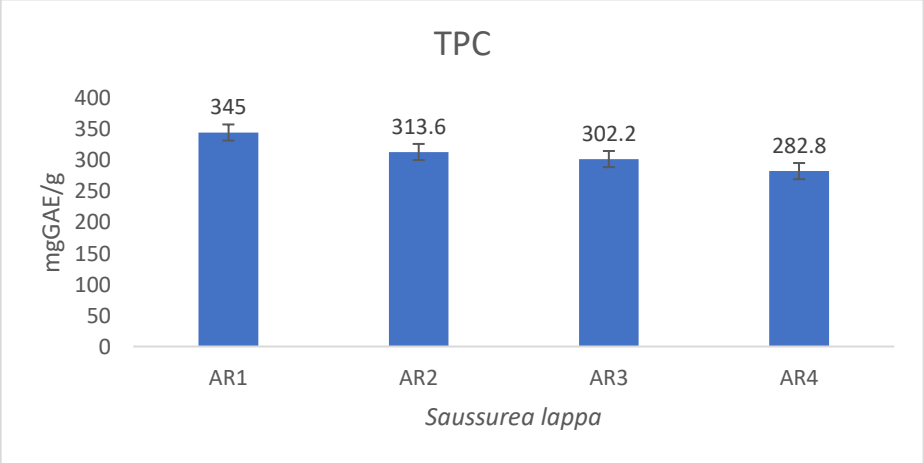
Compounds	Concentration ppm
Chlorogenic acid	166.31
Cinnamic acid	114.42
P-coumeric acid	54.37
Fenalic acid	52.18
Syringic acid	24.99
Vanillic acid	24.68
Sinapic acid	18.43
Quercetin	15.54
Gallic acid	14.72
Caffeic acid	13.43
M-coumeric acid	11.38



(Fig. A) HPLC analysis for the hydro - ethanolic extracts.

Total phenolic content

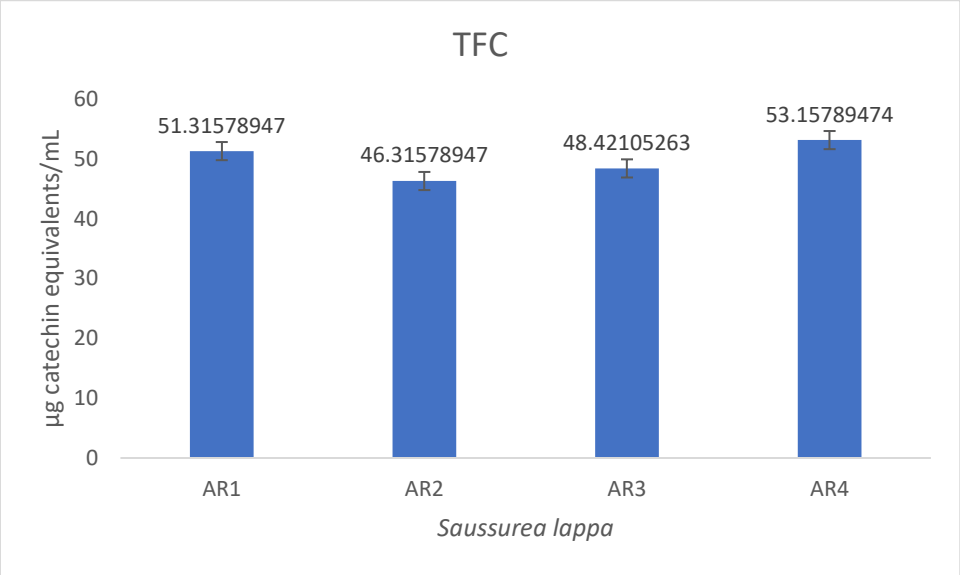
The graphical representation of the concentration and absorbance of gallic acid to calculate the total phenolic contents of Saussurea lappa as gallic acid equivalent as shown in Figure-B and the TPC is mentioned in Table 3. The entire phenolic contents of Saussurea lappa was analyzed in four concentrations. Highest value of TPC was found at concentration 80µL/mL which was 345.0 ± 0.41 mgGAE/g and lowest value was recorded in AR4 sample which was 282.8 ± 0.38 mgGAE/g.



(Fig. B) Total phenolic content of hydro-ethanolic extract of Saussurea lappa

Total flavonoids.

The Figure C was used to convert the *Saussurea lappa* roots total flavonoids (TF) into quercetin equivalent. The total flavonids content of *Saussurea lappa* was analyzed in four concentrations. Highest value of TFC was found at concentration 10µL/mL which was 53.1 ± 0.39 mgGAE/g and lowest value was recorded in AR2 sample which was 46.3± 0.42 mgGAE/g.
(Table 3).

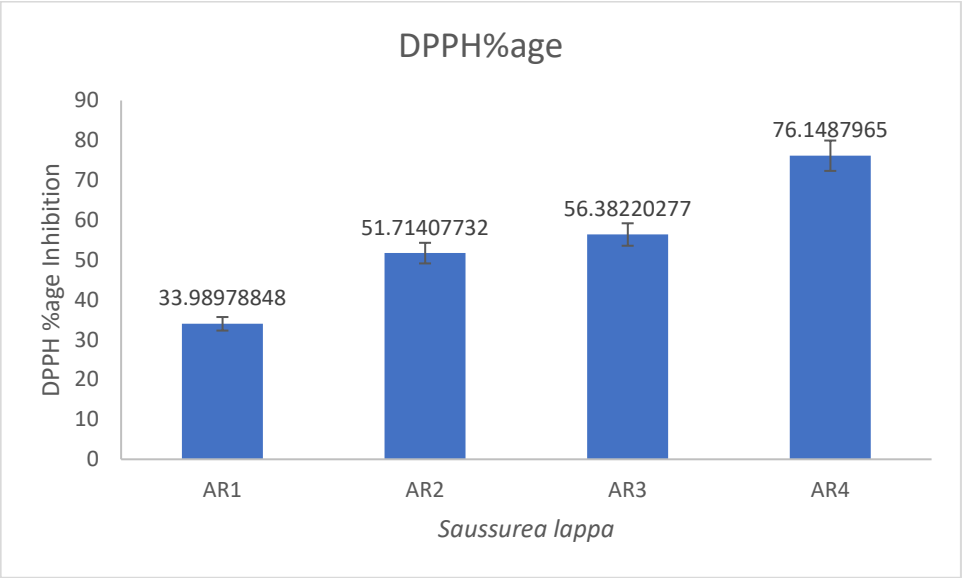


(Fig. C) Total flavonoids content of hydro-ethanolic extract of Saussurea lappa

A study by Sharma et al. (2011) assessed the TPC of Saussurea lappa. The results showed that the plant extract was rich in phenolic compounds. Phenolic compounds are known for their antioxidant properties, which contribute to the plant's ability to scavenge free radicals and protect cells from oxidative damage. The same study also evaluated the TFC of Saussurea lappa extract. The findings indicated that the plant extract was rich in flavonoids. Flavonoids are a subgroup of phenolic compounds and are known for their antioxidant and potential health-promoting properties. Results of my study also strengthen and proved by the above mention because in my findings Saussurea lappa contains considerable amounts of phenolic and flavonoid contents.

DPPH free radical scavenging assay.

DPPH is frequently used to measure how well Saussurea lappa neutralizes free radicals when assessing a substance's ability to do so. DPPH was utilized as a free radical-generating reagent. At 517 nm, antioxidants from Saussurea lappa were able to transform DPPH into yellow diphenyl-picryl hydrazine. The DPPH % age inhibition of Saussurea lappa was analyzed in four concentrations. The graphical presentation shown in Figure. D. Highest value of DPPH was found at concentration 10µL/mL which was 76.1 ± 0.32 mgGAE/g and lowest value was recorded in AR1 sample which was 33.9 ± 0.28 mgGAE/g. (table 3).



(Fig. D) DPPH free radical scavenging assay

Radical Scavenging Activity: A study conducted by Sharma et al. (2010) assessed the antioxidant activity of Saussurea lappa using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. The study found that the extract exhibited significant DPPH scavenging activity, indicating its potential as an antioxidant agent DPPH. In my study strengthen and proved by the above mention because in my findings Saussurea lappa contains considerable amounts of phenolic and flavonoid contents.

ABTS radical scavenging assay (TEAC assay).

Sodium persulphate converts ABTS to cationic radical which is bluish in color and absorbance was taken at 734nm. Most of the antioxidants are reactive to the ABTS radical cation. The bluish ABTS cation radical is converted to back during this reaction. The (TEAC) Trolox equivalent antioxidant capacity assay is the name of this test. The ABTS % age of inhibition Saussurea lappa extracts was analyzed in four concentrations. Highest value of ABTS was found at concentration 10µL/mL which was 51.0 ± 0.28 mgGAE/g and lowest value was recorded in AR1 sample which was 35.9 ± 0.34 mgGAE/g shown in figure E and Table 3.

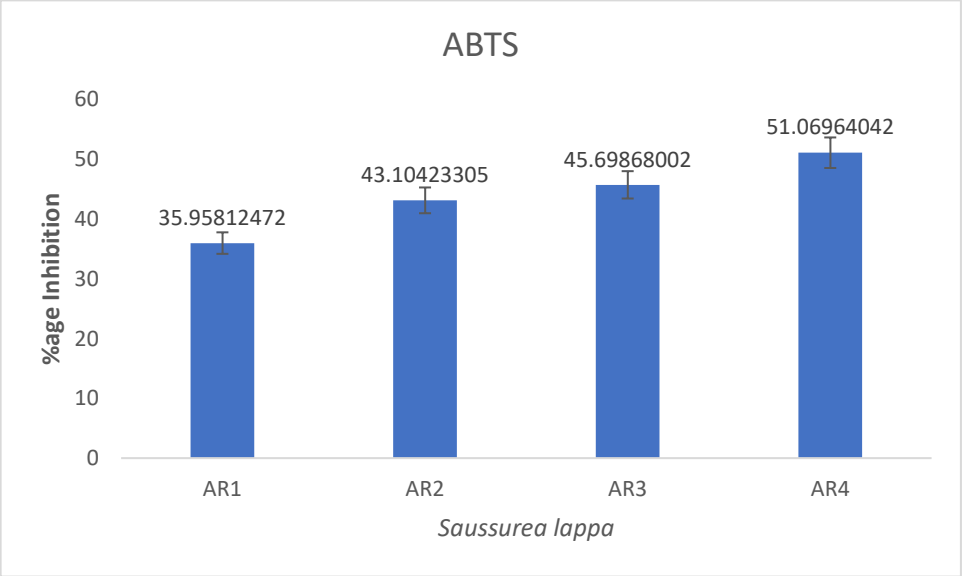
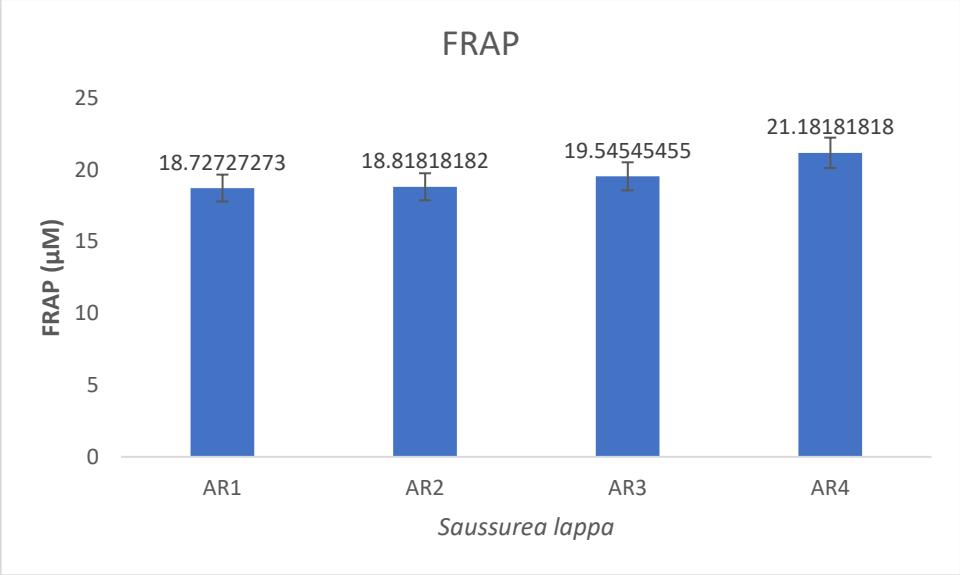


Fig. E) ABTS radical scavenging assay (TEAC assay)

FRAP assay.

The test solution's color has been changed from yellow to a variety of green and blue hues based on each compound's reducing power. The development of pearl Prussian blue at 700 nm, which indicates a higher reducing power is indicated by higher, was caused by the presence of antioxidants, which caused the conversion of the ferric (Fe+3) form to the ferrous (Fe+2) form. Table 3 displays the comparison of the FRAP test results for the alcoholic extracts to the standard (ascorbic acid) at 700 nm.

The FRAP %age inhibition of Saussurea lappa was analyzed in four concentrations. Highest value of FRAP shown in Table 3 was found at concentration 10 µL/mL which was 21.8 ± 0.38 mgEAAcid/mL (milligram equivalents to Ascorbic Acid per milli Litter) and lowest value was recorded in AR1 sample which was 18.7 ± 0.32 mgEAAcid/mL. As we increase the concentration of sample, FRAP values decreased in our study (Figure. F).

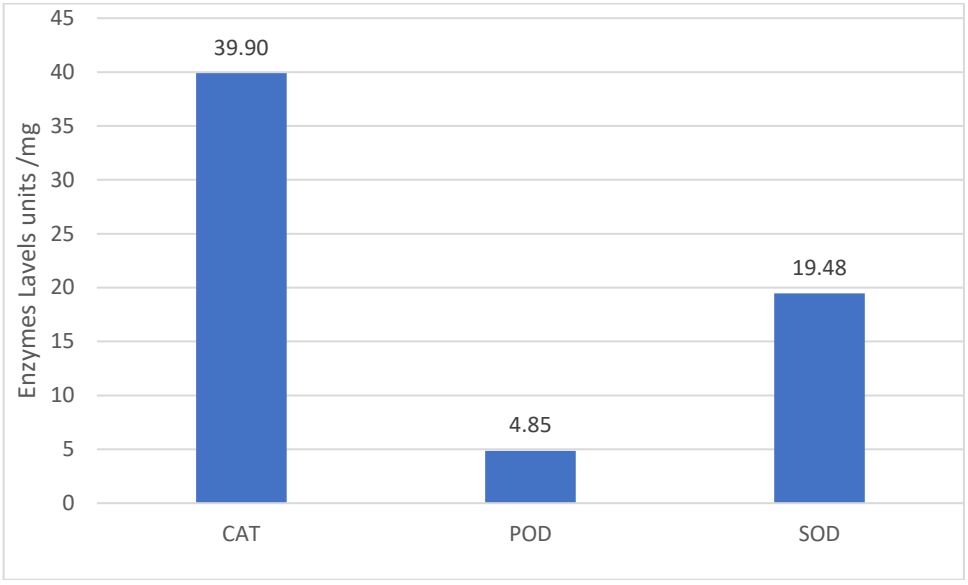


(Fig. F) FRAP %age inhibition of Saussurea lappa

Treatment	DPPH%age	TPC	TFC	FRAP	ABTS
AR1	33.98978848	345.0	51.3158	18.7273	35.9581
AR2	51.71407732	313.6	46.3158	18.8182	43.1042
AR3	56.38220277	302.2	48.4211	19.5455	45.6987
AR4	76.1487965	282.8	53.1579	21.1818	51.0696

Table. 3 Antioxidant enzymes (CAT, POD, SOD).

The total soluble proteins (TSPs) level was found 1.18 with SEM ± 0.07 mg/g FW in the roots of Saussurea lappa. The CAT contents were found 39.90 with SEM ± 0.09 units/mg as presented; The POD is used as a usual skin caring component in cosmetic products to remove the H2O2 from the tissues. The POD Enzymatic antioxidant was found 4.85 ± 0.04 units/mg which is the highest as found in the other drugs used for fertility. SOD contents are also known as the antioxidant defense in the body. Because they reduce oxidative stress the cause for diseases like atherosclerosis, heart attack, various age-related disorders, stroke and acute as well as chronic inflammatory conditions. The SOD concentration was found 19.48with SEM ± 0.04 units/mg as shown in Figure. G.



(Figure. G) Levels of Antioxidant enzymes (CAT, POD, SOD)

Antimicrobial Activity:

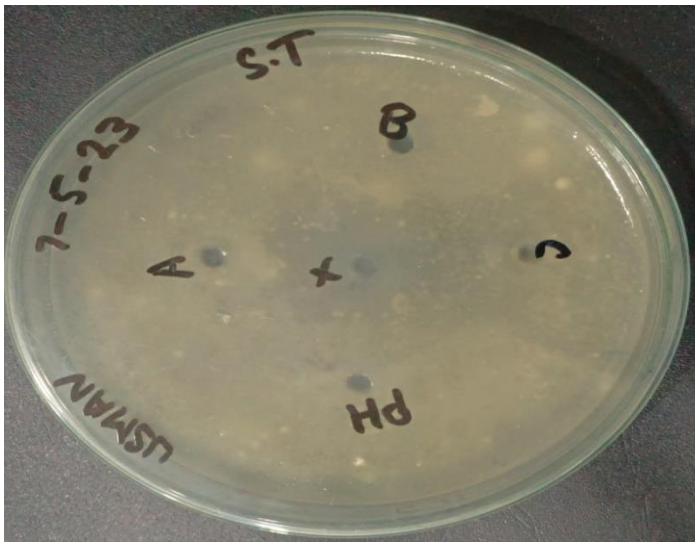
Antibacterial activity.

The antibacterial activity of the selected medicinal plant was calculated by the diameter (mm) of the zones of inhibition in the selected bacterial colonies. Ciprofloxacin is used as a standard drug. The zone of inhibition (Figure. H) of Saussurea lappa 22mm While the standard drug ciprofloxacin at 10µg concentration ZOI is 17mm shown in table 4. Our data express that the hydro-ethanolic extract of Saussurea lappa presented good antibacterial results as authenticated by standard.

Bacterial strain used: Salmonella typhi
Standard Antibiotic: Ciprofloxacin

Treatment	*Salmonella typhi*
	Positive C. 17mm
Saussurea lappa	22mm

Table .4



(Fig. H) Zone of inhibition (mm) at the conc. of 40mg of *Saussurea lappa* with antibacterial activity.

A study by Ali et al. (2010) assessed the antibacterial action of Saussurea lappa hostile to various bacterial strains. The outcomes sketched out that the extract exhibited inhibitory effects on the growth of incubated bacteria. This suggests that Saussurea lappa may contain bioactive compounds with antibacterial properties. Another research by Wali et al. (2018) investigated the antibacterial activity of Saussurea lappa against both gram-positive and gram-negative (e.g., Staphylococcus aureus) (e.g., Escherichia coli) bacteria respectively. The results showed that the plant extract demonstrated inhibitory effects against a broad spectrum of bacterial strains. Results of our study also strengthen and proved by the above mention because in my findings Saussurea lappa extracts has antibacterial activity against salmonella typhi.

Antifungal activity

Candida albicans fungal strain were used to check the antifungal activity of plant extract against ketoconazole standard drug. The zone of inhibition of Saussurea lappa against Candida albicans was detected, Ethanolic extract of Saussurea lappa produced a 15mm zone of inhibition at 40mg/ml concentration while the standard drug produced 24mm ZOI at 10µg dose. The Findings indicate that ethanolic plant extracts of Saussurea lappa showed antifungal activity. Research conducted by Ahmad et al. (2018) investigated the antifungal action of Saussurea lappa. The results indicated that the extract exhibited inhibitory effects against various fungal strains. This suggests that Saussurea lappa may contain bioactive compounds with antifungal properties.

Fungal strain used: Candida albicans

Standard Antibiotic: ketoconazole

Treatment	Candida albicans
	Positive C. 24mm
Saussurea lappa	15mm

Table .5

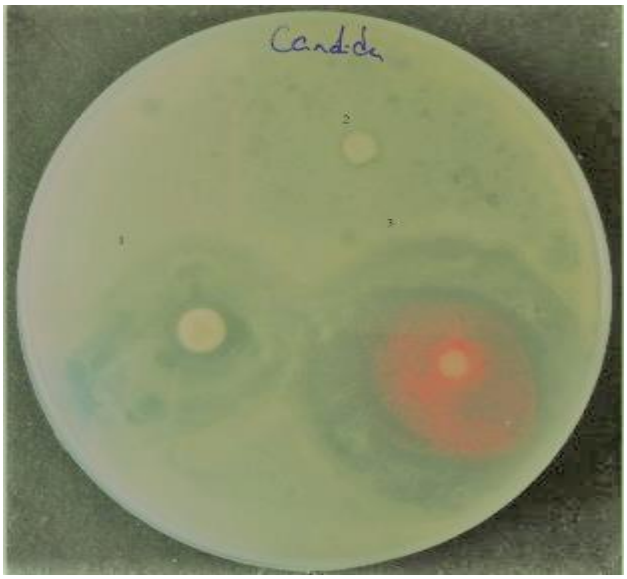


Fig.H. Zone of inhibition (mm) at the conc. of 40mg of *Saussurea lappa* with antifungal activity.

Conclusion:

antioxidant and antimicrobial study revealed that the extract exhibited the most potent free radical scavenging and reducing effects and antibiotic effect against salmonella typhi. Phenolic contents have a key role in antioxidant activity, as this study found a linear correlation among its antioxidant activity and phenolic contents. Based on the outcomes of in vivo studies on biological systems, the hunt for naturally occurring antioxidants, that would be effectively used in subsequent clinical trials, may open

new avenues. The study results have created an opportunity for researchers and clinical practitioners to treat oxidative stress-related diseases by natural drugs.

Conflict of Interest:

The authors have no conflict of interest.

Financial Statement:

The corresponding author has managed himself along with the available resources in the Department of Eastern Medicine, GC University Faisalabad.

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