

# Development of a Polyherbal Formulation for Asthma Management: Phytochemical Characterization and Pharmacological Investigation

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## ABSTRACT

One of the world health issues is respiratory diseases, including asthma and allergic airway inflammation, which are associated with a narrowing of the airways, inflammation, and immune malfunction. The aim of the current research was to examine the phytochemical, antiasthmatics, and immunomodulatory properties of the specific medicinal plant extracts and to generate a standardized polyherbal extract as a tool of controlling respiratory disorders. The phytochemical and pharmacological analysis of Ethanolic extracts of *Terminalia chebula*, *Vitex negundo*, *Curcuma longa* and *Aegle marmelos* was done.

The in-vivo antiasthmatics activity was measured by the means of haloperidol and clonidine induced catalepsy, milk induced eosinophilia and leukocytosis models and in-vitro measurement was done by goat tracheal chain preparation. Carbon clearance test, delayed-type hypersensitivity response, NBT reduction test, and neutrophil candidacidal assay were the methods used to determine immunomodulatory activity. The formulated polyherbal tablet was tested in terms of physicochemical properties and stability.

Findings showed that there was a profound decrease in catalepsy, eosinophil count, leukocyte count, and histamine induced bronchoconstriction in the treatment groups. The polyherbal preparation was more active than single extracts, and the effect was equal in comparison to standard medications. Immunomodulatory researches showed increased phagocytic activity and immune response. The formulation was also found to have acceptable physicochemical properties and stability. The paper concludes that the designed polyherbal formula has a strong antiasthmatic and immunomodulatory activity and could become an excellent natural treatment option in the respiratory diseases.

**Keywords:** Polyherbal formulation, Antiasthmatic activity, Immunomodulatory activity, Medicinal plants, Respiratory disorders

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## INTRODUCTION

Breathing diseases like asthma, chronic bronchitis, and inflammatory diseases of the airways are among the greatest health issues of millions of people worldwide [1]. These disorders have inflammation of the airways, bronchoconstriction, excessive secretion of mucus, and oxidative stress that collectively worsen pulmonary functioning and lower the quality of life [2]. It is believed that the increased frequency of respiratory disorders is caused by environmental pollution, allergens, smoking and genetic

predisposition [3]. Even though the traditional therapies such as bronchodilators and corticosteroids are effective, their chronic usage is commonly linked to any unwanted side effects foaming a desire to find safer and more efficient methods [4].

Oxidative stress is a major pathogenic agent of asthma and respiratory diseases of inflammatory nature. Overproduction of reactive oxygen species results in tissue injury and the activation of inflammatory mediators deteriorating airway hyperresponsiveness and inflammation [5]. Medical plants can be used as antioxidants to counter the effects of free radicals and

minimize oxidative stress, which offers therapeutic efficiency in respiratory illnesses [6]. Phenolic, flavonoid, tannin, and alkaloid medicinal plants have shown great anti-inflammatory, antihistaminic and bronchodilatory effects [7].

Plant-based medicine has a long history of respiratory and inflammatory treatment with traditional medicine [8]. The well-known ones amongst them are *Terminalia chebula*, *Vitex negundo*, *Curcuma longa* and *Aegle marmelos* which have antioxidant, anti-inflammatory, and immunomodulatory effects [9]. Bioactive compounds that include curcumin, gallic acid, flavonoids and terpenoids help attribute to their therapeutic promise in asthma as well as allergic disorders [10]. These medicinal plants should be scientifically verified by using contemporary observations of pharmacology and analytical methods to determine their effectiveness and safety [11].

Polyherbal preparations provide synergies in the effects of therapy as a result of the combination of various medicinal plants with divergent pathological pathways of respiratory diseases [12]. The formulations can be used to improve efficacy, toxicity, and patient compliance over single-herb therapy [13]. Phytochemical and chromatographic methods of standardization including UV spectroscopy, FTIR, TLC, HPTLC, and GC-MS are important in guaranteeing quality and consistency of herbal preparations [14]. Moreover, the scientific evidence of therapeutic potential is given by pharmacological assessment with antioxidant, antiasthmatics and immunomodulatory models [15].

## 2. MATERIALS AND METHODS

### 2.1 MATERIALS

The study was done using ethanolic extracts of *Terminalia chebula*, *Vitex negundo*, *Curcuma longa* and *Aegle marmelos*. Antioxidant studies were done with methanol, ethanol, distilled water, DPPH (2, 2-diphenyl-1-picrylhydrazyl), sodium nitroprusside, Griess reagent, ammonium molybdate, sulfuric acid, sodium phosphate, hydrogen peroxide, potassium ferricyanide, ferric chloride and ascorbic acid. TLC and HPTLC were carried out on precoated silica gel plates. All solvents and reagents used were of analytical grade.

### 2.2 METHODS

#### 2.2.1 UV-VISIBLE SPECTROSCOPY:

The ethanolic extracts of *Terminalia chebula*, *Vitex negundo*, *Curcuma longa*, and *Aegle marmelos* were subjected to the UV-Visible spectroscopic analysis to determine the major phytochemical groups. The extracts were properly diluted using their respective solvents to form clear solutions and scanned in the wavelength of 190-900 nm using UV-Visible spectrophotometer. The absorbance spectra were

observed and the  $\lambda_{max}$  values were recorded in order to identify the presence of phenolics, flavonoids and alkaloids and other conjugated phytochemicals in the extracts. [15].

#### 2.2.2 FTIR SPECTROSCOPY:

The FTIR spectroscopy method was used to determine functional groups in the ethanolic extracts. The samples were made as per the standard protocols and the spectra were measured in 400-4000  $\text{cm}^{-1}$  using a calibrated FTIR spectrophotometer. Functional group-specific characteristic peaks like hydroxyl group, carbonyl group, aromatic group, aliphatic group and ether group were observed to prove the existence of different phytoconstituents. [16].

#### 2.2.3 THIN LAYER CHROMATOGRAPHY (TLC):

Precoated silica gel plates were used as stationary phase to perform TLC analysis. The plates were preheated and then used. Ethanolic extracts were placed as spots with the help of capillary tubes and developed in appropriate solvent systems in a closed chamber. Plates were then dried and viewed under ultra-violet light and using detecting reagents after development. To obtain preliminary phytochemical profiling of the extracts, the value of the retention factor (Rf) of the separated components was obtained. [17].

#### 2.2.4 HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC):

HPTLC was used to perform a detailed chromatographic finger printing of the extracts. An automated sample applicator was used to apply samples in silica gel HPTLC plates in the form of bands. The optimization of mobile phase was used to develop plates in a twin-trough chamber. Plates were then dried and scanned under densitometer at specific wavelengths after development. Phytochemical profiles were compared by Rf values and peak areas to help in standardisation of extracts. [17].

#### 2.2.5 GC-MS ANALYSIS:

Analysis by GC-MS was done to determine volatile and semi-volatile phytochemical constituents in the ethanolic extracts. Analysis was done on samples through a GC-MS instrument that has a capillary column and carrier gas was helium. The temperature of the oven was set in such a way that it gave time to separate the compounds. Mass spectra were taken in the electron impact ionization mode, and compared to the NIST and Wiley spectral libraries to identify the compounds. [18].

#### 2.2.6 DPPH RADICAL SCAVENGING ASSAY:

The DPPH method was used to identify the free radical scavenging activity of extracts. The extracts

and standard antioxidant were put in a freshly prepared methanolic solution of DPPH and allowed to mix with various concentrations. The mixtures of the reactions were incubated at room temperature in the dark and the absorbance recorded at 517 nm with a UV-Visible spectrophotometer. The antioxidant activity was determined by percentage inhibition of DPPH radicals.

### **2.2.7 NITRIC OXIDE SCAVENGING**

#### **ASSAY:**

The nitric oxide scavenging activity was assessed by the use of sodium nitroprusside as a nitric oxide donor. After incubation, the extracts were incubated with sodium nitroprusside solution and Griess reagent was added. A UV-Visible spectrophotometer was used to measure the resulting chromophore at 550 nm. Nitric oxide scavenging potential of the extracts were determined through percentage inhibition. [20].

### **2.2.8 TOTAL ANTIOXIDANT CAPACITY (PHOSPHOMOLYBDENUM METHOD):**

The phosphomolybdenum reduction method was used to obtain total antioxidant capacity. Raw extracts were diluted with phosphomolybdenum reagent that included sulfuric acid, sodium phosphate, and ammonium molybdate and left to incubate under high temperature. The absorbance at 695 nm was then measured after cooling. Findings were represented in ascorbic acid equivalent that demonstrated total antioxidant capacity. [19].

### **2.2.9 REDUCING POWER ASSAY OF POLYHERBAL FORMULATION:**

It was ascertained that reducing power of the polyherbal formulation was achieved by mixing formulation with phosphate buffer and potassium ferricyanide and then incubating it. The mixture was centrifuged and ferric chloride was added to supernatant. The absorbance was read at 700 nm and absorbance read high meant more reducing power of the formulation. [19].

### **2.2.10 HYDROGEN PEROXIDE SCAVENGING ASSAY:**

Formulation hydrogen peroxide scavenging activity was assessed by combining varying amounts of formulation and hydrogen peroxide solution that was prepared in phosphate buffer. On incubation, UV-Visible spectrophotometer was used to measure absorbance at 230 nm. The activity was calculated as a percentage of scavenging when compared to the control absorbance. [21].

### **2.2.11 IN-VIVO ANTI-ASTHMATIC ACTIVITY STUDIES**

The antiasthmatic effects of the individual plant extracts and the resulting polyherbal formulation were

tested using the known pharmacological models that could mimic the key pathological characteristics of bronchial asthma including the release of histamine, eosinophilia, and inflammatory reactions. The test animals were arranged in control group, standard group and treatment group. The administration was in the form of extracts and polyherbal formulation at the dosage of 200mg/kg body weight, whereas the administration of standard drugs took place at recommended therapeutic doses. The observations were made and analyzed statistically to identify antiasthmatic potential [22,23].

### **2.2.12 HALOPERIDOL-INDUCED CATALEPSY MODEL**

The antihistaminic and antiasthmatic activity was measured by haloperidol induced catalepsy. Animals were also classified into various groups and treated with extracts, polyherbal formulation, or standard antihistaminic drug before the administration of haloperidol. In the case of catalepsy, haloperidol was injected intraperitoneally. The catalepsy period was determined in the bar test at certain time intervals. Decrease in time of cataleptic compared with disease control group showed protective and anti-histaminic action [22].

### **2.2.13 CLONIDINE-INDUCED CATALEPSY MODEL**

The antihistaminic activity that happens via the release of histamine was tested using clonidine-induced catalepsy. The animals were fed on test extracts or polyherbal formulation and then clonidine was administered. Cataleptic behavior was evaluated by the means of bar test and time of immobility was measured. Reduction in cataleptic duration relative to clonidine control group indicated great antihistaminic as well as antiasthmatics effect [22,23].

### **2.2.14 MILK-INDUCED EOSINOPHILIA**

Eosinophilia induced by milk was done in order to determine anti-allergic effects. Extraction and polyherbal formulation were used on the animals and the eosinophilia induced by milk subcutaneous injection. Blood samples were taken prior to and at induction and the eosinophil count was done on a hemocytometer. The decrease in the number of eosinophils in relation to the milk control group showed anti-allergic and antiasthmatics [23,24].

### **2.2.15 MILK-INDUCED LEUKOCYTOSIS**

Anti-inflammatory activity was assessed using milk induced leukocytosis model. Milk subcutaneously injected induced leukocytosis. Extracts or polyherbal formulation were administered using test groups before induction. Sampling of blood was done at a set time interval, and total leukocyte count was counted. The decrease in the number of leukocytes relative to control signified anti-inflammatory effect [24].

### 2.2.16 IN-VITRO ANTI-ASTHMATIC ACTIVITY

Goat tracheal chain preparation was used to test the in-vitro antiasthmatics of extracts and polyherbal formulation. Fresh goat trachea was bought and stored in chilled physiological saline. Preparation Tracheal rings were hooked up to an organ bath filled with Krebs-Henseleit solution at 37 °C and shaken with oxygen. Upon equilibration, histamine was put in to cause contraction. Standard drug and test samples were then added and relaxation responses taken. Percentage inhibition of contraction was determined [22,25].

### 2.2.17 CARBON CLEARANCE TEST

Carbon clearance test was also done to determine the immunomodulatory activity through measuring phagocytic activity of the reticuloendothelial system. Extracts and polyherbal formulation were used to treat animals in a predetermined period. Tail vein injections and blood samples were taken at a particular time interval using colloidal carbon ink. UV-visible spectrophotometer was used to measure optical density of blood samples and phagocytic index to calculate it [22,24].

### 2.2.18 DELAYED-TYPE HYPERSENSITIVITY (DTH) RESPONSE

Delayed-type hypersensitivity response was used to measure the cell-mediated immunity. The animals were sensitized using sheep red blood cells (SRBCs) and extract or polyherbal formulation. At the end of the sensitization period, the animals were challenged using SRBCs in the hind paw. A thickness of the paws was measured pre challenge and after challenge by the use of a digital vernier caliper. Growth in the thickness of the paws signaled the stimulation of cell-mediated immunity [23,25].

### 2.2.19 NBT REDUCTION TEST

The neutrophil activation was analyzed by nitroblue tetrazolium (NBT) reduction test. Blood samples were used to isolate polymorphonuclear cells which were then incubated with test extracts or polyherbal formulation. Incubation with NBT solution was done. Smears were made and studied in the microscope. The percentage of NBT-positive cells was calculated to establish the neutrophil activation [24].

### 2.2.20 NEUTROPHIL CANDIDACIDAL ASSAY

The candidacidal activity of neutrophil was evaluated to determine antimicrobial and immunostimulatory ability. Test samples were incubated with isolated neutrophils against *Candida albicans* suspension in the presence or absence of the test samples. Smears were stained after incubation. Killed *Candida* cell percent was calculated to calculate candidacidal activity [24,26].

### 2.2.21 EVALUATION OF POLYHERBAL FORMULATION

Ready-to-use polyherbal tablets/vati were assessed based on their physicochemical characteristics such as weight change, hardness, thickness, friability and disintegration period by standard pharmacopoeial methods. All tests were repeated three times and the results were shown in terms of mean  $\pm$  SD [28,29,30].

### 2.2.22 ACCELERATED STABILITY STUDY

Stability Accelerated stability study was achieved by placing the formulation at 40  $\pm$  2 °C and 75  $\pm$  5% relative humidity over a period of three months. Samples were appraised at specific periods of time in terms of physical appearance, hardness, friability, disintegration time and degradation evidence. Formulation stability was also determined using a comparison to the starting values and interpreted using standard pharmaceuticals and stability principles [28,29].

## RESULT:

Control with haloperidol resulted in a significant effect on cataleptic time as compared to control with a normal condition, which proved successful induction. Each of the individual extracts (200 mg/kg) decreased the catalepsy duration compared with haloperidol control and *Curcuma longa* exhibited the best inhibitory effect among individual extracts. The polyherbal preparation gave better inhibition (59.9%); and the effect was nearly the standard antihistaminic (66.7%).

Values expressed as Mean  $\pm$  SD (n = 6). N.A.: Not applicable.

**Table 5.20 Effect of Extracts and Polyherbal Formulation on Clonidine-Induced Catalepsy**

Treatment Group	Dose (mg/kg)	Duration of Catalepsy (sec)	% Inhibition of Catalepsy
Normal Control (Saline)	—	11.8 ± 1.3	N.A.
Clonidine Control	1.0	112.6 ± 5.8	0
Standard Antihistaminic	10	38.9 ± 3.4	65.5
<i>Terminalia chebula</i> Extract	200	70.4 ± 4.6	37.4
<i>Vitex negundo</i> Extract	200	65.2 ± 4.3	42.1
<i>Curcuma longa</i> Extract	200	62.8 ± 4.0	44.2
<i>Aegle marmelos</i> Extract	200	68.6 ± 4.5	39.1
Polyherbal Formulation	200	48.7 ± 3.7	56.8

**Table 5.21 Effect of Extracts and Polyherbal Formulation on Milk-Induced Eosinophilia**

Treatment Group	Dose (mg/kg)	Eosinophil Count (cells/mm <sup>3</sup> )	% Reduction in Eosinophils
Normal Control	—	92.4 ± 6.1	—
Milk Control	—	312.8 ± 14.5	0
Standard Antiasthmatic	10	118.6 ± 8.3	62.1
<i>Terminalia chebula</i> Extract	200	182.4 ± 10.6	41.7
<i>Vitex negundo</i> Extract	200	168.9 ± 9.8	46.0
<i>Curcuma longa</i> Extract	200	160.7 ± 9.2	48.6
<i>Aegle marmelos</i> Extract	200	174.2 ± 10.1	44.3
Polyherbal Formulation	200	132.5 ± 8.9	57.6

**Table 5.22 Effect of Extracts and Polyherbal Formulation on Histamine-Induced Contraction of Goat Tracheal Chain**

Treatment Group	Concentration (µg/mL)	Tracheal Contraction (%)	% Inhibition of Contraction
Histamine Control	—	100	0
Standard Antihistaminic (Chlorpheniramine / Salbutamol)	10	13.6 ± 2.1	86.4
<i>Terminalia chebula</i> Extract	200	47.7 ± 3.2	52.3
<i>Vitex negundo</i> Extract	200	41.4 ± 3.0	58.6
<i>Curcuma longa</i> Extract	200	38.1 ± 3.1	61.9
<i>Aegle marmelos</i> Extract	200	44.6 ± 2.9	55.4
Polyherbal Formulation	200	25.2 ± 3.4	74.8

**Table 5.23 Effect of Extracts and Polyherbal Formulation on Delayed-Type Hypersensitivity (DTH) Response**

Treatment Group	Dose (mg/kg)	Paw Thickness Increase (mm)	% Increase in DTH Response
Normal Control	—	0.32 ± 0.04	—
Standard Immunostimulant (Levamisole)	10	0.78 ± 0.06	143.8
<i>Terminalia chebula</i> Extract	200	0.52 ± 0.05	62.5
<i>Vitex negundo</i> Extract	200	0.56 ± 0.05	75.0
<i>Curcuma longa</i> Extract	200	0.59 ± 0.06	84.4
<i>Aegle marmelos</i> Extract	200	0.54 ± 0.05	68.8
Polyherbal Formulation	200	0.69 ± 0.06	115.6

**Table 5.24 Accelerated Stability Study of Polyherbal Formulation**  
**Stability Conditions: 40 ± 2 °C / 75 ± 5 % RH**

Parameter	Initial	After 1 Month	After 3 Months	Observation
Physical Appearance	Normal	No change	No change	Stable
Average Weight (mg)	498.6 ± 6.8	497.9 ± 7.1	496.8 ± 7.4	Within limits
Hardness (kg/cm <sup>2</sup> )	4.8 ± 0.4	4.7 ± 0.5	4.6 ± 0.5	Acceptable
Thickness (mm)	4.12 ± 0.09	4.11 ± 0.08	4.10 ± 0.09	Uniform
Friability (%)	0.62	0.65	0.68	< 1%
Disintegration Time (min)	12.4 ± 1.1	12.8 ± 1.2	13.2 ± 1.3	Within limits
Evidence of Degradation	Absent	Absent	Absent	Stable

Control with clonidine greatly enhanced catalepsy duration compared to control. The percent inhibition of standard antihistaminic was 65.5. Moderate inhibition was achieved in all extracts (200 mg/kg) and the polyherbal formulation achieved more inhibition (56.8) that the individual extracts and was very close to the standard drug response.

Values expressed as Mean ± SD (n = 6). N.A.: Not applicable.

The counts of eosinophils were significantly higher with milk control than with normal control, which is an indication of allergic inflammatory induction. The conventional antiasthmatic caused 62.1 percent decrease. All extracts had to decrease the number of eosinophils and the polyherbal formula had to decrease it more significantly (57.6%) than the individual extracts and was close to the effect of the common drug.

**Table 5.25 Effect of Extracts and Polyherbal Formulation on Milk-Induced Leukocytosis**

Treatment Group	Dose (mg/kg)	Total Leukocyte Count (cells/mm <sup>3</sup> )	% Reduction in Leukocytosis
Normal Control	—	6,120 ± 210	—
Milk Control	—	11,860 ± 420	0
Standard Anti-inflammatory Drug	10	6,980 ± 260	41.2
<i>Terminalia chebula</i> Extract	200	8,420 ± 310	28.9
<i>Vitex negundo</i> Extract	200	8,060 ± 295	32.0
<i>Curcuma longa</i> Extract	200	7,820 ± 280	34.1
<i>Aegle marmelos</i> Extract	200	8,210 ± 305	30.8
Polyherbal Formulation	200	7,240 ± 270	38.9

Values expressed as Mean ± SD (n = 6).

A significant increase in total leukocyte count was observed with milk control proving the induction of inflammation. The conventional drug decreased the leukocytosis by 41.2. Extraction of all reduced leukocyte count as compared to milk control and the polyherbal formulation had the highest reduction as compared to the test samples (38.9%), which was close to the standard drug response.

Values expressed as Mean ± SD (n = 6).

## 5.2 IN-VITRO ANTI-ASTHMATIC ACTIVITY (GOAT TRACHEAL CHAIN MODEL)

The relaxation was high with standard antihistaminic (86.4 ± 3.2%). Single extracts were moderately relaxing, with *Curcuma longa* being the most relaxed extract of them. The polyherbal mixture gave more relaxation (74.8 ± 3.4%) as compared to the

individual extracts and was close to the standard response.

Values expressed as Mean ± SD (n = 6).

Histamine caused complete contraction of control. The inhibition of the standard drug was high (86.4%). Extracts of all the extracts moderately inhibited contraction, and polyherbal formulation demonstrated more inhibition (74.8%) compared to that of individual extracts and approached the standard drug activity.

Values expressed as Mean ± SD (n = 6).

## 5.3 IMMUNOMODULATORY ACTIVITY STUDIES

The increase in phagocytic index was the most significant (104.8%), which confirmed the sensitivity of the model. Single extracts resulted in moderate increases and the polyherbal combination resulted in a high increase (85.7%), which was close to the normal immunostimulant effect.

Values expressed as Mean  $\pm$  SD (n = 6).

Maximum increase of paw thickness was observed with levamisole (143.8%), which confirms that the models were sensitive to it. extracts of all types led to modest increase in DTH response and the polyherbal preparation resulted in significant increase in the DTH response (115.6%), which is near the standard immunostimulant response.

Values expressed as Mean  $\pm$  SD (n = 6).

## 5.4 IN-VITRO IMMUNOMODULATORY STUDIES

The basal NBT was positive on control. Levamisole was a powerful activator (62.8  $\pm$  3.1). Every extract showed NBT positivity over and above control and the polyherbal extract showed significant activation (56.4  $\pm$  3.2), which was greater than the individual extracts and nearer to standard drug effect.

Values expressed as Mean  $\pm$  SD (n = 3).

**Table 5.3 Effect of Extracts and Polyherbal Formulation on Neutrophil Candidacidal Activity**

Treatment Group	Concentration ( $\mu$ g/mL)	% Candidacidal Activity	Observation
Control	—	21.6 $\pm$ 2.1	Basal killing activity
Standard (Levamisole)	10	68.2 $\pm$ 3.4	Strong antimicrobial response
<i>Terminalia chebula</i> Extract	200	46.3 $\pm$ 2.9	Moderate killing
<i>Vitex negundo</i> Extract	200	49.8 $\pm$ 3.1	Enhanced killing
<i>Curcuma longa</i> Extract	200	52.6 $\pm$ 3.3	Significant killing
<i>Aegle marmelos</i> Extract	200	47.5 $\pm$ 3.0	Moderate killing
Polyherbal Formulation	200	60.9 $\pm$ 3.5	Strong candidacidal activity

The candidacidal activity of levamisole was the most active (68.2  $\pm$  3.4). Extraction of all extracts raised killing activity relative to control and polyherbal extract formulation showed high candidacidal effect (60.9  $\pm$  3.5), which was more than single extracts and nearly comparable to the standard drug.

Values expressed as Mean  $\pm$  SD (n = 3).

## 5.5 EVALUATION OF POLYHERBAL FORMULATIONS

All tested physical characteristics of polyherbal tablet/vati formulation were within the pharmacopeial ranges. The weight and weight change indicated the same, hardness and thickness ensured that the mechanical integrity was acceptable and that there was uniformity of dimension, friability was less than 1 and disintegration time was within the recommended range.

Values expressed as Mean  $\pm$  SD (n = 6).

The appearance of the formulation remained unchanged and no sign of degradation was detected after 3 months under accelerated storage conditions. There were slight differences in weight, hardness, thickness, friability and disintegration time but this was all acceptable and favorable to physical stability in a stressing situation.

Values expressed as Mean  $\pm$  SD (n = 6).

## DISCUSSION

The current research indicated great anti-asthmatics and immunomodulatory ability of the chosen medicinal plant extracts and the resulting polyherbal extract. Antiasthmatic models in-vivo showed that all the extracts showed significant reduction of the

cataleptic duration, eosinophil count, and leukocyte count in comparison to disease control, which showed antihistaminic, anti-allergic and anti-inflammatory effects. Of the individual extracts, *Curcuma longa* was found to be relatively more active whereas a polyherbal formulation was found to be the most activity and generated effects more similar to the standard drug indicating that there was a synergetic interaction between therapeutic effects of the chosen plants.

Antiasthmatic testing in vitro with the use of goat tracheal chain preparation showed that there was a significant relaxation of tracheal smooth muscle and prevention of histamine induced contraction. Polyherbal preparation showed higher bronchodilatory effect compared to extracts, which enhance its efficacy to inhibit bronchoconstriction and inflammatory airway.

The studies on immunomodulatory showed improved phagocytic index, increased delayed-type hypersensitivity response, and neutrophil functions. Polyherbal formulation exhibited greater immunostimulatory effect than individual extracts, which suggested that there was synergistic immunological stimulation.

## CONCLUSION

The current research was able to establish the potential of the used medicinal plant extracts and polyherbal extract in the treatment of respiratory and inflammatory diseases. Phytochemical and pharmacological studies established that the individual extracts had great antioxidant, antiasthmatic and immunomodulatory effects. Of the samples studied, the synthesized polyherbal

formulation had a better efficacy than the individual plant extracts which implies that there is some synergism between the bioactive constituents interacting with each other.

In-vivo, in-vitro experiments showed a large decrease in catalepsy, eosinophilia, leukocytosis and histamine-induced bronchoconstriction and improvement of immune responses. The resulting polyherbal tablets had acceptable physicochemical characteristics and were stable when subjected to accelerated stability conditions. In general, the results indicate that the formulation of polyherbals that was developed is safe, stable, and therapeutically effective, and it can become one of the promising natural alternatives when managing asthma and other inflammatory conditions.

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