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Antioxidant activity of essential oil from *Lawsonia inermis* Linn from Pakistan

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Abstract

The present study was designed to assess the constituents of essential oil of *lawsonia innermis* Linn by GC-MS and measure the antioxidant ability by using DPPH stable radical. Essential oil was extracted from the leaves of *lawsonia Inermis Linn* by hydro-distillation using modified Dean Stark Apparatus, followed by drying with anhydrous sodium sulphate. GC-MS studies were prformed for constituents determination by comparison the retention indices and mass spectra with those obtained the library on National Institute of Standard and Technology (NIST). Antioxidant activity of the essential oil of the *lawsonia immerimis* was performed by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay. Light yellow colored oil with 0.4% yield was obtained. The chemical constituents were analyzed by GC-MS, which revealed 21 compounds, out of which 13 were identified and eucalyptol (18.141%). α-pinene (13.345%) and linalool (10.527%) were major components. The essential oil was tested for DPPH scavenging assay and it was found concentration dependant and maximum antioxidant activity 88% was observed for sample at 100 µl concentration while BHT showed activity 92% for the similar concentration. The present study suggested that essential oil of *lawsonia innermis* is a good source of natural antioxidant.

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Introduction

Lawsonia Inermis Linn generally temperate shrub of family *Lythraceae* consists of 25 genera and about 550 species representing in Pakistan by 7 genera *Lawsonia*, *Woodfordia*, *Rotala*, *Lagerstroemia*, *Heimia*, *Lythrum* and *Ammonia*, and 13 species commonly called henna[1]. Its tropical and subtropical habitats, justifies its presence in Pakistan, India, Morocco, Iran, Egypt, the Middle East and North Africa, and Australia. Important component of *Lawsonia Inermis* Linn is Lawsone or 2-hydroxy-1, 4-naphthoquinone containing 0.5-2% concentration of dry leaves,[2] besides, it also contains mannite, tannic acid, mucilage, gallic acid, napthoquinone and other components such as carbohydrates and flavonoids[3]. *Lawsonia Inermis* Linn is a medicinal plant and has been used against tuberculostatic[4] and as antispasmodic and soothing effect on the uterus[5]. Henna leaf alcoholic extract shows mild antibacterial activity, producing leaves with fragrant oils and tanning agents[6]. Leaves used wounds, ulcers, cough, bronchitis, lumbago, rheumatalgia, infections, diarrhea, dysentry, leucoderma, scabies, boils, anemia, hemorrhage, fever, hair, hair falling greyness[7]. Poultices leaves a variety of cancers is to reduce Lawsonia Inermis Linn disinfectant properties are investigated by a variety of workers from around the world[7]. It has also been used in cosmetics, flavors and natural environmental balancer.

The value of medicinal plants in the field of chemistry depends on the impact of construction of compounds. The phytochemical analysis of plants helps to determine new drug development and their connection with some other secondary metabolites. The use of natural products along with their therapeutic role is a very ancient part of human civilizations. The phytochemical study of plant is important also for pharmaceutical study[8]. Plants produce a variety of toxic substances. against disease-causing micro-organisms which are use for the formulation of drugs.For example, myrrh (Commiphora) latex and poppy capsule (Papaver somniferum) use in 2600 BC,[9] function as traditional medicines[10] and herbal supplements.Some errors cause serious infection and toxicity. Resolution discovery of antibiotics to fight the virus in the 20th century noted[11]. Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism and environmental factors, such as air pollutants or cigarette smoke. ROS are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions.[1-6] The shift in the balance between oxidants and antioxidants in favor of oxidants is termed “oxidative stress.” Regulation of reducing and oxidizing (redox) state is critical for cell viability, activation, proliferation, and organ function. Aerobic organisms have integrated antioxidant systems, which include enzymatic and non-enzymatic antioxidants that are usually effective in blocking harmful effects of ROS.[7-12] However, in pathological conditions; the antioxidant systems can be overwhelmed. Oxidative stress contributes too many pathological conditions and diseases, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma[13-16].

The aim of this study is to distill essential oil from leaves of *lawsonia inermis linn* and determine its chemical constituents, its antioxidant activity

Materials and methods

Plant Material

Lawsonia Inermis Linn was gathered from the plants growing in the gardens of the Pakistan Council of Scientific and Industrial Research, Laboratories, Lahore. The leaves were separated from the stalk dried under shade and then powdered to be used for the development of essential oil.

Extraction of essential oil

The powdered leaves (900 gm in 1200mL water) were hydro distilled in the Dean Stark apparatus[17] for about 24 hours at 100 ^{0}C to obtain a yellowish volatile oil. The oily layer after removal from water using a separating funnel was further dried by anhydrous sodium sulphate. The percent yield was calculated based on the dried weight of the plant material which comes to be 0.4% and stored until analysis.

The oil was then subjected for GC/MS analysis. The oil was also subjected for antioxidant, antifungal and antimicrobial activity.

GC-MS Analysis

Essential oil extracted from leaves of *Lawsonia innermis* Linn was analyezed for its chemical constituents by GC-MS. Agilant 5973-6890 gas chromatograph–mass spectrometry system, operating in EI mode at 70 ev equipped with a split-splitless injector was used. Helium was used as a carrier gas at the flow rate of 1 ml/min, while HP-5MS (30m, 0.25mm, 0.25um) capillary column was used. The initial temperature was programmed at 50-100°C at the rate of 5°C/min and then 100-250°C at the rate of 3°C/min followed by a constant temperature at 260°C for a period of 20 minutes. Sample (2µl) was injected to the column programmed at 200°C and resolution of components was attained. Identification of components was performed by matching their retention indices and mass spectra with those obtained the NIST library[18-19].

DPPH free radical Scavenging activity

The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. DPPH accepts hydrogen from an antioxidant. DPPH is one of the few stable and commercially available organic nitrogen radicals. The antioxidant effect is proportional to the disappearance of DPPH in test samples. Monitoring DPPH with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH shows a strong absorption maximum at 517nm (purple)[20]. The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by the decrease of UV absorption at 517nm. The 0.43 gm of DPPH was taken in 90mL of methanol and 3mL was added to the sample test tubes labeled as L1, L2, L3, L4, and L5 containing 20µL, 40µL, 60µL, 80µL, and 100µL of oil. The solution mixtures were kept in dark for 30 minutes and then absorbance was recorded using spectrophotometer and % inhibition was calculated using the formula.

$$\% \text{ inhibition} = \frac{\text{Absorbance of standard- Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

Results & discussion

The essential oil of leaves of lawsonia innermis was extracted through hydrodistillation and light yellow colored oil with 0.4% yield was obtained. GC-MS analysis was performed and thirteen components were identified which corresponds to 69.44% of the total essential oil (Table 1). Eucaloptol was the majour compontent (18,141%), followed by α-pinene (13.345%), linalool (10.527%), 2,6-octadien-1-ol, 3,7-dimethyl acetate (5.636%), 2,6-octadien-1-ol, (3.195%), α- caryophyllene, (2.867%), 1,2-dimethoxy-4-[2-propenyl], benzene (2.129%), 9-isopropyl-1-methyl-2-methylene-5-oxatricyclo [5,4,0,0 {3,8}] undecane (1.493%), nerolic acid (2,6-octadienoic acid) (1.408%), α- terpineol (1.205%), 1,6-octadien-3-ol, 3,7-dimethyl, formate (0.834%) and trans-pinocarveol (0.811%). The composition of essential oil of leaves of *l*awsonia *i*nermis *linn* was subjected to GC/MS and total of 13 compounds were identified which are listed in table.

These chemical constituents are also found in reported literature[21-23] but in different concentrations.

Chemical constituents of essential oil from *Lawsonia Inermis Linn*

S.no#	Compound Name	Molecular Formula	Molecular weight	%composition	Retention time
1	α- pinene	$C_{11}H_{16}$	136	13.345	4.148
2	Eucalyptol	$C_{10}H_{18}O$	154	18.141	5.453
3	Linalol	$C_{10}H_{18}O$	154	10.527	7.118
4	Trans- pinocarveol	$C_{10}H_{15}O$	151	0.811	7.919
5	α- terpineol	$C_{10}H_{18}O$	154	1.205	8.417
6	2,6-octadien-1-ol	$C_{10}H_{18}O$	154	3.195	9.784

S.no#	Compound Name	Molecular Formula	Molecular weight	%composition	Retention time
7	Nerolic acid (2,6-octadienoic acid)	$C_{11}H_{18}O_2$	182	1.408	10.683
8	2,6- octadien-1-ol, 3,7-dimethyl, acetate, (E)	$C_{12}H_{20}O_2$	196	5.636	11.678
9	α- caryophyllene	$C_{15}H_{24}$	204	2.867	12.485
10	1,2-dimethoxy-4-[2-propenyl], benzene	$C_{11}H_{14}O_2$	178	2.129	12.909
11	1,6-octadien-3-ol, 3,7-dimethyl, formate	$C_{11}H_{18}O_2$	182	0.834	13.223
12	9-isopropyl-1-methyl-2-methylene-5-oxatricyclo [5,4,0,0 {3,8}] undecane	$C_{15}H_{24}O$	220	1.493	13.750
13	Durohydroquinone	$C_{10}H_{14}O_2$	166	7.856	14.465

The DPPH radical scavenging activity of essential oil of *Lawsonia Inermis* and standard BHT are shown in Fig. 1 and Fig. 2 respectively. It is clear from the results that essential oil and standard BHT. DPPH scavenging activity is concentration dependent manner. The scavenging ability of essential oil is dependent on the presence of polyphenol compounds, most especially phenols that have the ability to donate the hydrogen atoms in their hydroxyl group[24].

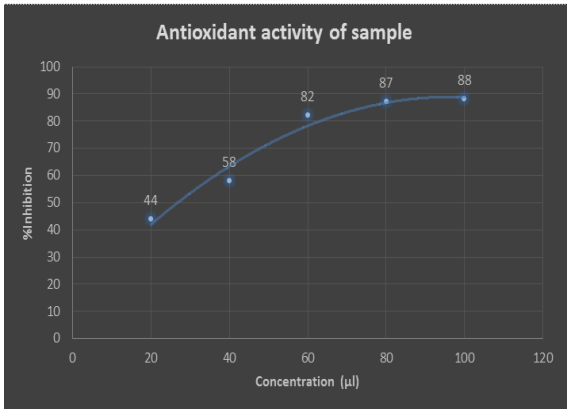

Fig. 1. Antioxidant activity of essential oil of leaves of lawsonia innermis.

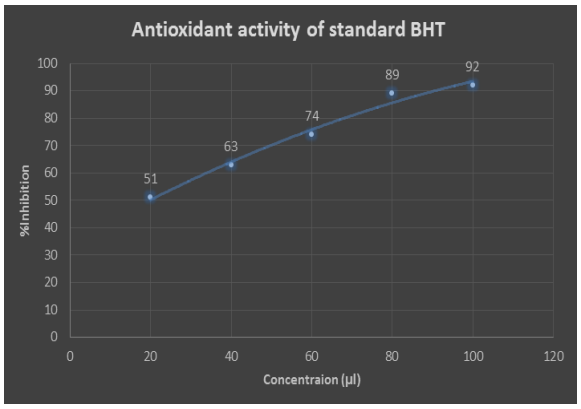

Fig. 2. Antioxidant activity of standard BHT.

The degree of high phenolic compounds having free hydrogen and acts as hydrogen donor, it is a radical scavenger. Its antioxidant activity is proportional to the disappearance of DPPH, in the test tubes. The color turned from purple to yellow by absorbance of hydrogen. Two principle mechanisms of action have been proposed for antioxidants[25]. The first is a chain-breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the systems.

The second mechanism involves removal of ROS/reactive nitrogen species initiators (secondary antioxidants) by quenching chain-initiating catalyst. Antioxidants may exert their effect on biological systems by different mechanisms including electron donation, metal ion chelation, co-antioxidants, or by gene expression regulation. An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable, resulting in the leakage of ions and other cell contents[26].

Conclusion

Light yellow colored essential oil with 0.4% yield was obtained from the leaves of *lawsonia innermis Linn* through hydro-distillation and analyzed by GC-MS for its chemical constituents. Thirteen constituents were identified with the help of NIST and major contents were eucalyptol (18.141%). α-pinene (13.345%) and linalool (10.527%) respectively. Essential oil was evaluated for antioxidant activity by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay.

It was found concentration dependent and maximum antioxidant activity 88% was observed for sample at 100 µl concentrations while BHT showed activity 92% for the similar concentration. The present study suggested that essential oil of *lawsonia inermis* is a good source of natural antioxidant.

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