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IN VITRO-INVESTIGATION OF ANTI-OXIDANT AND ANTI ASTHMATIC ACTIVITY OF POLYHERBAL INDIAN MEDCINAL PLANTS

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Abstract:
Objective: For the purpose to determine the existence of a variety of secondary metabolites, the phytochemical profile of the extracts will be evaluated.
Material & methods: Medicinal plant that has anti-asthmatic properties through the utilisation of an in vitro model, *M. Viminalis* (Leaves) and Ruta graveolens (Shoot) were able to inhibit histamine-induced contractions in isolated goat tracheal chain preparations.
Result & Discussion: It was discovered that the methanolic extract of M. *viminalis* (leaves) and Ruta graveolens has anti-inflammatory and antibacterial effects (Shoot). performs exceptionally well in comparison to ethanolic extracts of M. viminalis (leaves) and Ruta graveolens in terms of its ability to inhibit the contraction that is brought on by histamine in these tissue preparations (Shoot). Histamine was administered in several dosages of 30 μg/ml during the DRC procedure. All of the extracts of *M. Viminalis* (Leaves) and Ruta graveolens (Shoot) were prepared using an in vitro isolated goat trachea chain.
Conclusion: Drugs that treat asthma have been produced from medicinal plants that are indigenous to regions that are not in the Western hemisphere. The tracheal chain of a goat is more sensitive than the tracheal chain of a guinea pig. Additionally, the tracheal chain of a goat is easier to handle and prepare than the tracheal chain of a guinea pig. It is possible to discover H1 receptors, in addition to M3 and B2 receptors, hidden within the tracheal muscle of a goat. The smooth muscle of the bronchi contracted as a consequence of the activation of H1 receptors, which caused the bronchi to shorten. During the course of the present investigation, the antiasthmatic effect of the methanolic extract of *M. viminalis* (leaves) and R. graveolens (shoots) was demonstrated by a shift to the right in the histamine dose response curve (DRC).
Keywords: In-Vitro study, Poly herbal, Phyto chemical screening

Introduction

In asthma, the severity of the condition can range from a slight wheeze that happens only sometimes to a severe obstruction of the airway that arises suddenly and poses a significant risk to the patient's life. This condition manifests itself extremely early in a person's life and is linked to other atopic symptoms like eczema and hay fever[1][2][3]. It is a condition that affects the skin. Asthma in children is a significant public health problem that frequently leads to hospitalisations and bears a large price tag for the essential medical care necessary to treat the condition. The hyperresponsiveness of the airways is the defining characteristic of this disorder, and it can be brought on by a wide range of the situations that are around the individual. There is a substantial mortality rate associated with asthma, and this is true even if treatment for the condition is delayed for a short length of time[4]. When it is in its chronic state, asthma is one of the conditions that is among the most challenging to manage because of its severity. It is estimated that there are 17 million cases of asthma in the United States alone, which is a remarkable increase in prevalence of 75% over the course of the past two decades. This is a significant increase. Approximately one in twenty persons and approximately one in thirteen children are affected by asthma in today's current population. Since 1980, there has been a dramatic rise in the number of instances of asthma that have been detected in children who are younger than 5 years old. The current trend is really concerning. There has been a seventy-five percent rise in the number of childhood asthma cases among children of school age. It is

estimated that somewhere between 15 million and 20 million people in India suffer with asthma. Other estimates place the number somewhere in the middle. It is possible that the number of deaths that occur per 100,000 people in that age bracket could range anywhere from 0.1 to 0.8, as indicated by the data that was collected on mortality rates in developed nations. When treating asthma attacks, the lowering of asthma symptoms should be your primary focus while you are treating asthma episodes. When it comes to the treatment of asthma, traditional Indian medicinal practises such as Ayurveda, Siddha, and Unani all prescribe the utilisation of a wide range of herbs. Both preventative and therapeutic methods are included in these customs and practices. It is possible for the leaves of M. viminalis, which are members of the Myrtaceae family, to be suspended from the branches of this little tree or shrub in a number of different ways or configurations. In spite of the fact that its popular name is used by a large number of people, the breathtaking plant that is commonly referred to as bottlebrush is actually only native to the most arid and coldest regions. In addition to this, it is frequently found in open areas such as parks and botanical gardens [1-3]. It is possible to use the leaves of the M. viminalis plant as a substitute for tea. These leaves have a flavour and aroma that are comparable to which of mint. For culinary purposes, these leaves can also be utilised. Multiple components of *M. viminalis* have been shown to exhibit antibacterial and antihelminthic activity [8]. These activities have been observed in a number of different components. It has been used as a hot beverage, which is referred to as "tea" in the area, and it has been demonstrated to be useful in treating stomach disorders, diarrhoea, and eczema [9]. As a part of our larger inquiry into the compositional, nutritional, and antioxidant potential of the medicinal flora of Pakistan [10–16], we conducted research into the antioxidant capacity and oil content of the *M. viminalis* plant. This research was carried out as part of our investigation. Within the scope of our investigation, we discovered that the *M. viminalis* plant possesses a substantial quantity of phenolic chemicals, which are recognised for their capacity to reduce inflammation.

Plant Materials

Melaleuca Viminalis (Leaves)* and *Ruta graveolens (Shoot)

DNA extraction and analysis from plant tissue samples collected for analysis. A generous donation of *M. viminalis* (leaves) and R. graveolens (stems) was made by the Central Nursery of the Forest Research Institute in Dehradun, India.

Fig 1: ***Melaleuca viminalis***

Fig 2: ***Ruta graveolens***

Preparation of extract:

During the process of screening and identifying phytochemical components, established techniques were utilized for the chemical analyses that were performed on the powdered specimens and extracts of the medicinal plants. These experiments were carried out in accordance with the established protocols.

Ethanol Extract:

Following the drying of the marc in the air and the subsequent addition of ethanol to a Soxhlet apparatus, it is possible to obtain the extract. For the purpose of conducting additional analysis,

the crude extract that was obtained was stored in a container that was designed to prevent air from escaping and was maintained at a temperature that was lower than 10 degrees Celsius. For the purpose of carrying out the experiment, the extracts were combined with a solution of CMC that contained a concentration of 0.5%.

Preliminary Phytochemical Screening

An investigation into the extract in its natural state, from both a physical and a chemical point of view Through careful examination of the various parts of *M. viminalis* (Leaves) and Ruta graveolens (Shoot), we were able to determine whether or not these two components were located within the sample.

Test for Alkaloids

Following the process of combining very small amounts of dried extract and diluted HCl solution through stirring, the mixture was subsequently filtered. A more in-depth examination revealed that the material that was filtered included indications of the presence of alkaloids.

a. **Mayer's test**: The production of a precipitate that is white in colour occurs as a result of the treatment of alkaloids with Mayer reagents.
b. **Dragendroff' s test**: When alkaloids are put through the Dragendroff test, a precipitate that is orange-yellow in colour is produced.
c. **Wager's test:** Alkaloids form a rusty-brown precipitate when mixed with other chemicals.
d. **Hager's reagent:** If there are alkaloids, a yellow precipitate will form in the Hager test.

Test for Carbohydrates

A minute quantity of extract was dissolved in distiller's water in order to ascertain the amount of carbohydrates present in the mixture. After subjecting the resulting solution to filtration, the component that was left over was subjected to analysis.

a. **Molisch' s test:** When a millilitre of strong sulfuric acid and a few drops of Molisch's reagent were carefully poured down the test tube slides, a ring of reddish-violet colour developed on the filter that had been treated.
b. **Fehling's Test:** When the Fehling reagents are mixed together in quantities that are equivalent to one another, the result is a colour that resembles bricks.

Test of Glycosides

a. **Borntranger's test:** During the process of hydrolysis, a small quantity of the extract was subjected to a water bath containing diluted hydrochloric acid for a period of several hours. There is a reddish brown colour that can be seen at the interface between the two layers, which is an indication that glycosides are present.
b. **Keller – Killiani test:** A millilitre of glacial acetic acid was mixed with a little quantity of the extract after the combination had cooled. Later on, a few drops of ferric chloride were added to the mixture. Following that, the components were blended together and merged. Following this, one millilitre of concentrated hydrogen sulphide was affixed to the side of a test tube, and the contents were transferred into the test tube. Following that, the tube was shook. A coating that, when exposed to light after being left alone for a period of time, changes colour from a reddish-brown to a blue-green hue is one way that glycosides can be recognised from other compounds.

Test for Saponins:

a. **Foam test:** A graded cyclinder was used to shake the mixture for fifteen minutes after twenty millilitres (mL) of distilled water was added to the extract. After that, the mixture

was shaken. The existence of saponins was demonstrated by the production of foams with a thickness of approximately one millimetre.

Test for Phenolic Compounds and tannins

Within a separate container, a portion of the extract and a little amount of water were mixed together. The phenolic chemical test should be investigated, and the results should be found.

Add 10% W/v lead acetate solution: Observed for white color precipitates.

Test for Amino acid and protein:

Following the dissolution of a little amount of extract in a few millilitres of distilled water, the following reagents were added to the mixture in order to complete the process.

a. **Millon's reagents:** The colour red, in its literal sense The precipitate distinctly demonstrates the existence of proteins as well as amino acids.
b. **Ninhydrin reagents:** It is possible to determine whether or not amino acids and proteins are present by searching for the colour purple.

Test for Flavonoids:

a. **Shinoda Test:** Following the dissolution of the extract in ethanol, a little amount of metallic magnesium or zinc was added to the mixture with the intention of enhancing its properties. The addition of a few drops of strong hydrochloric acid resulted in the appearance of a pink hue.
b. **Ammonia Test:** The ethanol had a very small amount of extract added to it, and then the mixture was agitated. It was noted that the colour of a piece of filter paper changed from white to orange as it went through a process in which it was first soaked in an alcoholic solution and then subjected to treatment with ammonia.

Bronchodilator activity

Preparation of isolated goat tracheal chain shows inhibition of histamine-induced contraction. (Test Tube Procedure)

Almost immediately after the goats were slain, their tracheas were removed and placed in separate plastic bags for storage or disposal. The trachea was first chopped into rings, and then those rings were made into a necklace by being strung together. The trachea was kept in a bath of Kreb's solution that was maintained at 370.5 degrees Celsius, and it was accessible to oxygen at all times. For the purpose of determining the histamine dose response curve (DRC), both pure Kreb's solution and Kreb's solution that contained ethanolic extracts of *M. viminalis* (leaves), Ruta graveolens (shoots), and an isolated fraction (F1BP) of the extract were utilised. This was done in order to facilitate a comparison of the two different versions of Kreb's response. When there were no pharmacological extracts present, the DRC of histamine was measured as a percentage of the maximal contractile reaction on the ordinate. Additionally, the concentration of histamine on the abscissa was also recorded.

Statistical analysis

By the utilization of one-way analysis of variance (ANOVA), the groups were compared to the control, and Dunnett's test was utilized to determine whether or not there was statistical significance. It was decided that the threshold for significance was $P>0.001$ as the cutoff point.

Result & Discussion
Preliminary Phytochemical Screening

Table: 1 Phytochemical screening of Leaves of *M. Viminalis (Leaves) Ruta graveolens (Shoot)*.

Phytochemical screening of Leaves of *M. Viminalis (Leaves) Ruta graveolens (Shoot)*.		
Phyto-constituents	**Ethanol**	
Carbohydrates	+	*M. viminalis*
Amino acid	+	
Glycosides	-	
Alkaloids	+++	
Flavonoids	++	
Tannins	+	
Saponins	+++	
Steroids	+++	
Resins	-	
Carbohydrates	+	*Ruta graveolens*
Amino acid	+	
Glycosides	+++	
Alkaloids	-	
Flavonoids	-	
Tannins	++	
Saponins	+	
Steroids	+	
Resins	-	

+++ Most present, ++ Less present, + present, - Absent

All extracts of *M. viminalis* (leaves) and *R. graveolens* were tested in a preliminary animal study using an in vitro isolated goat trachea chain preparation.

The leaves of *M. viminalis* and the shoots of Ruta graveolens were extracted with methanol, and the results showed that the extract possessed anti-inflammatory and antibacterial properties. demonstrates a favourable comparison to ethanolic extracts of *M. viminalis* (leaf) and Ruta graveolens (flower) in terms of its ability to counteract the contraction that is caused by histamine in these tissue preparations (Shoot). In the Democratic Republic of the Congo, multiple administrations of histamine at a concentration of 30 g/ml were utilised. The relationship between the maximal contractile response and the negative log molar concentration of histamine (30 g/ml) shows that the action of histamine on contraction is reduced with increasing dosage. Using a 200 g/ml concentration of a methanolic extract of *M. viminalis* (leaves) and *Ruta graveolens* (shoots), the contraction of a goat tracheal chain preparation was significantly reduced ($p>0.001$).

Table: 2 All extracts of M. viminalis (leaves) and R. graveolens were tested in a preliminary animal research using an in vitro isolated goat trachea chain preparation (Shoot).

Dose of histamine 30 µg/ml	% Maximum contraction (mean ± SEM)				
	Control group	M.V	R.G	F1BP	Standard
0.1	17.8±0.90	10.78±1.32	12.56±1.45	12.46±0.61	9.21±0.52
0.2	41.03±1.69	26.96±1.32	26.78±1.62	24.14±2.19	21.19±0.74
0.4	32.51±1.28	38.49±1.25	35.64±1.21	46.62±1.56	34.87±0.54
0.8	73.45±0.89	46.21±1.40	40.14±1.58	59.23±1.32	42.18±1.14

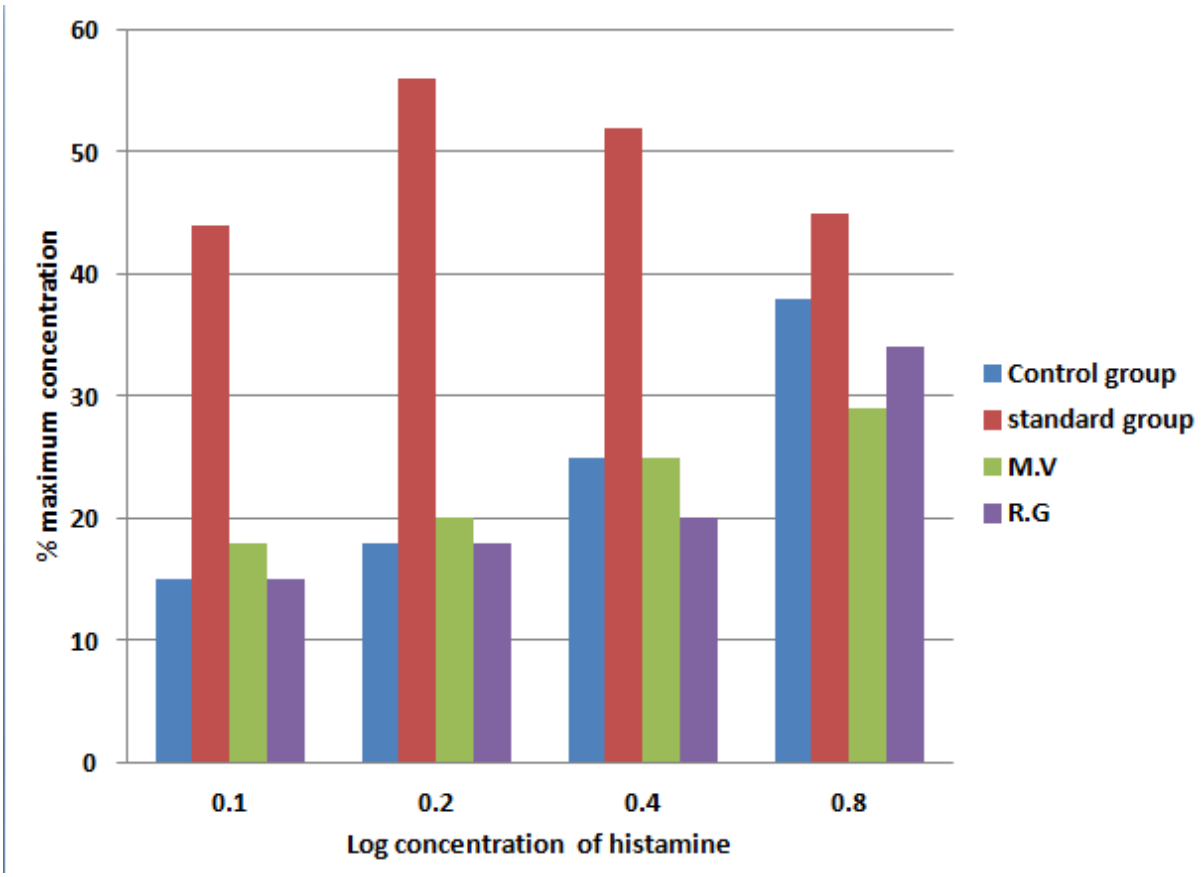

Fig 3. *M. viminalis* (leaf) and *R. graveolens* (flower) extracts were prepared in vitro using isolated goat trachea chains.

Conclusion

Therapeutic herbal treatments for asthma have a lot of potential and an exciting future ahead of them. Inhaled corticosteroids, phosphodiesterase inhibitors, leukotriene modifiers, and 2-adrenoceptor agonists are the components of asthma treatment that are currently considered to be the most essential. In certain clinical settings, the effectiveness of currently available medications has been established. It is vital to develop new drugs and treatment methods because existing ones have limits, such as the inability to treat all instances of a disease or the presence of undesired side effects. Ingestion of herbal medicines is yet another strategy that can be utilised for the treatment and management of asthma. Ayurvedic medicine, traditional Chinese medicine, and the Unani medical system have all documented these practices since ancient times. Traditional Chinese medicine is located in China. New asthma remedies have been developed with the use of medicinal herbs that are native to regions of the world that are not the Western world. The tracheal chains of goats are slightly more fragile than the tracheal chains of guinea pigs, and they are also much simpler to handle and prepare for surgical procedures. When examining the tracheal muscle of a goat, it is feasible to locate H1 receptors, M3 receptors, and B2 receptors. The stimulation of H1 receptors was responsible for the contraction of the smooth muscle of the bronchial passages. In order to reach the conclusion that a methanolic extract of M. viminalis (leaves) and R. graveolens (shoots) have antiasthma qualities (DRC), a rightward shift in the histamine dose response curve was utilized.

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