

Phyto-Chemical Analysis, In-Vitro Antioxidant Potential and GC-MS of *Lallemantia royleana* Seeds

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Abstract- Unani medicine is now becoming a strong part of medicinal system and it is now popularly practiced by a large segment of the population. This medicinal system originated in Greece and was brought to the subcontinent by Muslim scholars. However, the unani medicine currently practiced in India is vastly different from its Greek roots. *Lallemantia royleana* (Benth.) is an important folk medicine which is used in number of ailments. This study was conducted to assess the anti-oxidant activity and phyto-chemical analysis of methanolic extracts of *L. royleana* seeds. The antioxidant activity was determined by using different anti-oxidant methods via 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay and hydrogen peroxide scavenging assay. The present study shows that *L. royleana* seeds possess significant anti-oxidative property therefore, it can be a good remedy for the diseases caused by oxidative stress. The seeds extract contains Alkaloids, Anthraquinones, Flavonoids, Glycosides, Phlobtannins, Tannins and Terpenoids. The antioxidant activity found in DPPH method is more effective than hydrogen peroxide scavenging method. The results of GC-MS analysis showed that at least 21 compounds are present in methanolic extract of *L. royleana*. The compounds which were identified through mass spectrometry (MS) are attached with gas chromatogram (GC). Thus the *in-vitro* studies clearly indicate that the methanolic extract of *L. royleana* seeds show significant antioxidant activity and also a better source of natural antioxidant, which might be helpful in preventing the process of various oxidative stresses.

Index Terms- Anti-oxidant activity, BHT, DPPH, H₂O₂, GC-MS, *lallementia royleana*, phyto-chemical.

I. INTRODUCTION

Lallementia royleana is commonly known as Lady's mantle in english and Tukhm-e-balanga in hindi (1) and in urdu language it is referred to as Balangu shirazi (2). It is an annual herb belonging to the family Labiatae (3). It is cultivated throughout Western Asia, India, Pakistan, and northern of Iraq, for its highly medicinal and mucilaginous seeds which are used widely as a remedial agent in various diseases. (4). The part taken for the study are seeds of the plant. Seeds are more common part of folk medicine and are ethno-botanically well-established worldwide for the treatment of abscesses, inflammation and respiratory problems, and also used in drinks due to sedative effects (5,6,7). The studies had showed the presence of carbohydrates, fibre, oil, protein and tannins (2,8,9).

Other studies toward natural remedies confirmed the fact that plant and food medicine are good source of natural and safe healing therapies (10,11).

The mucilaginous seeds are traditionally used as curative agents against various diseases. The poultices of moistened seeds are found to be useful in abscesses, boils and inflammations. The seeds are also known to cure fever, common cold, act as soothing agents for stomach warmth and intestinal troubles. The roots of the plant are known to cure coughing. The seeds are used as an added palatable ingredient in cooling drinks during summers mixed with brown sugar. The plant seeds are also used as sedative and considered to be cephalic astringent, cardiac tonic and carminative (2).

In Chinese medicine, *L. royleana* is one of the major ingredients of an ointment used in the treatment of skin tumours. In Ivory Coast, Burkina Faso, Gabon and Tanganyika, different parts of the plant are used for the treatment of intercostals pain, rheumatic pain and fever. The leaf and root decoctions are used to treat pneumonia. The plant is also used in preparation of herbal brain tonics in India (12).

It has been also reported that the plant extract along with the extract of *Valeriana wallichii*, exhibits anti-microbial activity (Chitralkha *et. al.*, 1964). The physico-mechanic properties and chemical composition of *L. royleana* seed showed that the seeds contain average amounts of dry matter (92.75%), ash (3.63%), crude protein (25.60%), crude fat (18.27%), crude fiber (1.29%), NDF (30.67%) and ADF (47.80%) (13).

The seeds are a good source of fibre, protein and oil. The factor in its physiological fiber behaviour is usually because of its high viscosity and gel like character in water. The property in turn is related to the functions associated with its high molecular weight polysaccharides (14). The compounds such as Linoleic acid, oleic acid, palmitic acid, stearic acid and beta-sitosterol are present in the seeds. The gums of the seeds are composed of L-arabinose, D-galactose, L-rhamnose, pentosans, proteins, uronic anhydride. It also contains all the amino acids which are present in plant (15).

Humans have an inbuilt anti-oxidant system, which reacts with reactive species and neutralizes them. Thus the natural anti-oxidant system includes enzymes like catalase, superoxide dismutase and glutathione, which protect the body from free radical species and prevent oxidative stress (16). The synthetic anti-oxidant like butylated hydroxyl toluene and butylated hydroxyl anisole are carcinogenic in nature. So, there arises a need for natural anti-oxidant (17). With this background, the aim of the present study was to determine the possible phyto-

chemical and anti-oxidant activity of methanolic extract of *L. royleana* seeds.

II. MATERIALS AND METHODS

A. Plant Material

Seeds were purchased from Unani practitioners. The seeds were then taxonomically identified from the Herbarium Department at NBRI, Lucknow, India.

B. Preparation of the Plant Extract

Plant extracts were prepared by the method of Alade and Irobi (1993) with minor modifications (18). Briefly, 30 g of powdered seeds were soaked in 100 ml of methanol for 72 h in dark. The mixture was stirred every 24 h using a sterile glass rod. At the end of extraction the extract was passed through Whatman filter paper No. 1 (Whatman, UK). The alcoholic filtrate obtained was concentrated *in vacuo* at 40°C and stored at 4°C until further use.

C. Preliminary Phyto-chemical Screening

The preliminary phyto-chemical investigation was carried out for methanolic extracts of seeds of *L. royleana* for the detection of various phyto-constituents by using standard procedures to identify the constituents (19, 20,21,22,23). The results are shown in Table No. 1.

i. Test for the presence of Alkaloids (Wagner's test)

Wagner's reagent was prepared by dissolving 2 g of iodine and 6 g of KI in 100 ml of water. The plant extract was prepared by taking 500 mg of plant material in 500 ml of methanol for 20 minutes, on a water bath. The extract was then filtered off and allowed to cool. The 2 ml plant extract was taken and treated with few drops of Wagner's reagent. A reddish brown coloured precipitate indicates the presence of alkaloids.

ii. Test for the presence of Anthraquinone (Borntrager's test)

About 0.5 g of the extracts was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10 percent ammonia were added to the mixture and heated. Formation of rose-pink colour indicates the presence of anthraquinones.

iii. Test for the presence of Flavonoids

The crude powder of dried plant was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution and observed a yellow colouration of solution was observed.

iv. Test for the presence of Phlobatannins

An aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid (HCl) to observe the deposition of red precipitate.

v. Test for the presence of Glycosides (Fehling's test)

The crude plant powder of 0.5 g was dissolved in 5 ml of methanol. The 2 ml of this solution was taken and to it 10 ml of 50% HCl was added. The mixture was heated in a boiling water bath for 30 min. To the mixture 5 ml of Fehling's solution was added and the mixture was boiled for another 5 min to observe a brick red precipitate as an indication for the presence of glycosides.

vi. Test for the presence of Saponins (Frothing test)

About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy layer of small bubbles) showed the presence of saponins.

vii. Test for the presence of Steroids (Salkowski test)

The 1 ml of plant extract was taken and to it few drops of concentrated sulphuric was added. The presence of red colouration indicates the presence of steroids.

viii. Test for the presence of Tannins (Ferric chloride test)

The presence of tannins was tested in 0.5 g of the crude plant powder and was stirred with 10 ml of distilled water. The extract was filtered and ferric chloride reagent was added to the filtrate, a blue-black precipitate was taken as an evidence for the presence of tannin.

ix. Test for the presence of Terpenoids (Salkowski test)

The presence of terpenoids was tested in 0.2 g of the extract of the plant sample and mixed with 2 ml of chloroform and concentrated sulphuric acid (3 ml H₂SO₄) was added carefully to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

D. Anti-oxidant Activities

Anti-oxidant activity of methanolic seed extract of *L. royleana* was determined *in vitro* by super oxide scavenging activity by DPPH free radical scavenging activity and H₂O₂ radical scavenging activity.

i. Determination of Hydrogen Peroxide (H₂O₂) Scavenging Activity-

Chemicals and reagents which were required to perform H₂O₂ test hydrogen peroxide and di-sodium hypo phosphate were purchased from Merck Co. (Germany).

a. Protocol for Estimation of H₂O₂ Scavenging Activity-

The protocol used for hydrogen peroxide assay was the procedure done by Ruch *et. al.*, (1989) (24) method with minor modification. A solution of hydrogen peroxide (20 mmol/ L) was prepared in phosphate buffer (pH 7.4). The test samples and standard (BHT) were prepared at different concentrations ranging from 1.95 to 1000 µg/ml and was added to the hydrogen peroxide solution (20 mmol/ L, 2 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of samples was calculated using the following formula:

$$\% \text{ scavenged } [H_2O_2] = [(A_0 - A_1)/A_1] \times 100$$

Where A₀ was the absorbance of the control,

And A₁ was the absorbance of the sample.

ii. DPPH free radical scavenging activity-

Chemicals which were required for DPPH test were DPPH and methanol purchased from Merck Co. (Germany).

a. Protocol for estimation of DPPH scavenging activity-

1. DPPH radical scavenging activities of all the fractions were determined by the method of Blois (1958) (25) with minor modifications. The crude plant extracts were prepared at different concentrations (1000 µg/ml to 1.95 µg/ml) in methanol, initially 10 µl of plant extracts was mixed with 200 µl of 100 mM DPPH (dissolved in methanol). The reaction mixtures were incubated for 30 min at 37° C under dark condition. Butylated hydroxy

toluene (BHT) was used as control, while methanol was used as blank. The DPPH radical scavenging activity was determined by measuring the absorbance at 490 nm spectrophotometrically (26). The percentage inhibitions were calculated by the formula given

$$\text{Scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control,

2. And A_1 was the absorbance of the sample.

E. Characterization of Crude Extract by Gas chromatogram and mass spectrometer (GC-MS)

The methanolic crude extracts were characterized by GC-MS technique. The analysis was done at Advanced Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi on payment basis. The instrument used for analysis was GCMS-QP2010 Plus. Analytical conditions for Gas chromatogram and mass spectrometer are given below.

GCMS-QP2010 Plus (Shimadzu)

Column Rts- 5 MF (30 m x 0.25 mm internal diameter x 0.25 μ m film thickness

Mode Electron ionization (EI)

Source temperature 230° C

Scan time 0-60 min

Transfer line temperature 280° C

Injection mode Split mode 10:0

Injection temperature 250° C

Injection volume 1 μ l

Carrier gas Helium; Flow rate: 1.5 ml/min

Oven temperature 80° C- 310° C

i. Identification of Components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST05) and WILEY8 spectral database having more patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST05 and WILEY8 library. The Name, Molecular weight, Molecular formula were obtained.

III. RESULTS AND DISCUSSION

The phyto-chemical analysis of methanolic seeds extract showed the presence of alkaloids, anthraquinones, flavonoids, glycosides, phlobotannin tannins and terpenoids while steroids and saponins were not present. The result of the phyto-chemical screening is given in Table 1.

The crude methanolic extract of *L. royleana* seeds showed prominent IC_{50} value of 140.53 ± 4.22 μ g/ml by DPPH method and 576.5 ± 0.00 μ g/ml by Hydrogen peroxide method and the standard BHT showed an IC_{50} value of 43.40 ± 1.30 μ g/ml by DPPH method and IC_{50} value of 26.16 ± 0.351 μ g/ml by hydrogen peroxide method. The DPPH assay observed high antioxidant activity as compared to hydrogen peroxide methods. The result of the anti-oxidant activity is given in Table 2. The results of GC-MS analysis showed that at least 21 compounds are present in methanolic extract of *L. royleana*. The compounds which were identified through mass spectrometry are attached with GC. The mass spectra of these compounds were matched with those found in the NIST05 and WILEY 8 spectral database. The fragmentation of major compound was found in 9, 12, 15-

Octadecatrienoic acid, (Z, Z, Z)-74.54% (retention time: 15.442 min), n-Hexadecanoic acid 13.77% (retention time: 14.206 min) and 1, 2, 3-propanetriol (retention time: 4.943 min) 1.27%. The active principles along with their retention time, area, area percent and compound name in the methanolic extract of *L. royleana* are given in Table 3. The chromatogram of GC-MS is given in Figure 1.

IV. CONCLUSION

The present study showed significant phyto-chemical compounds and anti-oxidant potential of *Lallementia royleana* methanolic seeds extract. Based on study, it can be concluded that the plant extract can be used for the treatment of several diseases caused by oxidative stress. Some texts are available on the genus *Lallementia*. The studies made on the plant opened new way to research about the special effects of *Lallementia*. The efficacy and activity of plants extract needs to be further studied and evaluated to understand the mechanism of action for the plant.

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S.No.	Active principle	Phyto-chemical Analysis	Result
1.	Alkaloids	Wagner's Test	+
2.	Anthraquinones	Borntrager's Test	+
3.	Flavonoids	Sodium Hydroxide (NaOH) Test	+
4.	Phlobatanins	Hydrochloric Acid (HCl) Test	+
5.	Glycosides	Fehling's Test	+
6.	Saponins	Frothing Test	-
7.	Steroids	Salkowski Test	-
8.	Tannins	Ferric chloride (FeCl ₃) Test	+
9.	Terpenoids	Salkowski Test	+

Table No. 1: Qualitative Phyto-chemical Tests

S.No.	TEST PERFORMED	<i>L. royleana</i> IC ₅₀	BHT IC ₅₀
1.	DPPH Method	140.53 ± 4.22	43.40 ± 1.30
2.	H ₂ O ₂ Method	576.5 ± 0.00	26.16 ± 0.351

Table No.2- IC₅₀ value of different anti-oxidant activity of seed extract of *L. royaleana*

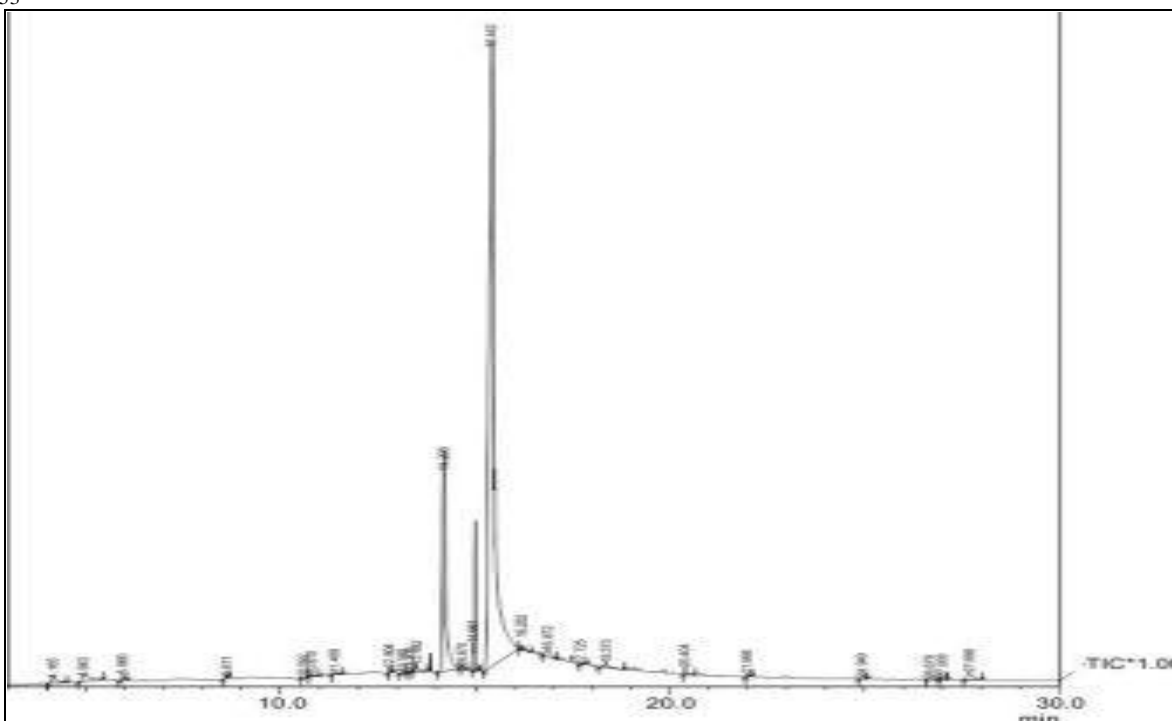


Figure No. 1: Chromatogram of *Lallelantia royleana* methanolic seeds extract

Peak	R. Time	Area	Area%	Name
1	4.165	5843004	0.76	1-Butane, 4-Isothiocyanato-
2	4.943	9758315	1.27	1,2,3-Propanetriol
3	5.960	1649384	0.22	Undecane
4	8.611	748205	0.10	Cyclohexasiloxane, dodecamethyl-
5	10.592	571484	0.07	trans-Z-.alpha.-Bisabolene epoxide
6	10.810	1418485	0.19	Phenol, 3,5-bis(1,1-dimethylethyl)-
7	11.409	1615616	0.21	3',5'-Dimethoxyacetophenone
8	12.804	1759571	0.23	Tetradecanoic acid
9	13.140	541339	0.07	Isopropyl Myristate
10	13.492	4015724	0.52	1,2-Benzenedicarboxylic acid, Bis(2-Methylpropyl) Ester
11	14.206	105549348	13.77	n-Hexadecanoic acid
12	14.670	2020914	0.26	Eicosanoic acid
13	14.961	29815108	3.89	9,12-Octadecadienoic acid, methyl ester
14	15.442	571182242	74.54	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
15	18.373	4291323	0.56	Tetratriacontane
16	20.404	3003773	0.39	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(2)]
17	21.998	1373739	0.18	9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-
18	24.940	1565530	0.20	7-(Trimethylsilyloxy)-3-[4-(Trimethylsilyloxy)Phenyl]-4H-1-
19	26.679	1099323	0.14	7-(Trimethylsilyloxy)-3-[4-(Trimethylsilyloxy)Phenyl]-4H-1-
20	27.009	1546408	0.20	2-Butanone, 4-(2,2-Dimethyl-6-Methylenecyclohexylidene)-
21	27.698	6488647	0.85	Tetrahydroabietic acid

Table No. 3: The table shows peak results of *L. royleana* methanolic seed extract