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Original Article

Comparison of the Anti-asthmatic Potential of Himalayan Plants *Lonicera obovata* Royle and *Morina longifolia* Wall: An *in vivo* Study

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ABSTRACT

Background: Asthma is a very common and chronic disease in which there is an inflammation of the lungs. Inflammatory airways and narrowing, spasms, over-secretion of mucus, bronchoconstriction, and eosinophilic airway inflammation are generally seen in the asthmatic condition. Globally, its prevalence is rising constantly. The extracts of *Lonicera obovata* and *Morina longifolia* of the Caprifoliaceae family were traditionally used by the Himalayan local people to treat asthmatic and inflammatory conditions. **Objectives:** The goal of this experimental study was to identify the phytochemicals of the plants through GC-MS. To investigate and compare the anti-asthmatic potential of methanolic whole plant extracts of *L. obovata* and methanolic root extracts of *M. longifolia* through *in-vivo* experiments. **Materials and Methods:** The anti-asthmatic potential of extracts was determined by the method of leukocytosis and eosinophilia induced by milk in the Swiss albino mice model. **Results:** Various secondary active phytoconstituents were detected through the GC-MS screening method, some of which have anti-allergic (anti-asthmatic) and antihistaminic properties. The *in vivo* experiment result showed that in the case of increased leukocyte count, *M. longifolia* shows better results than *L. obovata*, and in the case of increased eosinophil count, *L. obovata* shows better results than *M. longifolia* to reduce the increased count. **Conclusion:** The anti-asthmatic potential of plant extracts could be due to the presence of the phytoconstituents like 1-Monolinoleoylglycerol trimethylsilyl ether in the *L. obovata* extracts and Octadec-9-enoic acid and Cis-vaccenic acid in both *L. obovata* and *M. longifolia* extracts. Hence, through the experiments, the unexplored anti-asthmatic properties were revealed and justifies the traditional usage of both plants to treat asthma.

Keywords: Anti-asthmatic, Eosinophilia, *in vivo*, leukocytosis, *Lonicera obovata*, *Morina longifolia.*

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INTRODUCTION

Asthma is a non-communicable and long-lasting body disorder that leads to lung inflammation, breath shortness, tightness of the chest, wheezing, and cough.[1,2] The disordered physiological processes associated with asthma are leukocytes (mainly eosinophils) infiltration, reversible obstruction of airways/ bronchi, remodeling of bronchi wall, hyperresponsiveness and hyperreactivity of smooth muscle cells of airways (due to narrowing of airways), mucus hyperproduction by goblet cells, and chronic inflammation of airways.[3] The prevalence of asthma has been increasing day by day. Around 300 million people suffer worldwide and 250,000 people die annually from asthma.[4] In the prevalence, mortality, severity, and incidence of asthma, there is a great variation among regions, sex of the organism, and stages of life, affecting people of all age groups in millions. The prevalence of the asthmatic condition is high in children as compared to adults and mortality is higher in adults than children. Its prevalence is higher in female adults. It affects people worldwide with prevalence rates more in well-paid countries than in the least developed countries. Relatively to other chronic diseases, asthma has high morbidity rates and low mortality rates. It worsens the quality of life. It is a complex multi-factor medical ailment that occurs due to interactions between hereditary susceptibility, host factors like obesity, infection, and environmental factors like pollution, allergens, pollens, etc.[5] Th2 cytokines-IL-4, IL-5, and IL-13 play a significant function in the disordered pathological processes in asthma.[6] The trigger point in asthma is the affinity between elevated serum IgE and inflammatory mediators IL-4, IL-5, and IL-13 that are generated by Th2 cells in pulmonary tissue.[7] As reported, more than 50 different types of mediators are known to be involved in the development of asthma.[6] Some known mediators that are involved in asthma are histamine, leukotrienes, chemokines, prostaglandins, etc. The Chemokines show affinity towards inflammatory cells like eosinophils, T-lymphocytes,

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macrophages, etc. The interleukin-4 and Interleukin-13 which are extravasated from Th2 cells give rise to airway constriction, hyper-responsiveness, mucus overproduction, and eosinophilia in the airways caused by Interleukin-5.[8] Hematological parameters can help in the diagnosis and prognosis of asthma disease. Eosinophils, neutrophils, epithelial cells, mast cells, macrophages, etc are the cells that take part as an important role in the onset of asthma. Granulocytic parameters like eosinophilia and neutrophilia are characteristic aspects of allergic diseases like asthma. Eosinophils counter asthma and infection.[9]

In allopathy, generally, asthma is treated by using bronchodilator drugs (for example β2-adrenoceptor agonists and leukotriene receptor antagonists) and corticosteroids that inhibit eosinophil function. Though these are very effective against asthma but have numerous adverse effects on the body. Sedation and neuropsychiatric symptoms are the main side effects of β2-adrenoceptor agonists and leukotriene receptor antagonists. Osteoporosis, weight gain, Candidiasis, skin lesions, osteopenia, hoarseness, and cushingoid features are the side effects of corticosteroid therapy.[3,10] The traditional medicine system is well-known everywhere in the world. In the traditional system, there was a usage of medicinal plants in treating many ailments. US Food and Drugs Administration certified that 34% of new medicines were derived from natural product derivatives between 1981 and 2010.[11] The medicinal plants that are used to treat asthma must have the properties like smooth-muscle relaxant, anti-histaminic, immunomodulatory, and anti-inflammatory.[7]

Lonicera obovata of the Caprifoliaceae family is commonly known as Blueberry honeysuckle or small-leaved honeysuckle. It is a dwarf perennial shrub bearing dark bluish-purple fleshy berries which are spherical in shape and born in pairs. It is distributed in Asian countries globally and in Himachal Pradesh, Uttarakhand, Jammu and Kashmir, and Sikkim in India and it grows at an altitude of 7000-14000 feet approximately.[12] Traditionally, local people of Kinnaur district of Himachal Pradesh, India used the whole plant powder of *L. obovata* mixed with other plants such as *Aconitum violaceum, Rhododendron anthopogon, Gentiana nubigena, Meconopsis aculeata*, and *Polygonum tortuosum* twice a day for the treatment of asthma.[13] *Morina longifolia* is a member of the Caprifoliaceae family and its common name is Himalayan Whorlflower. It is an evergreen perennial and glabrous herb having dark green leaves with spines, distributed in the Himalayan region (Kashmir) up to Bhutan and found at a height of 2400-4200 meters. This medicinal plant was used by the traditional medical system. It was reported that this plant is used to treat wounds caused by maggot infestation.[14] *M. longifolia* essential oil exhibits antimicrobial, antibacterial, and antifungal properties. This plant is aromatic in nature and due to this property, it is used in agarbatti and dhoop making and aromatic flowers used for unconsciousness.[15] Traditionally people used flowers and leaves of this plant to digest food faster and for curing wounds and increasing appetite. This plant is also rich in antioxidant compounds.[16] As previously informed, the roots of this plant treat the condition of boils and have antiallergic properties.[17]

The purpose of this experimental work was to find out the bioactive phytoconstituents which are having anti-asthmatic properties and to determine and compare the anti-asthmatic potential of plants *Lonicera obovata* and *Morina longifolia* by the method of leukocytosis and eosinophilia induced by milk in the albino mice model.

MATERIALS AND METHODS

Collection of plants and their authentication

L. obovata whole plant was collected from the Churdhar peak, Sirmour district, Himachal Pradesh in the month of July 2019. *M. longifolia* root part was collected from the Hatu peak, Narkanda, Shimla, Himachal Pradesh in August 2019. Their identification was done by B.S.I., Northern Regional Centre, Dehradun, Uttarakhand, India. The identification number is BSI/NRC/Tech./Herb.(Ident.)/2019-20/328.

Extraction method

The plant's parts were washed properly with water, shade-dried, and ground in a mixer. Methanol (95%) solvent was used to prepare the extract of the plants. The maceration procedure was followed for extraction in which the methanol was poured into a flask on the coarse plant powder until all the plant material was dissolved in the solvent. The flask was kept in a shaker for continuous stirring to make sure for complete extraction for 3-4 days. Then it was sieved with filter paper (Whatman) and kept for drying out. The solvent was evaporated and a layer of extract remained in the Petri plate and set aside at a very low temperature for further usage.

Chemicals purchase

The chemicals used for the experiments are Diethyl ether, Dexamethasone, Methanol, Chloroform, Sulphuric acid, Sodium Hydroxide solution, Ferric Chloride, Fehling A and Fehling B reagents, Hydrochloric acid, Potassium Iodide, and Mercuric Chloride. These were purchased from G.K. Entreprises, Chandigarh.

Qualitative screening for phytochemicals

Methanolic extracts of *L. obovata* (whole plant) and *M. longifolia* (root) were used for qualitative screening of phytochemicals. Different test methods were used for the screening of various phytochemicals to know their presence in the extracts.[18]

Analysis of extracts for their secondary metabolites by Gas Chromatography-Mass Spectrometry

The characterization of plant secondary metabolites was done by a GC Clarus 500 Perkin Elmer system encompassing an AOC-2Oi autosampler and gas chromatograph attached with a mass spectrometer. It was equipped with a capillary column 30 ×0.25 mm ID × 1EM df, eV. The temperature of the oven was set from 110°C to 280°C for 5 min. to 10 min. hold time. The volume that was injected into the gas chromatograph was 10 µL. The carrier gas Helium was utilized. The run time for the procedure was 30 min. The low mass and high mass values were taken at 40 m/z and 650 m/z. With the aid of Retention Time (R_t), CAS number, molecular formula, and weight, unknown secondary metabolites were revealed when these were compared from the NIST databases.

Animal model

Male albino mice (20-25 g) of the Swiss strain were procured for the *in-vivo* experiment to evaluate the anti-asthmatic properties of plants. Proper food and distilled water was provided to mice and ensured about standard conditions like temperature and sunlight in the lab. IAEC sanctioned the experimental protocol and the sanction number is PU/45/99/CPCSEA/IAEC/2019/316.

Acute toxicity determination

For determination of the acute toxicity of plant extracts, the OECD guideline number 423 was followed up. The oral dosage of 500, 1000, 1500, and 2000 mg/kg plant extract concentrations was introduced to the animals. Mice were observed for 1, 4, and 24 hr of extracts administration.[19]

In-vivo experiment for the anti-asthmatic potential of plants

The anti-asthmatic potential of plant extracts was determined by the assay of leukocytosis and eosinophilia in mice induced by milk. The mice were separated into five categories, each category having 6 mice. Mice were given anesthesia by the diethyl ether chemical before blood was collected from the retroorbital plexus. The first group (control) was introduced with 10 mL/kg distilled water orally, the second group was injected with 4 mL/kg boiled and cooled milk subcutaneously, the third and fourth group was administered with 100 and 200 mg/kg plant extracts intraperitoneally and the fifth group administered with 50 mg/kg dexamethasone drug intraperitoneally. After 30 min of administration of plant extracts and dexamethasone, the 4 mL/kg milk was injected subcutaneously into the third, fourth, and fifth groups. The total leukocyte number and total eosinophil number were counted in each group before extract treatment and after 24 hr of milk administration and the difference was calculated.[20]

Statistical method

The values were represented by mean ± Standard error of the mean using Excel 2016. With the help of SPSS version 16.0 software, ANOVA and Dunnet's (*post hoc* test) were used. The statistical significance measurement value (denoted by *P*) was chosen as less than 0.05.

RESULTS

Qualitative screening for phytochemicals

The qualitative screening disclosed that glycosides, flavonoids, tannins, steroids, phenols, and terpenoids were found in both the whole plant extract of *L. obovata* and the root extract of *M. longifolia*. Saponins are absent in both plant extracts. Alkaloids are present in *M. longifolia* but are absent in *L. obovata* as presented in Table 1. These phytochemicals are of great medicinal importance like tannins and terpenoids have analgesic and anti-inflammatory properties, and tannins also have astringent, antioxidant, antidiarrheal, antifungal, antiviral, antibacterial, cytotoxic and antiparasitic properties.[21,22] Saponins have the properties to treat infections caused by fungi and yeast. Flavonoids help in preventing allergies, cancer, and inflammation and exhibit antioxidant, antimicrobial, and antiviral potential. Cardiac glycosides help to cure heart failure.[21] Alkaloids have various activities like anticancer, analgesic, antihypertensive, antimalarial, and antiarrhythmic. Phenols possess potential against inflammation and cancer.[22]

Analysis of extracts for their secondary metabolites by Gas chromatography-mass spectrometry method

In the *L. obovata* extract, 16, and in the *M. longifolia* 13 bioactive phytochemicals were detected by the screening method of GC-MS. Figure 1 and Figure 2 represent the graphical record of *L. obovata* and *M. longifolia* extracts showing relative concentrations of the bioactive phytoconstituents at different peak heights. Table 2 and Table 3 show the list of both plants' identified phytoconstituents with their percentage of peak area, Retention Time (R_t), and molecular formula. The biological properties of some identified phytoconstituents of *L. obovata* extract are the following: 2,2-Dimethoxybutane exhibits antidermatophytic,[23] and antimicrobial properties.[24] 9-Octadecenal, (Z) has anti-inflammatory, anticancerous, and antimicrobial activities and reduces the risk of heart attack and atherosclerosis.[25] i-Propyl 9-tetradecenoate has antimicrobial potential.[26] 1-Monolinoleoylglycerol trimethylsilyl ether exhibits antiasthmatic, anti-inflammatory, antioxidant, anti-diabetic, anticancerous, and anti-arthritic properties and has potential against microbes, prevent liver damage, and is diuretic.[27] 9-Octadecenoic acid (Z)-, phenylmethyl ester possesses anticancer, antimicrobial, and antiviral activities; is a photoprotective agent and defends skin barriers.[28] It is a 5α-reductase inhibitor, prevents inflammation, repels insects,

Table 1: Qualitative analysis of bioactive phytoconstituents of *L. obovata* and *M. longifolia*.

Phytochemicals	Qualitative method	*L. obovata* extract	*M. longifolia* extract
Alkaloids	Mayer's reagent	-	+
Phenolic compounds	$FeCl_3$ test	+	+
Tannins	$FeCl_3$ test	+	+
Terpenoids	Salkowski's test	+	+
Glycosides	Fehling's test	+	+
Flavonoids	Sodium hydroxide test	+	+
Saponins	Foam test	-	-
Steroids	Salkowski test	+	+

+ indicated presence and – indicated absence of phytochemicals

Table 2: Bioactive phytoconstituents of *L. obovata* extract.

Compound name	Retention time (minutes)	Molecular formula	Peak area (%)
2,2-Dimethoxybutane	3.35	$C_6H_{14}O_2$	0.14
Dimethylsulfoxide-D6	3.82	C_2D_6OS	91.20
9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl) oxy]methyl]ethyl ester, (Z,Z,Z)	20.46	$C_{27}H_{52}O_4Si_2$	6.81
9-Octadecenal, (Z)	21.69	$C_{18}H_{34}O$	0.07
Cis-Vaccenic acid	21.80	$C_{18}H_{34}O_2$	0.06
9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)-1-[[(trimethylsilyl) oxy]methyl]ethyl ester, (Z,Z,Z)	21.95	$C_{27}H_{52}O_4Si_2$	0.07
i-Propyl 9-tetradecenoate	22.70	$C_{17}H_{32}O_2$	0.07
9-Octadecenoic acid, 2,2,2-trifluoroethyl ester	22.85	$C_{20}H_{35}F_3O_2$	0.10
1-Monolinoleoylglycerol trimethylsilyl ether	22.97	$C_{27}H_{54}O_4Si_2$	0.03
9-Octadecenoic acid (Z)-, phenylmethyl ester	23.29	$C_{25}H_{40}O_2$	0.35
Octadec-9-enoic acid	23.93	$C_{18}H_{34}O_2$	0.05
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	24.08	$C_{21}H_{40}O_4$	0.31
9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)-1-[[(trimethylsilyl) oxy]methyl]ethyl ester, (Z,Z,Z)	24.58	$C_{27}H_{52}O_4Si_2$	0.07
6-Octadecenoic acid	24.80	$C_{18}H_{34}O_2$	0.12
i-Propyl 9-tetradecenoate	25.45	$C_{17}H_{32}O_2$	0.39
9-Octadecenoic acid (Z)-, phenylmethyl ester	25.91	$C_{25}H_{40}O_2$	0.14

and has hypocholesterolemic effects.[29] 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester inhibits proliferation in Keloid fibroblasts,[30] and induces testosterone hydroxylase.[31] The phytoconstituents identified in the *M. longifolia* extract also show biological properties like Cyclopentaneundecanoic acid, methyl ester has antimicrobial activity.[32] 2-Methyl-Z,Z-3,13-octadecadienol exhibits herbicidal, pesticidal, and insecticidal properties.[30] 6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z) possesses anticancer, antioxidant, antimicrobial, and pesticidal properties and treats inflammation.[33] Both the plants have common phytoconstituents with some properties such as Cis-Vaccenic acid exhibits activities like antiallergic, anti-inflammatory;[34] antibacterial, hypolipidemic;[35] antioxidant, tyrosinase inhibition,[36] and anti-cancerous.[37] 6-Octadecenoic acid has antioxidant potential,[38] and has antimicrobial and anti-aging properties, and treats cancer.[39] 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl) oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (ZZZ) possesses anti-inflammatory, antioxidant, antidiabetic,[40] and anticancerous properties.[41] Octadec-9-enoic acid prevents coronary heart disease, and inflammation and repels insects. It is a hepatoprotective and hypocholesterolemic agent and also exhibits antihistaminic and anticancer properties.[42]

Table 3: Bioactive phytoconstituents of *M. longifolia* extract.

Compound name	Retention time (minutes)	Molecular formula	Peak area (%)
Dimethylsulfoxide-D6	3.83	C_2D_6OS	43.82
Dimethylsulfoxide-D6	13.07	C_2D_6OS	3.92
Dodecanamide	16.64	$C_{12}H_{25}NO$	38.80
Cyclopentaneundecanoic acid, methyl ester	17.64	$C_{17}H_{32}O_2$	0.72
2-Methyl-Z,Z-3,13-octadecadienol	19.22	$C_{19}H_{36}O$	1.31
Cis-Vaccenic acid	19.35	$C_{18}H_{34}O_2$	2.28
6-Octadecenoic acid	21.93	$C_{18}H_{34}O_2$	0.53
6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)	22.56	$C_{25}H_{36}O_2$	2.30
6-Octadecenoic acid	23.92	$C_{18}H_{34}O_2$	0.48
Cis-Vaccenic acid	24.09	$C_{18}H_{34}O_2$	1.22
14-Octadecenal	25.40	$C_{18}H_{34}O$	2.54
9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl) oxy]methyl]ethyl ester, (Z,Z,Z)	25.92	$C_{27}H_{52}O_4Si_2$	1.11
Octadec-9-enoic acid	29.31	$C_{18}H_{34}O_2$	0.97

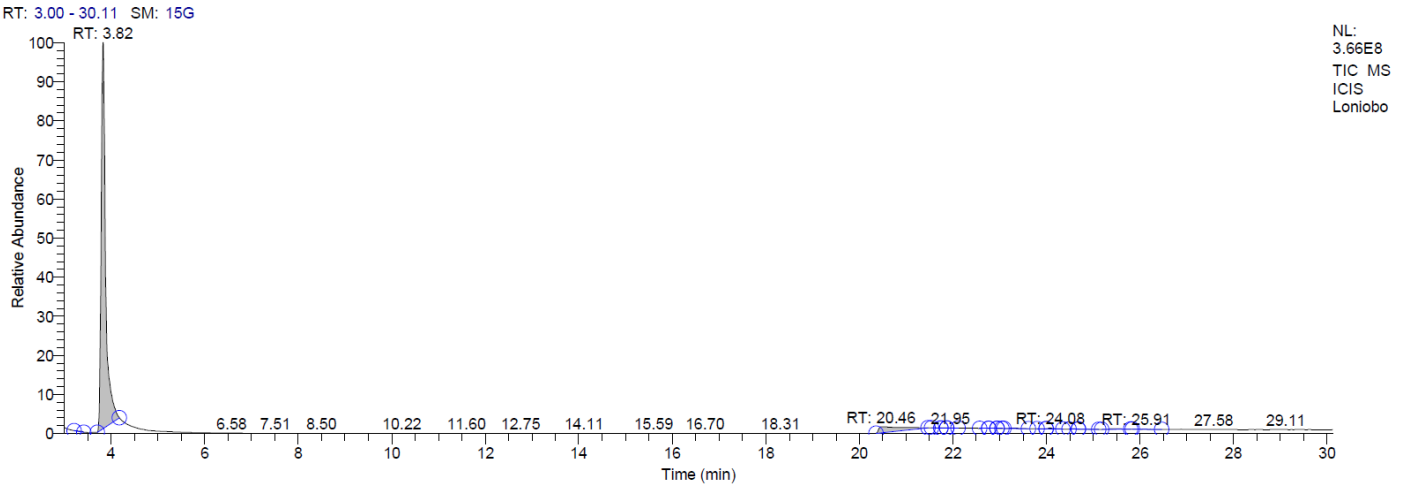

Figure 1: Chromatogram showing bioactive phytoconstituents of *L. obovata* extract by the method of Gas Chromatography-Mass Spectrometry.

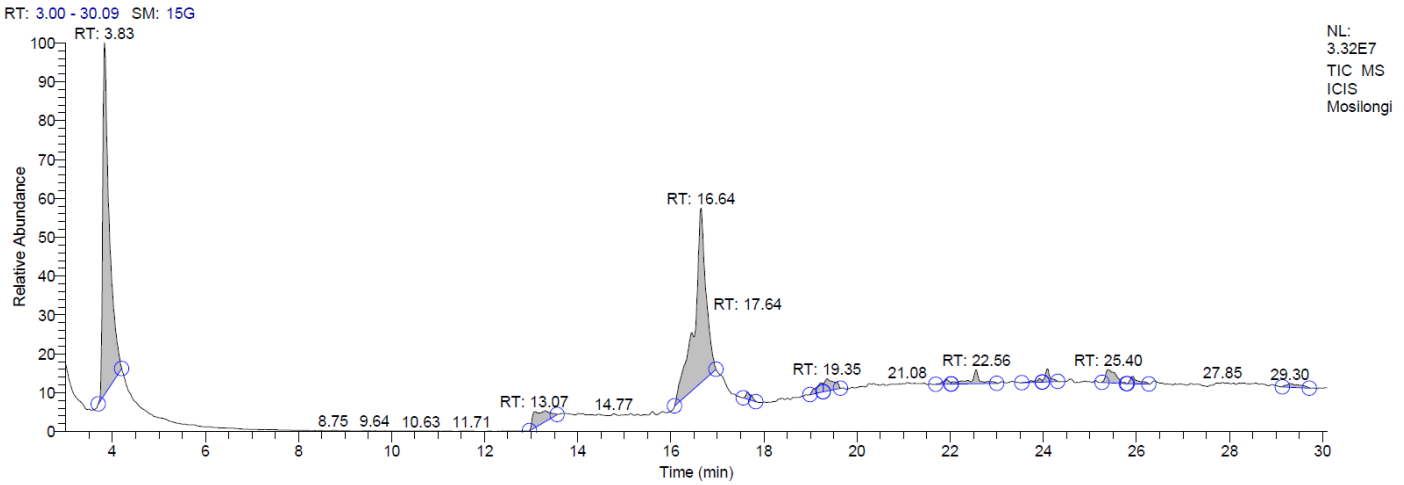

Figure 2: Chromatogram showing bioactive phytoconstituents of *M. longifolia* extract by the method of Gas Chromatography-Mass Spectroscopy.

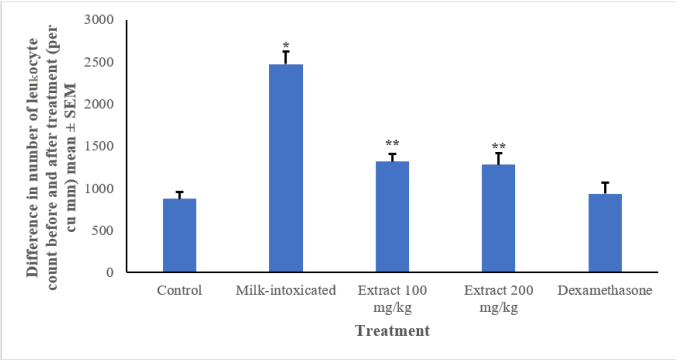

Figure 3: Histogram showing consequences of plant extract of *L. obovata* on increased leukocyte by milk in mice. *p<0.001, **p<0.05 when comparison was done with the control.

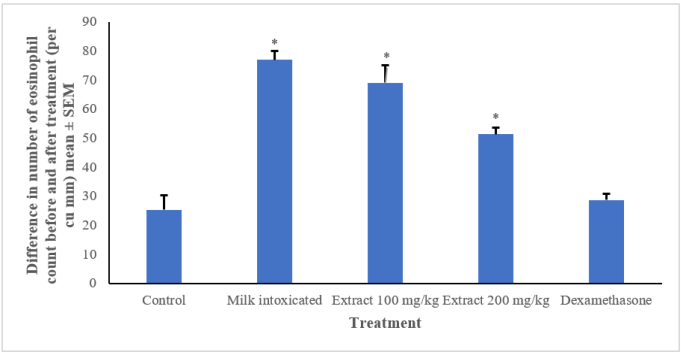

Figure 4: Histogram showing consequences of plant extract of *L. obovata* on increased eosinophils by milk in mice. *p<0.001 when comparison was done with the control.

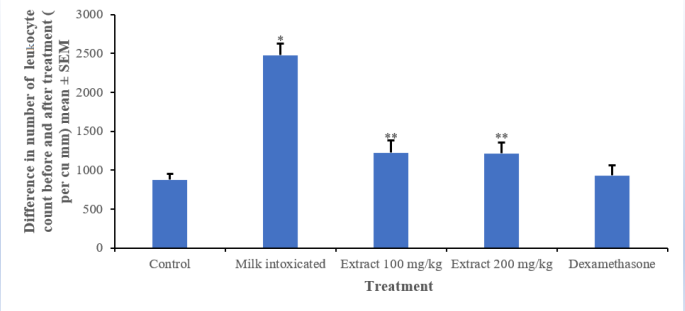

Figure 5: Histogram showing consequences of root extract of *M. longifolia* on increased leukocyte in mice. *p<0.001 and **p<0.05 when comparison was done with the control.

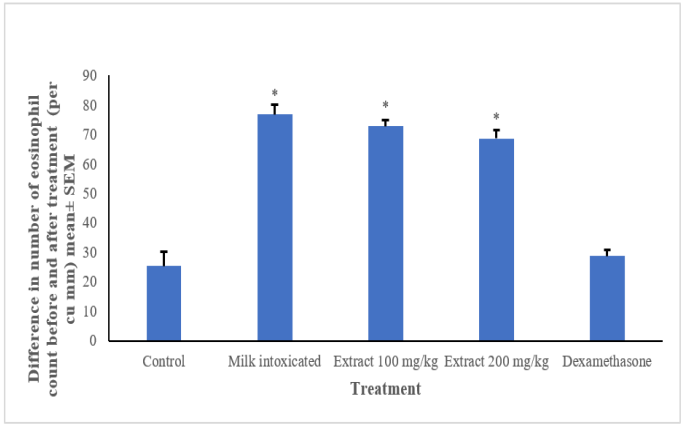

Figure 6: Histogram showing consequences of root extract of *M. longifolia* on increased eosinophils in mice. *p<0.001 when comparison was done with the control.

Acute toxicity determination

No mortality was noticed among mice that were administered with extracts in both plants till the concentration of 2000 mg/kg in the observation time span of 14 days. No behavioral changes, skin reactions, changes in breathing, toxicity signs, etc. observed. So, the Lethal Dosage (LD_{50}) was more than 2000 mg/kg.

In-vivo experiment for the anti-asthmatic potential of plants

In this assay, the count of leukocytes and eosinophils was increased in a significant manner (p<0.001) in the albino mice by the injection (subcutaneous) of 4 mL/kg milk allergen after 24 hr. The increased count was decreased in a significant way (p<0.05) by the administration (intraperitoneal) of extracts at 100-200 mg/kg in the case of both plants, as when compared to the standard dexamethasone (reference drug). In the experiment, the count reduction by the plant extracts was dose-dependent. In the case of *L. obovata*, the increased leukocytes count (2475±148.18) by the milk allergen was significantly (p<0.05) reduced to (1280±133.29) and the increased eosinophil count (76.83±3.13) was also significantly (p<0.001) reduced to (51.33±2.27) by the extracts at a higher concentration of 200 mg/kg when compared to their reference drug as presented in Figure 3 and 4. In *M. longifolia*, increased leukocytes count (2475±148.18) was significantly (p<0.05) reduced to (1216.66±138.24) and increased eosinophil count (76.83±3.13) was also significantly (p<0.001) reduced to (68.66±2.76) by the plant extracts at 200 mg/kg concentration when compared to their reference drug as presented in Figure 5 and 6. Here, when a comparison was made between these two plants, in the case of increased leukocytes count, *M. longifolia* extracts showed better results to reduce the increased count, and in the case of increased eosinophil count, *L. obovata* showed better results in reducing the increased count.

DISCUSSION

Medicinal plants have different types of secondary phytoconstituents which possess various properties to treat and cure diseases. Plant secondary metabolites like flavonoids, phenolic compounds, alkaloids, terpenoids, and saponins help in the treatment of asthma. Secondary metabolites like glycosides, lactones, polysaccharides, and terpenes have immunomodulatory potential. In asthma and inflammation, the key cytokines are regulated by the potent compounds flavonoids and phenols.[10] In our research work, flavonoids, phenolic compounds, and terpenoids are present in both plants and alkaloids are present in *M. longifolia* extracts as pointed out by the preliminary phytochemical analysis. The active phytoconstituents having anti-asthmatic properties were identified in the whole plant extract of *L. obovata* and root extract of *M. longifolia* by the

GC-MS screening. The anti-asthmatic potential of plants *L. obovata* and *M. longifolia* was determined by using the mice model and a comparison was made with the reference drug in both plants.

The term eosinophilia is related to many respiratory problems, one of which is asthma. In the eosinophilia condition, eosinophils increase irregularly (more than 4%) out of the total leukocyte number. Eosinophilia is responsible for the shed off of the epithelial layer of airways, constriction of bronchi, and further spread of inflammation in the respiratory tract.[43] Eosinophil count in the blood can be used as a prognostic biomarker (in the prediction of asthma in advance) but not in the diagnosis as confirmed by the Global Initiative for Asthma. Leukocytosis (high white cell count of blood than normal) is a common characteristic of asthma. A study was conducted by the authors to know the anomalies in the hematological profile of patients suffering from asthma, in which they found out that the White blood cell count was higher when compared with healthy control.[9] In asthma, the severity and seriousness of impaired lung function are directly linked with leukocyte and eosinophil count in the blood. Thus, the blood count of leukocytes and eosinophils can be used as a marker for the severity of the impaired function of the lungs.[6] It is reported that the leukocytes and eosinophils count will increase after 24 hr when the milk allergen is parenterally administered (subcutaneously) in albino mice. This traumatic state can be normalized by the use of adaptogens or drugs that reduce stress. Besides leukocytosis and eosinophilia, inflammatory mediators such as histamine, cytokines, etc. promote ongoing inflammatory conditions.[43]

The present research work disclosed that both plants *L. obovata* and *M. longifolia* possess good anti-asthmatic potential, as this potential of both plants is also traditionally claimed. To the best of my information gathered, there is no published research work on the anti-asthmatic potential of *L. obovata*. Though the antiallergic activity of *M. longifolia* is mentioned in the literature, but not been experimentally proven as per my knowledge. There is also no published work on screening for the secondary active phytochemicals of *L. obovata* and roots of *M. longifolia* by the GC-MS method. Here, the research work disclosed that the secondary phytoconstituents like 1-Monolinoleoylglycerol trimethylsilyl ether in the *L. obovata* extracts and Octadec-9-enoic acid and Cis-vaccenic acid in both *L. obovata* and *M. longifolia* extracts have anti-asthmatic properties as confirmed through the GC-MS study. Therefore, it was appropriate to explore the traditionally claimed anti-asthmatic properties of these plants in an animal model.

CONCLUSION

Some active phytoconstituents with anti-asthmatic properties were identified in the whole plant extracts of *L. obovata* and *M. longifolia* root with by GC-MS, then further by the experiments performed, it justifies that both the plants were traditionally used for the cure of asthma disorder. These plants significantly reduced the elevated count of leukocytes and eosinophils in the mice model. No work has been done on these plants for their anti-asthmatic potential, so there was a need to explore these plants. A novel herbal anti-asthmatic drug can be prepared from these plants bypassing the side effects of steroid-containing allopathic drugs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GC-MS: Gas Chromatography-Mass Spectrometry; ***L. obovata*:** *Lonicera obovata*; ***M. longifolia:*** *Morina longifolia*; **Th2:** T-helper 2 cells; **IL:** Interleukin; **IgE:** Immunoglobulin E; **B. S. I.:** Botanical Survey of India; **RT:** Retention Time; **CAS:** Chemical Abstracts Service; **NIST:** National Institute of Standards and Technology; **IAEC:** Institutional Animal Ethical Committee; **OECD:** Organization of Economic Co-operation and Development; **ANOVA:** Analysis of Variance; **LD:** Lethal Dose; **CSIR:** Council of Scientific and Industrial Research.

SUMMARY

Methanolic whole plant extract of *L. obovata* and methanolic root extract of *M. longifolia* were prepared and analyzed by qualitative phytochemical screening method and Gas Chromatography-Mass Spectrometry method. Then, the anti-asthmatic potential of both plants was determined and compared on the mice model by the assay of milk-induced leukocytosis and eosinophilia. Through this research work, it is found that there are 16 active phytoconstituents identified in *L. obovata* and 13 phytoconstituents identified in *M. longifolia*. Both plants show a significant reduction in the elevated count of leukocytes and eosinophils induced by the milk, thus showing anti-asthmatic properties.

REFERENCES

1. Shahzad N, Alzahrani AR, Ibrahim IAA, Soni K, Shahid I, Alsanosi SM, *et al. In vivo* pharmacological testing of herbal drugs for anti-allergic and anti-asthmatic properties. J Pharm Bioallied Sci. 2021;13(4):380-6. doi: 10.4103/jpbs.jpbs_454_21, PMID 35399803.
2. Hammad H, Lambrecht BN. The basic immunology of asthma. Cell. 2021;184(6):1469-85. doi: 10.1016/j.cell.2021.02.016, PMID 33711259.
3. Kim H, Ahn YT, Kim YS, Cho SI, An WG. Antiasthmatic effects of *Schizandrae fructus* extract in mice with asthma. Pharmacogn Mag. 2014;10(Suppl 1):S80-5. doi: 10.4103/0973-1296.127348, PMID 24914313.

4. Oghale OU, Idu M. Phytochemistry, anti-asthmatic and antioxidant activities of *Anchomanes difformis* (Blume) Engl. leaf extract. Asian Pac J Trop Biomed. 2016;6(3):225-31. doi: 10.1016/j.apjtb.2015.12.007.
5. Dharmage SC, Perret JL, Custovic A. Epidemiology of asthma in children and adults. Front Pediatr. 2019;7:246. doi: 10.3389/fped.2019.00246, PMID 31275909.
6. Ninave PB, Patil SD. Antiasthmatic potential of *Zizyphus jujuba* Mill and Jujuboside B. Possible role in the treatment of asthma. Respir Physiol Neurobiol. 2019;260:28-36. doi: 10.1016/j.resp.2018.12.001, PMID 30521862.
7. Khazdair MR, Anaeigoudari A, Kianmehr M. Anti-asthmatic effects of *Portulaca oleracea* and its constituents, a review. J Pharmacopuncture. 2019;22(3):122-30. doi: 10.3831/KPI.2019.22.016, PMID 31673441.
8. Hall S, Agrawal DK. Key mediators in the immunopathogenesis of allergic asthma. Int Immunopharmacol. 2014;23(1):316-29. doi: 10.1016/j.intimp.2014.05.034, PMID 24933589.
9. Solomon Y, Woldu B, Mesfin N, Enawgaw B. Selected hematological abnormalities and their associated factors among asthmatic patients in Northwest Ethiopia: A cross-sectional study. BMC Pulm Med. 2022;22(1):228. doi: 10.1186/s12890-022-02020-z, PMID 35698065.
10. Gandhi GR, Leão GCS, Calisto VKDS, Vasconcelos ABS, Almeida MLD, Quintans JSS, *et al*. Modulation of interleukin expression by medicinal plants and their secondary metabolites: A systematic review on anti-asthmatic and immunopharmacological mechanisms. Phytomedicine. 2020;70:153229. doi: 10.1016/j.phymed.2020.153229, PMID 32361292.
11. Leal LKAM, Silva AH, Viana GSdB. *Justicia pectoralis*, a coumarin medicinal plant have potential for the development of antiasthmatic drugs? Rev Bras Farmacogn. 2017;27(6):794-802. doi: 10.1016/j.bjp.2017.09.005.
12. Indiabiodiversity.org. Biodiversity informatics platform. v4.2.1; updated 2022 Dec 10. Available from: https://indiabiodiversity.org/species/show/230307.
13. Devi G. Ethno-medicinal plants of tribal District Kinnaur, Himachal Pradesh. Botany D. Chandigarh, India: Faculty of Science, Panjab University; 2015.
14. Kumar A, Varshney VK, Prasad R, Rawat MSM, Stashenko EE. *In vitro* antioxidant, antifungal and antibacterial activities of essential oil of *Morina longifolia* Wall. leaves. J Biol Act Prod Nat. 2013;3(3):183-93. doi: 10.1080/22311866.2013.833384.
15. Joshi RK. Genus Morina: Valuable aromatic plants for herbal drug. VRI-PM. 2013;1(2):81-4. doi: 10.14259/pm.v1i2.34.
16. Yousuf S, Morenikeji O, Kutchy NA, Singh AP. Elucidating pharmacological activities of volatile chemotypes in essential oil of *Morina longifolia*. FASEB J. 2022;36(S1). doi: 10.1096/fasebj.2022.36.S1.R6083.
17. Gairola S, Sharma J, Bedi YS. A cross-cultural analysis of Jammu, Kashmir and Ladakh (India) medicinal plant use. J Ethnopharmacol. 2014;155(2):925-86. doi: 10.1016/j.jep.2014.06.029, PMID 24952280.
18. Roy A, Bharadvaja N. Qualitative analysis of phytocompounds and synthesis of silver nanoparticles from *Centella asiatica*. ITAG. 2017;1(2):88-95.
19. Lalitha P, Sripathi SK, Jayanthi P. Acute toxicity study of extracts of *Eichhornia crassipes* (Mart.) Solms. Asian J Pharm Clin Res. 2012;5(4):59-61.
20. Taur DJ, Patil RY. Evaluation of antiasthmatic activity of *Clitoria ternatea* L. roots. J Ethnopharmacol. 2011;136(2):374-6. doi: 10.1016/j.jep.2011.04.064, PMID 21575696.
21. Nandagoapalan V, Doss A, Marimuthu C. Phytochemical analysis of some traditional medicinal plants. Bio Disc. 2016;7(1):17-20.
22. Chanda S, Ramachandra TV. A review on some Therapeutic aspects of Phytochemicals present in Medicinal plants. Int J Pharm Life Sci. 2019;10(1).
23. Ishak AH, Shafie NH, Esa NM, Bahari H, Ismail A. From weed to medicinal plant: Antioxidant capacities and phytochemicals of various extracts of *Mikania micrantha*. Int J Agric Biol. 2018;20(3):561-8. doi: 10.17957/IJAB/15.0522.
24. Rubab M, Chellia R, Saravanakumar K, Mandava S, Khan I, Tango CN, *et al*. Preservative effect of Chinese cabbage (*Brassica rapa* subsp. pekinensis) extract on their molecular docking, antioxidant and antimicrobial properties. PLOS ONE. 2018;13(10):e0203306. doi: 10.1371/journal.pone.0203306, PMID 30281596.
25. Thangaraj M, Saravana BP, Thanasekaran J, Joen-Rong S, Manubolu M, Pathakoti K. Phytochemicals of algae, *Arthospira platensis* (Spirulina) *Chlorella vulgaris* (chlorella) and *Azolla pinnata* (azolla). GSC Biol Pharm Sci. 2022;19(2):023-43. doi: 10.30574/gscbps.2022.19.2.0167.
26. Kadhim MJ. *In vitro* antifungal potential of *Acinetobacter baumannii* and determination of its chemical composition by gas chromatography-mass spectrometry. Pharm Chem. 2016;8(19):657-65.
27. Tyagi T, Agarwal M. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. J Pharmacogn Phytochem. 2017;6(1):195-206.
28. Pereira L, B. N. Biologically active components for cosmeceutical use extracted from *Chaetomorpha aerea*. Tradit Med Res. 2022;7(4):35. doi: 10.53388/TMR20220326002.
29. Prabha S, Kumar J. Gas chromatographic and Mass Spectroscopic (GC-MS) analysis of rhizome of *Acorus calamus* Linn. for identification of potent antimicrobial bio-active compounds. J Sci Res. 2021;13(1):263-73. doi: 10.3329/jsr.v13i1.48452.
30. Anyasor GN, Funmilayo O, Odutola O, Olugbenga A, Oboutor EM. Chemical constituents in n-butanol fractions of Castus afer ker Gawl leaf and stem. J Intercult Ethnopharmacol. 2014;3(2):78-84. doi: 10.5455/jice.20140112010648, PMID 26401352.
31. Sivakumaran G, Prabhu K, Rao MR, Jones S, Sundaram RL, Ulhas VR, *et al*. Gas chromatography-mass spectrometry analysis of one Ayurvedic oil, Ksheerabala Thailam. Drug Invent Today. 2019;11:2661-5.
32. Ahuchaogu AA, Igara CE. Determination of bioactive components of *Prunus avium* L. leaf. J Pharmacogn Phytochem. 2019;8(4):2668-79.
33. Al-Rubaye AF, Kadhim MJ, Hameed IH. Characterization of antifungal secondary metabolites produced by *Klebsiella pneumoniae* and screening of its chemical compounds using GC-MS. Int J Curr Pharm Rev Res. 2017;8(2):141-8. doi: 10.25258/ijcprr.v8i02.9198.
34. Korinek M, Tsai YH, El-Shazly M, Lai KH, Backlund A, Wu SF, *et al*. Anti-allergic hydroxy fatty acids from *Typhonium blumei* explored through ChemGPS-NP. Front Pharmacol. 2017;8:356. doi: 10.3389/fphar.2017.00356, PMID 28674495.
35. Qadir A, Aqil M, Ali A, Ahmad FJ, Ahmad S, Arif M, *et al*. GC-MS analysis of the methanolic extracts of *Smilax china* and *Salix alba* and their antioxidant activity. Turk J Chem. 2020;44(2):352-63. doi: 10.3906/kim-1907-5, PMID 33488162.
36. Panda P, Dash P, Ghosh G. Chemometric profile, antioxidant and tyrosinase inhibitory activity of Camel's foot creeper leaves (*Bauhinia vahlii*). Nat Prod Res. 2018;32(5):596-9. doi: 10.1080/14786419.2017.1326487, PMID 28508671.
37. Huda JAT, Imad HH, Salah AI, Mohammed YH. Biochemical analysis of *Origanum vulgare* seeds by fourier-transform infrared (FTIR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). J Pharmacogn Phytother. 2015;7(9):221-37. doi: 10.5897/JPP2015.0362.
38. Alaribe CS, Oladipupo AR, Ojo-Nosakhare O, Kehinde O, Ogunlaja AS. GC-MS analysis and mitochondrial Functionality Potential of the Fruits of *Tetrapleura tetraptera* by Cupric Reducing Antioxidant Capacity Assay. J Phytomed Ther. 2020;19(1):338-47. doi: 10.4314/jopat.v19i1.2.
39. Verma R, Tapwalb A, Kumara D, Puria S. Phytochemical profiling and biological activity of *Leucas lanata* Benth. an important ethnomedicinal plant of Western Himalaya. Ecol Environ Conserv. 2020;26:169-75.
40. Rao MR, Anisha G, Prabhu K, Shil S, Vijayalakshmi N. Preliminary phytochemical and gas chromatography-mass spectrometry study of one medicinal plant *Carissa carandas*. Drug Invent Today. 2019;12(7):1629-30.
41. Al Bratty M, Makeen HA, Alhazmi HA, Syame SM, Abdalla AN, Homeida HE, *et al*. Phytochemical, cytotoxic, and antimicrobial evaluation of the fruits of miswak plant, *Salvadora persica* L. J Chem. 2020;2020:1-11. doi: 10.1155/2020/4521951.
42. Ramya B, Malarvili T, Velavan S. GC-MS analysis of bioactive compounds in *Bryonopsis laciniosa* fruit extract. Int J Pharm Sci Res. 2015;6(8):3375. doi: 10.13040/IJPSR.0975-8232.6(8).3375-79.
43. Vikhe S, Nirmal S. Antiallergic and antihistaminic actions of *Ceasalpinia bonducella* seeds: Possible role in treatment of asthma. J Ethnopharmacol. 2018;216:251-8. doi: 10.1016/j.jep.2017.12.007, PMID 29247697

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