

# Characterization of Essential Oils of *Saussurea lappa* Clarke, Their Effect on Pathogens and Possible Implication for the Treatment of COVID-19

## Abstract

COVID-19 virus is one of the chest diseases that affect the respiratory system because of contamination through Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). It is utilized as anti-inflammatory, anti-viral, antibacterial, lipid lowering, analgesic and antipyretic. Therefore, *Saussurea lappa* clarke extracts can be used in the management of COVID-19. In this work, the original oil was extracted from *Saussurea lappa* (*S. lappa*) clarke by using hydro distillation apparatus and then subjected to gas chromatography mass spectrometric analysis. Sums of twenty one composites were recognized and major compounds that were detected are the following: Costunolide (9.29%), ehydrocostus lactone (47.54%) and  $\alpha$ -curcumene (4.33%). Anti-oxidant action against the standard 2 of crude extracts of the *S. lappa* was checked, 2-Diphenyl-1-Picryl-Hydrazyl (DPPH). The antibacterial activity against *E. coli* and fungal strains were also assessed, and the plant extract showed greater results than the standard drug. The cytotoxicity of *S. lappa* essential oil was superior to crude extracts and significant results were obtained regarding metal chelation and minimum inhibitory concentration. During the qualitative and quantitative analysis, the compound  $\beta$ -pinene and  $\delta$ -terpineol showed more percentage peak areas of 2.49% and 4.33% respectively than the previously reported percentage peak area. The iron chelation potential of the fundamental oil of *S. lappa* was determined using Gas Chromatography Mass Spectrometric (GC-MS), which also showed remarkable results. Hence, the fundamental oil of *S. lappa* possesses excellent antibacterial activity, low cytotoxicity, iron chelation capacity and thus recommended for medicinal use against pathogens and for the potential treatment of COVID-19. It has also been shown that  $\alpha$ - and  $\beta$ -pinene type terpenes have broad health properties and can be used to treat COVID-19 as well as other medical diseases. However, clinical trials are needed to corroborate the efficacy for COVID-19 treatment.

**Keyword:** Essential oil • *Saussurea lappa* • GC-MS analysis • Biological activities • Anti-fungal and antibacterial •  $\alpha$  and  $\beta$ -pinene • COVID-19 treatment

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## Introduction

*S. lappa* Clarke is a member of the family Compositae identified as the costus. It is a perennial herb that cultivates to an elevation of 1 to 2 meters, its stem is erect, the root is long and has a distinctive aroma, the leaves vary in size, including small front and large basal, flowers arranged, fruits hairy and arched. This plant is of great medical importance as it is used as an anti-

cancer, to cure ulcers, viral diseases, bacterial infections, fungal infections, anti-arthritis, stomachache [1].

*S. lappa* clarke's medicinal importance is due to the presence of secondary metabolites, the most significant of which are dehydrocostus, lappadilactone, lactone cynaropicrin, germacrenes for instance 4.11 (13)-trien-12al, germacra-1 (10), germacra-1 (10), 4.11 (13)-trien-12-ol as

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well as (+)-germacrene A, germacra-1(10),4,11(13)-trien-12-oic acid, dehydrocostus, lappadilactone. Which has medical activity in the treatment of many diseases, so scientists seek to extract these active substances for use in the medical field.

*S. lappa* grows between 2500 and 3500 metres above sea level in India, China and Pakistan. In addition to the Kishenganga valley, Jammu, Kashmir, the Western Ghats and cultivation in Tamilnadu and Uttar Pradesh, it has also expanded to other locations such as the Kishenganga valley, Jammu, Kashmir, and the Western Ghats [2].

Essential oils are the natural products obtained from the raw material of plants from different techniques *i.e.* steam distillation or dry distillation [3-4]. Essential oil is separated from the aqueous phase by different methods [5].

These techniques have some merits and demerits. The quality of essential oils depends on the methods of extractions. Steam distillation is the most popular technique for acquiring essential oils. Because it is the most suitable and widely accessible method for extracting essential oils, the hydro-distillation process was chosen. It is an easily useable method and it gives pure oil that is extracted from this technique. It is preferred because of its economic importance and faster method. Steam distillation is an old technique. In modern times, an advanced microwave combination method is used in the extraction of many plants, which is faster and easier than the previous techniques [6].

The distilled oil is colorless; having an aroma and flavor inferior to the cold pressed oil and it does not contain impurities because the extraction of oil is usually conducted at high temperatures. Essential oils have been used for their pharmacological activities such as antibacterial, anti-fungal, anti-oxidant, anti-malarial [7-10]. Anti-proliferative activities [11]. Moreover, essential oils were assessed in hepatic oxidative damage and renal failure in rats, in different foodborne pathogens and food spoilage bacteria [12,13].

Furthermore, they find applications in different industries such as perfumes, aromatherapy, spices, nutrition and cosmetics. Around three hundred species of the same family are found in the genus *Saussurea*, including sixty one species found in India. One of the well identified varieties in this type is *Saussurea lappa* [14].

The major goal of this work was to use the GC-MS method to investigate the bioactivity of essential oils isolated from *S. lappa* roots. In addition to determining the anti-oxidant properties and measuring the percentage of flavonoids, total phenol, and cytotoxic action, in addition to the anti-fungal and antibacterial effect.

## Materials and Methods

### Sample collection

*S. lappa* roots were obtained from two higher altitude locations in Azad Jammu and Kashmir, which is located among longitude 73-75° E also latitude 33-36° N, it covers a region of 13,297 kilometers square.

### Preparation of vegetable material

Roots of *S. lappa* were assembled from two places of the higher altitudes in Pakistan (Kashmir and Azad Jammu). University of Azad Jammu and Kashmir, plant taxonomist department of botany was recognized this plant. The roots were dried in a circulating atmosphere oven at 30°C -40°C for 4 h. 15 gm of the dried roots were well ground using an electrical grinder (ANEX AG-694) to a fine powder. Samples were accumulated in bottles in the freezer at 5°C until examined, for further studies (Figure 1).



Figure 1: Preparation of *S. lappa* powder from roots.

### Isolation of essential oil

To obtain essential oil, the dried roots of *S. lappa* were exposed to a hydro-distillation process for 2-3 hours. 50 grammes of *S. lappa* powder were soaked in 500 ml of distilled water, and then stirred for 2-3 hours at 70°C on a shaker. The suspension was clean using Whatman No. 1. The fundamental oil was dried above anhydrous sodium sulphate as well as accumulated at 4°C.

### Gas Chromatography Mass Spectrometric (GC-MS) analysis

Trimethylsilyl (TMS) was derived by steps developed by Moldoveanu and David [15,16]. The dry extract was re-suspended at 10  $\mu$ L and 50  $\mu$ L of N, O-Bis (trimethylsilyl) Trifluoroacetamide (BSTFA), then keep warm for 60 minutes at 70°C for 60 minutes in a dry block hotter. Gas chromatography (7890B) and mass spectrometer detector are used in this GC MS system (5977A). The GC has an HP-5MS shaft (internal diameter was 30 m x 0.25 mm and film breadth was 0.25 m), a split ratio of 50:1, a carrier flow rate of 1.0 mL/min, furthermore an injected fluid volume of 0.5 L. The detector and injector are kept at 250°C while the thermal regime is set for 1 minute to 50°C, then to 300°C at an 8°C/min rate for 20 minutes. To obtain mass spectra, used Electron Ionisation (EI) at 70 eV for this purpose. The temperature of this mass was 230°C, while the temperature of the Quad was 150°C. The individual components were determined by comparing them to spectra stored in NIST mass spectral library data and wiley.

### Total flavonoids

To 0.5 mL of the extract, 0.1 mL of potassium acetate (1 M) as well as 0.1 mL of 10% aluminum chloride were added, followed by 4.3 mL of 80% methanol for the total volume of 5 mL. Absorption was estimated at 415 nm using a UV-VIS double beam spectrophotometer in opposition to a blank was consisting the relevant solvent but no extracts. The whole flavonoids in the extract were quantified using a standard curve then articulated in milligrams of quercetin equivalent per gram [17].

### Total phenolic content

The phenolic content of root fractions (acetone/water (80:20)) was identified using gallic acid as well as the Folin ciocalteu reagent as a standard. A diluted sample was gathered with 0.125 ml Folin ciocalteu reagent and 0.5 mL distilled water for 6-7 min. before mixing 1.25 mL of 10% sodium carbonate. After 90 minutes of incubation in a dark note, an absorbance of 765 nm was obtained after adding 3 mL distilled water then keeping the solution at room temperature. The plant's total phenolic content was calculated in milligrams of gallic acid equivalents per-gramme of dry weight (mg GAE/g DW) [18].

### Metal (iron) chelation study

Many phytochemicals present in natural plants,

such as phenols and flavonoids are responsible for chelating toxic metals and for preventing lipid peroxidation. When there is iron overloading Iron chelation therapy is used, more iron may be stored in body liver as well as bone marrow. This morequantity of iron can harm these organs. Cheaters should be specific and have a proper administration [19].

### Anti-oxidant activity by method of DPPH

In the DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl) test, a 100 mL aliquot of various concentrations of that extract was added for 5 mL of a 0.004 % of DPPH methanolic solution. After incubation for 30 min., the absorption at 517 nm was estimated against the blank solution using a spectrophotometer [20]. As the control substance, ascorbic acid was used. The test was recurring 3 instances; moreover the percentage of inhibition was determined by using the subsequent equation:

$$\text{Equation 1: } I\% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

The concentrations of the essential oil used were measured from the graph of percentage inhibition.

### Cytotoxicity

A 3 mL sample of freshly prepared heparinized bovine blood was obtained from the University of agriculture in Faisalabad Pakistan. The specimen of blood patients was centrifuged at 1000 x g for 5 min., then discarded of the plasma. Following that, the cells were rinsed 3 occasions through (5 mL) of cold (4°C) sterile isotonic solution. Furthermore, utilized was Phosphate-Buffered Saline (PBH) with a pH of 7.4. For each test, erythrocytes were kept at a density of 108 cells/mL. Human cells (108 cells/mL) were combined with 100 litres of each chemical individually. The chemical was given to the cells at various concentrations before being consisted up to 1 mL with PBS. For 30 min incubated of the samples at 37 °C. After incubation for 10 min., samples were agitated and put in ice for 5 minutes. The material was then centrifuged at 1000 x g for 5 min. From each tube, (100 L) of the supernatant was collected and diluted ten times in cooled (4°C) PBS. Triton X-100 (0.1 percent v/v) was utilized as a positive control in the experiment. PBS was utilized as the negative control. Both controls were subjected to the same procedure. Micro quant was used to measure absorbance at 576 nm. Each sample's percent RBC lysis was

recorded.

**Equation 2:** % of dead cells=Dead cells/total cells x 100

#### Antibacterial assay by disc diffusion method

The method used for the antibacterial activity of the plant extracts was agar disc diffusion. *S. lappa* plant extract was used against the *E. coli* strain [21]. Uncontaminated water was merged with twenty eight g/L of nutrient agar (Oxoid UK). The combination was homogeneously distributed using autoclaved at 120°C for 15 to 20 min. A 100 µL/100 mL of inoculum was merged with the medium before moving to the petri plates and the medium was placed inside the petri plates. On the growing medium, little filter paper discs consisting 100 litres of fraction were placed. The medium was combined with a bacterial strain loop crammed with uncontaminated culture and shaken for 22-24 hours at 37°C. The inocula were held at 4 degrees Celsius. For further research, inoculate with 1107 spore's mL was employed.

#### Anti-fungal assay by disc diffusion method

A pure culture of the fungi was placed inside the petri plates and slanted through the assistance of a potato dextrose agar medium. The growth medium of fungi including *Candida glabrata*, *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus solani*, was transmitted to the petri plates for production and sterilization. The temperature was maintained at 30°C for 48 h in the incubator for the fungal medium growth. On growth medium of fungal growth, strains filter paper discs were positioned flat, as well as fraction of

100 µL was applied to every plate moreover again incubated in the petri plates. The discs illustrated anobvious zone when essential oil was applied to them, indicating anti-fungal activity. From using a zone reader, the inhibition zones were calculated in mm [22]. The sterilization was done for three hours in hot air to grow the fungal strains and stored these strains at 30°C for 4 h.

#### Statistical analysis

The extraction of various compounds was done and analyzed using the gas chromatography mass spectrometric technique. The free radical scavenging action was evaluated using the crude extract of *S. lappa* with other pharmaceutically relevant activities such as cytotoxicity, antimicrobial, and minimum inhibitory concentration was assessed. The data was statistically evaluated using SPSS software's one-way analysis of variance and T-test. To facilitate the discussion, the mean SD of antibacterial actions of the sample as well as gel filtration fractions of the therapeutic plant were computed and the percentage demonstration is given visually [23].

### Results and Discussion

#### GC-MS analysis

The original oil of the *S. lappa* was obtained using hydro-distillation technique, and it was subjected to analysis of GC-MS. About fifteen components were identified during this analysis. The major compounds identified are listed in Table 1, where costunolide (9.29%), dehydrocostus lactone (47.54%), and  $\alpha$ -curcumene (4.33%).

S. no	Compound	RIC	RIR	Area %	Identification
1	$\beta$ -Pinene	980	979	2.49	RI-MS
2	$\alpha$ -Phellandrene	1002	1003	0.25	RI-MS
3	(D)-Limonene	1025	1024	0.35	RI-MS
4	$\beta$ -Phellandrene	1031	1028	0.23	RI-MS
5	1,8-Cineol	1033	1030	1.37	RI-MS
6	$\gamma$ -Terpinene	1054	1056	0.14	RI-MS
7	$\delta$ -Terpineol	1163	1679	4.33	RI-MS
8	$\alpha$ -Terpineol	1187	1189	0.36	RI-MS
9	trans-Cinnamaldehyde	1267	1271	0.51	RI-MS
10	$\alpha$ -Copaene	1374	1371	0.63	RI-MS
11	$\beta$ -Elemene	1388	1388	0.31	RI-MS
12	Dihydro- $\alpha$ -ionone	1405	1425	1.21	RI-MS
13	$\alpha$ -curcumene	1485	1510	0.12	RI-MS



14	Dehydrocostus lactone	1868	-	47.54	RI-MS
15	Costunolide	1917	-	9.29	RI-MS
<b>Note:</b> SR#: identify; RIC: Reconstructed Ion Current; RIR: Rietveld Reference Intensity Ratio; RI-MS: Retention Index- Mass Spectrophotometer					

These results were similar to that reported by Liu and colleagues, in which hydro distillation was used to obtain the original oil of *S. lappa* roots, then analysed utilizing Gas Chromatography (GC) with GC-Mass Spectrometry (MS) [24]. The original oil of *S. lappa* roots was found to contain 39 different components. The essential oil contains more sesquiterpenoids (79.80 % than monoterpenoids (13.25 %). Dehydrocostus lactone (46.75 %), costunolide (9.26 percent), 8-cedren-13-ol (5.06 %), as well as curcumene were the main composites in *S. lappa* essential oil (4.33 percent). Costunolide along with dehydrocostus lactone were segregated from *S. lappa*, the original oil using bioactivity directed fractionation. On the other hand, Deabes, et al. decided that the obtained data indicated the presence of 14 compounds. Five of them represent over 80% of the constituents. Butanedioic acid, 2TMS plagiaristic documented the maximum proportion with 20.4% pursued through androstan-17-one, 3TMS derivative as well as L-(-)- sorbofuranose, caffeic acid, D-(-)- fructofuranose, pentakis (trimethylsilyl) ether (isomer 1), 3-ethyl-3-hydroxy-, (5. alpha.-), pentakis (trimethylsilyl) ether with area percentage 18.4%, 16.5%, 14.4% and 11.4%, respectively.

While Srinivasan, et al. identified the chemical composites in the essential oil from costus furthermore found that n-hexadecanoic acid to be the main ingredient in every scrutinized essential oil accompanied by further fatty acids, hydrocarbons with mono-, di- and sesquiterpenes. Sesquiterpene lactones are composite members

most distinctive secondary metabolites (Asteraceae). Numerous plant families, including the Acanthaceae, Magnoliaceae, Amaranthaceae, Apiaceae, and Costaceae, include diverse chemical structures and biological reactions, including antitumorogenic, insect's antifeedant, regulating of plant growth, cytotoxic properties, antibacterial and anti-fungal [25].

However, the gas chromatography analysis in this study showed some differences compared to other previous studies, and the reason for this may be the time of harvest, storage places for roots, climatic conditions, degree of salinity and humidity [26].

**Flavonoid contents**

Flavonoids are naturally present in plants, and they showed antibacterial, anti-inflammatory, antiviral, anti-allergic and anticancer activities [27]. *S. lappa* aqueous extract was analysed to quantitatively determine phytoconstituents such as flavonoids. The essential oil extract contained the most flavonoids and demonstrated the mechanism of action through the chelation process and free radical scavenging. Flavonoids have been shown to have a significant correlation with phenolic content through analysis. Flavonoids in different methanol extracts of *S. lappa* ranged from 4.2-22.4 mg/g of extract.

These consequences are parallel to the results of Elgharabawy, et al., which showed that the amounts of flavonoids in the ethanolic fraction of costus were found to be 6.377 mg CE/g (Table 2) [28].

S No.	Part of plant	Flavonoid contents (mg/g extract)
1	<i>Saussurea lappa</i> (Root extract)	5.53
2	<i>Saussurea lappa</i> (Root original oil)	6.78

**Phenolic content**

Table 3 illustrates the total phenolic contents of ethanolic extract as well as solvent fractionated *S.lappa* is samples of *S. lappa* were investigated in n-butanol, ethanol, n-chloroform, hexane. The findings were presented as mean and Standard Deviations (SD) values from the triplicate analysis. The total phenolic content

(mg GAE/g extract) of the specimens trended in the following direction: n-butanol (0.0440.61 mg)> chloroform (0.0330.12 mg)> ethanol ex. (0.0260.07 mg)> n-hexane fr. (0.02.360.14 mg) The total phenolic content of the n-butanol soluble fraction from *S. lappa* roots was increased than that of the further ethanol extracts and solvent fractions. Elgharabawy, et al. reported that the total phenols content in

costus extracts were significantly high, with 3.502 mg/g of GAE, compared to other studies. The phenolic compounds antioxidant activity was thought to be because of their redox features, which were significant in neutralising as well

as absorbing free radicals, triplet oxygen with quenching singlet, and decomposing peroxides. The flavonoid compounds anti-oxidant activity is thought to be because of their scavenging or chelating process [29].

**Table 3: Total phenolic extraction yield of ethanol extract and *Saussurea lappa* root soluble fractions**

Extracts of plant	Total phenolic (mg GAE/g)
n-Butanol fraction	0.044 ± 0.61
Chloroform fraction	0.033 ± 0.15
Ethanol extract	0.026 ± 0.08
n-Hexane fraction	0.020 ± 0.16

**Anti-oxidant activity by DPPH method**

Table 4 is reported the anti-oxidant assay of the plant extract of *S. lappa*. The ability of a substance to donate hydrogen for the conversion of DPPH into the non-radical form DPPH can be monitored by a spectrophotometer (517 nm). Ascorbic acid was used as a standard reagent. Table 5 shows the anti-oxidant activities of the original oil of *S. lappa*. Extracts roots of *S. lappa* showed strong scavenging activity, higher than plant extracts. The reason for these findings is

in agreement with the observation reported by Kyung-Mi and colleagues, since the n-butanol soluble fractionated *S. lappa* C.B. Clarke (1,000 ppm) demonstrated the greatest inhibitory effort on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) reducing as well as radical power, with (92.98 and 0.38 percent), correspondingly. As a result, findings of this work suggest that the *S. lappa* C.B. Clarke plant might aid in the prevention of antioxidative stress [30].

**Table 4: Assay of anti-oxidant on root extracts of *Saussure lappa***

S. No	Concentrations of extracts µg/mL	Percentage scavenging by root oil.
A	0.521	-
B	0.216	59.34
C	0.193	63.45
D	0.127	76.23
E	0.098	83.2
F	0.072	85.12

**Table 5: DPPH scavenging activity percentages of essential oil of the root *S. lappa***

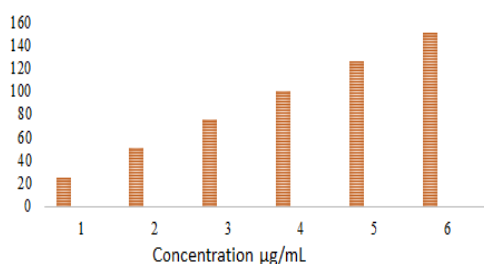
S. No.	Absorbance (517 nm)	DPPH scavenging activity percentages
A	0.324	—
B	0.231	69.05
C	0.125	71.56
D	0.095	75.23
E	0.078	77.65
F	0.064	79.34
Mean ± S.D	0.1528 ± 0.0921	62.13 ± 7.67

**Metal (iron) chelation study**

The iron chelating study of *S. lappa* root extracts is shown in Table 6 and Figure 2. In this study, the chelating ability of *S. lappa* root extracts was

demonstrated. The chelating ability increased proportionally as the extract concentration increased.

S. No	Concentration of extract in µg/mL	Percentage chelation by root extracts
1	25	1.26
2	50	1.37
3	75	1.48
4	100	1.59
5	125	1.67
6	150	1.76



**Cytotoxic effect**

The cytotoxic effect of *S. lappa* essential oil is reported in Table 7.

Figure 2: Chelating activity percentages by root extracts of *S. lappa*

Samples name	Cytotoxicity (% by hemolytic activity)
<i>S. lappa</i> root extracts	0.93
<i>S. lappa</i> root essential oil	4.23

**Antibacterial activity**

There was thought that *S. lappa* (Kuth) could exhibit the activity of antibacterial against *Escherichia coli* without using a drug cocktail. Figure 3 depicts *Saussurea lappa*'s antibacterial activity against *Escherichia coli*. Table 8 shows the inhibition zones measured in millimetres. The concentration (mg/mL) was computed on the x-axis and the zones of inhibition (mm) on the y-axis.

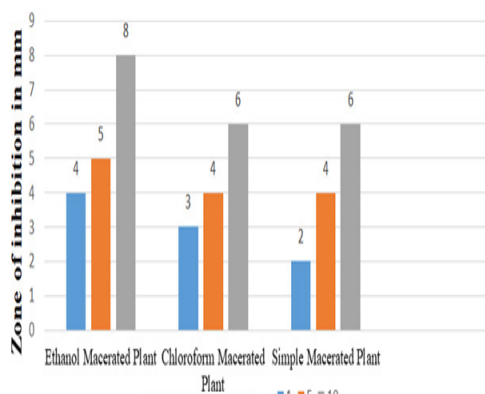


Figure 3: Chloroform, ethanol and simple macerated *S. lappa* with *E. coli*

	Ethanol macerated plant			Chloroform macerated plant			Simple macerated plant		
Concentration (mg/mL)	1	5	10	1	5	10	1	5	10
Zone of inhibition (mm)	4	5	8	3	4	6	2	4	6

*S. lappa* extract has an antibacterial effect in a dose dependent manner. Where different concentrations from different solvent extracts were used, the antibacterial effect increased with an increase of extract concentration, the

best antibacterial effect against *E. coli* was using 10 mg/mL from Ethanol Macerated Plant; the zone of inhibition was 8 mm. These results agree with Vukovic reported the antibacterial activity of different extracts of *Costus speciosus*

against pathogenic strains of bacteria, including *Staphylococcus aureus*, *E. coli*, *Shigella*, *K. pneumonia*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Salmonella*.

In addition to in agreement with Deabes, et al., decided that the highest activity of *S. costus* extract was recorded against *E. coli* with MIC value of 0.3 mg/ml [31].

This is because the plant contains triterpenoids, flavonoids, steroids, and sesquiterpene lactones, which are known to have antibacterial biological activity.

### **The potential use of essential oils— $\alpha$ - and $\beta$ -pinene for treating COVID-19**

*S. costus* contains myrcene, which acts on ACE receptors<sup>15</sup> and may be altered by virus entry into cells. Oleic acid anti-leukotriene-D415 works, so it is used to expand the bronchi. It is also used as an anti-fungal, antibacterial and anti-viral [32].

Pinene is the main terpene found in many plants, especially *Cannabis sativa*. Pinene with the chemical formula (C<sub>10</sub>H<sub>16</sub>) has two structural isomers that differ slightly from each other and are called  $\alpha$ -pinene and  $\beta$ -pinene.  $\alpha$ -pinene is one of the main cannabis terpenes. Terpenes are a large family of organic compounds that plants and some insects produce. Terpenes often have a strong aroma.  $\alpha$ -pinene has a pine scent that is reminiscent of a fresh forest aroma.  $\alpha$ -pinene is found in hemp, in the oils of conifers. Rosemary is another known source of  $\alpha$ -pinene, as are eucalyptus oil and orange peel oil [33].

It is also produced by many herbs such as parsley, basil, dill, sage, hops, cumin, hemp, lavender, yarrow, Korean mint and *Nigella sativa* [34].

These two isomers have different biological actions, directing to different applications, for instance, fungicides, flavors, fragrances, antimicrobial, as well as antiviral [35].  $\alpha$ - and  $\beta$ -pinene are constituents of medicines for treating kidneys and liver diseases [36]. They are also used as antimicrobial agents for their membrane toxicity.  $\alpha$ - and  $\beta$ -pinene have also been studied for possible treatment of breast cancer and leukemia [37].

A clinical trial with  $\alpha$ -pinene was performed utilizing a capsule production called Gelomyrtol forte (one capsule=0.3 g of mirtol, 75 mg of 1,8-cineol, 20 mg of  $\alpha$ -pinene and 75 mg of limonene) in the patients with chronic disease lungs respiratory failure. Respiratory failure

can be acute or chronic. The former occurs suddenly within minutes or hours its effects can be reversed. The prognosis of patients with chronic respiratory failure, characterized by a gradual deterioration of the respiratory function that increases over a longer period, is much worse; its effects are often irreversible. The most characteristic symptom of respiratory failure is shortness of breath. Treatment of acute respiratory failure includes the opening of the airway. Gelomyrtol forte gave positive treatment results [38].

Pine syrup under laboratory conditions has a strong anti-cancer effect, antibacterial, antifungal, anti-viral [39-44]. It has an antithrombotic effect that stimulates cytoprotective activity against H<sub>2</sub>O<sub>2</sub> [45-47]. It has anti-inflammatory, analgesic, anti-oxidant properties, as well as an expectorant and phlegm dissolving effect, being natural support in the upper respiratory tract infections with a runny nose and cough. It helps you to unplug a clogged nose when combined with eucalyptus oil perfectly. Alpha-pinene makes breathing easier, which expands the airways [48-51].

In a recent hypothesis, Adamski and colleagues speculated on using forest products containing  $\alpha$ - and  $\beta$ -pinene (pine and larch syrup and amber) for the potential treatment of COVID-19. Amber is a fossilized resin of conifers that grew 50 million years ago in the form of yellow or brown nuggets. The healing properties of amber have been known for a long time. Succinic acid is a universal biostimulant and an inseparable component of all living organisms. It is made in human beings cells and is responsible for energy exchange, which plays an important role in immunological processes [52].

Succinic acid accelerates the respiration processes in biological cells, thus reducing the amount of “unused” oxygen contained in them, thus reducing the likelihood of the appearance of free radicals and protecting the mitochondrial apparatus against damage. Cells protect against degeneration, and the body against disease and early ageing myself. Succinic acid has been proven to be the best and fastest acting natural anti-oxidant (anti-oxidant) that prevents the pernicious influence of free radicals [53].

Spirit tincture on amber is said to boost immunity and relieve cold, runny nose, and fever symptoms, as well as rheumatic and muscular problems [54-58].



### Recipe for effective COVID-9 treatment

**Indications about the administration are the following:** Drink a tablespoon of pine syrup and a tablespoon of larch syrup. After drinking these syrups, take a 5-10 min break. After the break, drink a small teaspoon of amber tincture you can add 15 drops of propolis. After drinking the tinue, make an hour break drink the syrups 3 times daily. Amber tincture to prepare the tincture, you need small natural amber stones, e.g. 50 g of natural amber, 0.25 L of spirit. Wash the amber and crush it in a mortar; pour amber into a jar and pour 0.25 L of spirit. The dish should be put aside in a warm and dark place for a minimum of 10 days. The jar should be shaken every few days. After 10 days, the tincture should turn golden in color. Pine syrup can be bought at a pharmacy or herbal store or made yourself [59].

**A way to make pine syrup:** The syrup can be made from needles, a pine flower, or green cones, if you make green cones cut the cones into pieces. Take a 5 L jar, pour a 1 cm layer of cones on the bottom and cover with sugar. Then, another layer, cones, and sugar. Pour 1 kg of sugar into a 5 L jar. Leave the filled jar in the warm for a period of 1 month until the syrup crystallizes well. After a month, the syrup can be poured into a bottle or leave it in the jar. It is possible to throw cones into tea, drinking this tea occasionally. Similarly, it is possible to obtain syrup from needles or pine flower. Flower syrup takes longer to crystallize. Larch syrup is difficult to buy at a pharmacy or store, so you should make it yourself. We take a 5 L jar, green larch cones or needles. Sprinkle the cones with layers of sugar in the jar in a similar way as with pine syrup. Close the filled jar and leave it for up to 1 month in a warm place. It is similar to the needles, we cover them with sugar, but the syrup crystallization time is even longer up to 2 months. After a month, we have a wonderful and delicious larch syrup [60].

**Make the ginger syrup as follows:** cut the ginger into small pieces and throw it into a jar, sprinkle it with sugar in a small jar. The next day we have the syrup ready. Squeeze half a lemon into the ginger syrup, making it taste better. Lemon, onion and wild garlic syrup cut lemon, onion and garlic into pieces and sprinkle with sugar. After a few hours, the syrup is ready. It is suggested to drink all these syrups every hour. Twice a day to drink flaxseed with warm milk reduces the irritated mucosa in the esophagus. Notably, we also highlight the role of vitamin D, which regulates the activity of enzymes

responsible for neutralizing free radicals and strengthens the anti-oxidant defense system.

Vitamin D also regulates the function of endothelial cells and the action of surfactants in the lungs. Pulmonary surfactant is a complex of lipid compounds and proteins that change the alveoli function.

*In vitro* replication of SARS-CoV-2 was inhibited by the action of nitric oxide, which inhibited the growth of viral proteases. Nitric oxide synthase is important for healthy endothelial function and has anticoagulant activity.

Nitric oxide has been found to hinder viral protein and RNA synthesis. Nitric oxide has been found to inhibit viral protein and RNA synthesis. Organic sources produce nitric oxide and nitrite, which are found in green leafy vegetables, beetroot and seaweed.

The treatment of COVID-19 with forest products has been fruitful. Five day therapy restores the patient to normal functioning, which was not achieved by conventional medicine. Similar results are presented in their works.

Beekeeping products are a valuable remedy for the coronavirus [61,62]. Propolis, royal jelly contains large amounts of enzymes, pollen, honey, but also tinctures and larch juice, as well as fruits and vegetables containing large amounts of melanin. At each stage of the disease, the patient should be in motion, use active body massage. Artificial respiration should be used because it activates the alveoli, which, as mechanoreceptors, polarize the protein structures in the alveoli and release the electric field that directs the respiration process. Bee's milk royal jelly contains a complete set of essential amino acids, carbohydrates, enzymes, lipids, natural hormones, minerals, phosphorus compounds and acetylcholine. Royal jelly largely contains gamma globulin, which stimulates the immune system and effectively fights infections. It is an effective antibacterial and antiviral agent [63].

Propolis reduces the intensity of pathogens such as the bacterium *P. larvae* (pneumonia diphtheria, which is characteristic of the Coronavirus) [64,65].

In combating parasites and pathogens, bees change their nutrient intake to activate their immune system. Some species of bees starve to death in order to destroy the pathogenic organism [66].

**Free radicals, solution waves and spin waves**

**treat COVID 19:** Elementary particles have electric charge, mass and spin. The electric charge is expressed by the electric field in the space surrounding the particle; The magnetic moment (spin) is expressed in the magnetic field of a rotating particle [67].

In biological systems, the spin wave most often results from the functioning of free radicals, such as: “hydroxyl, superoxide, hydroxide and nitric oxide radicals”. Free radicals are atoms that do not have a pair. Free radicals are formed in the body from many natural chemical reactions and are an integral part of life. Free radicals from proteins, lipids, nucleic acids and sugars present in cells arise as a result of chemical reactions with free oxygen radicals, or from the single electron oxidation of molecular oxygen. Free radicals play an important role in the proper functioning of a biological cell. They participate in many processes, among others. In muscle contractions, hormone secretion, in the functioning of the immune system, in the regulation of vascular tone. The destructive effect begins only when the radicals are produced in excess. It is an inflammatory focus [68].

In the foci of inflammation, free radicals are produced in large amounts by leukocytes in order to destroy microbes. Free radicals easily oxidize proteins, nucleic acids and lipids of cell membranes. Radical reactions with the participation of amino acids present in proteins can lead to damage: protein aggregation it is a non-specific assembly of proteins into fibrils that trigger cellular toxicity, cross linking, fragmentation, loss of enzymatic properties and changes in the conformation of molecules [69].

The excess of free radicals is inadvisable, so they should be deactivated in living organisms. This process can occur in two ways: By adding the missing electron (reduction), or by taking the overactive electron (oxidation). The consequence of oxidative stress, in the absence of efficient cell repair mechanisms, is the oxidation of cell membranes, modification of protein functions and DNA damage. A mutation arises, which may initiate the development of diseases such as: cardiovascular system, lungs, eyes, hepatitis, pancreatitis, diabetes, damage to the nervous system, etc. [70].

In living organisms, the spin wave closely cooperates with the solution wave, which has coded programs for the proper functioning of the cell and the maintenance of homeostasis. Homeostasis consists in maintaining the balance

of the internal environment of the human body in relation to external conditions. Solutions are also responsible for supplying the human body with vital nutrients such as vitamins, minerals, elements, fatty acids and amino acids. They regulate blood pH, osmotic pressure, as well as the partial pressure of carbon dioxide and oxygen in the blood. High intensity of the spin wave disrupts the solution function and can lead to cell death. Disruption of this mechanism can lead to loss of alveolar activity, muscle dysfunction, or other dysfunctional cell or tissue functions. The task of the solutions is to restore the homeostatic balance of the body and the proper functioning of the biological cell [71,72].

Solutions are formed in nonlinear optical centers and in Bose Einstein concentrates, which are generated by the biological system using laser light. Strong laser waves, a degree of non-linearity and a high concentration of atoms in the Bose Einstein condensate influence the formation of multidimensional solutions. Currently, the greatest degree of non-linearity is achieved by organic substances, in which electrons appear to be traveling long distances [73].

Solution is a solitary wave of unchanging shape, located in time and space. There are light, water and sound solutions that can interact strongly with other solutions, but they remain unchanged after collision, only phase shift occurs. The form and structure remain unchanged. This means that they interpenetrate each other without losing their identity [74].

The movement of solutions is influenced by the density and thickness of the biological membrane in the cell, as it determines the size of the piezoelectric effect from which the electric field flows, interacting with solutions. Solutions can spread without distortion over very long distances [75,76].

The action of solutions in the human biological system provides the basis for seeing human psychobiological structures in a different light than that presented by biology and psychology. The spin and solution waves create a different image in relation to the electromagnetic waves received by the eyesight receptor. The science to date recognizes only the action of an electromagnetic wave on the sense of sight. It can be concluded that we are dealing with the second center that creates the structure of the world image and is responsible for health and the development of diseases in humans. The diseased biological tissue contains an excess of

negative free radicals that generate a wide range of spin waves that disrupt the information of in genesis. Solutions constitute a barrier that limits the intensity of the spin wave [77,78].

Solutions contain the age old structure of the cosmos, consisting of the laws and patterns responsible for the functioning of biological cells. For Jung, these are the archetypes that determine human behavior solutions can spread throughout the universe without distortion and they don't disappear. They exist from the beginning of life to the present.

The cosmos is densely filled with a solution network, carrying content and meaning. The brain has the ability to generate and receive solution fields that take an active part in human life processes and determine human health, disease and personality development [79].

Spin waves disrupt this structure; solutions restore it again and are responsible for the self-regulation of biological processes [80-83].

Closing the description of the action of free radicals, it should be noted that the crude extract of *Saussurea lappa* has a significant effect on scavenging free radicals in the biological system and also has a high pharmaceutical activity, active phytonutrients, potentially useful for various therapeutic purposes against pathogens. *S. lappa* essential oil has excellent antimicrobial activity, low cytotoxicity, iron chelating capacity and is therefore recommended for use in medicine against pathogens and we suggest possible use in the treatment of COVID 19. For medicine, this is a new challenge in the search for plants that contain a high amount of solutions [84]. Today in medicine we look at products with high or low pH, but soon medicine will be looking for products with significant amounts of solutions. The new generation vaccine against COVID-19, will not, like traditional vaccines, not introduce the whole virus into our body, but rather it will introduce information that will "trick" the body into thinking that it has the virus in it, and as a result will make antibodies. It is possible thanks to the control of quantum information processes, e.g. by means of an electromagnetic wave, solution wave, electric field, acoustic wave,

spin wave, or bioplasm.

## Conclusion

Plants have medicinal importance, as many plants are therapeutic plants utilised to heal many ailments. The current research was conducted to examine the probable employ of *Saussurea lappa* as a resource of anti-oxidant mediators. Different solvents fractionated from *Saussurea lappa* roots were examined for their anti-oxidative efficiency's. *S. lappa* was collected from the Himalayan region, and crude extract of the *S. lappa* was collected at room temperature, while the extraction of various compounds was done and analyzed using the gas chromatography-mass spectrometric technique. The principal composites in *S. lappa* essential oil were costunolide (9.29%),  $\alpha$ -curcumene (4.33%) and dehydrocostus lactone (47.54%). The free radical scavenging action was evaluated using the crude extract of *S. lappa*, and other pharmaceutically relevant activities such as cytotoxicity, antimicrobial and minimum inhibitory concentration were assessed with significant results. The findings of this assessment indicated that *S. lappa* has different pharmacologically active phyto constituents potentially useful for various medicinal purposes against pathogens.

The essential oil of *S. lappa* possesses excellent antibacterial activity, low cytotoxicity, iron chelation capacity and thus recommended for medicinal use against pathogens and we suggest a possible application for a potential treatment of COVID-19. It has also been shown that  $\alpha$ - and  $\beta$ -pinene type terpenes have broad health properties and can be used to treat COVID-19 as well as other medical diseases. However, clinical trials are needed to corroborate the efficacy regarding a possible application for the COVID-19 treatment.

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## Competing Interests

The author declares that they have no competing interests

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