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1. ABSTRACT

Plants have been one of the important sources of medicines since the beginning of human civilization. Medicinal plants play a major role and constitute the backbone of the traditional medicine. According to the WHO survey 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs.

Bronchial asthma is a syndrome, characterized by increased responsiveness of trachea and bronchi to various stimuli and manifested by acute, recurrent and chronic attacks of widespread narrowing of airways. The past decade has witnessed phenomenal increases in the incidences of asthma, asthma-related deaths and hospitalization. Typically, all asthma patients with active disease have hyper responsive (hyper reactive) airways, manifest as an exaggerated bronchoconstrictor response to different stimuli which can be allergenic, environmental, occupational, infections, pharmacological, exercise related and emotional. Asthma is characterized by a complex inflammatory response involving resident cells (e.g. mast cells, macrophages, nerves), recruited cells (e.g. lymphocytes, eosinophils, monocytes) and structural cells (e.g. epithelium, airway smooth muscle, fibroblast). This bronchoconstrictive response associated with acute inflammation results in recurrent episodes of wheezing, dyspnea, and shortness of breath that last for a day or so.

Currently available drugs for the treatment of asthma include $\beta_2$ agonists, anticholinergics, corticosteroids, mast cell stabilizers, phosphodiesterase inhibitors, PAF inhibitors, leukotriene modifiers, TXA$_2$ inhibitors etc. For many patients, these drugs have been helpful in symptomatic relief. But, none of the existing treatment is curative and symptoms return soon after treatment is stopped. Also, it has been reported that prolonged treatment produced various adverse effects. Muscle tremor and hypokalemia are major adverse effects of $\beta_2$ agonists. Inhaled ipratropium bromide had bitter taste and causes dryness of
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mouth and throat, cough and nausea. Adverse effects of corticosteroids include fluid retention, increased cell mass, increased appetite, weight gain, osteoporosis, capillary fragility, hypertension, peptic ulceration, diabetes, cataract, and psychosis. The efficacy of currently used anti-asthmatic drugs is compromised in several ways. Individual oral agents act only on a part of the pathogenic process of bronchial asthma and hence they may not prevent all complications of bronchial asthma. This emphasizes the urgent need for newer and better therapeutic approaches.

Ayurveda is an example of a long standing tradition that offers a unique insight into comprehensive approach to asthma management through proper care of the respiratory tract. Medicinal plants contain a wide range of chemical compounds that could serve as ‘leads’ for the development of novel agents. Large numbers of medicinal plant preparations have been reported to possess anti-asthmatic effects. Majority of herbal products found to be useful in the management of asthma in the experimental studies have yet to undergo clinical trials. In the light of above facts, indepth studies are required to establish and make best use of plant resources for the treatment of bronchial asthma.

Medicinal plants selected for the present investigation are:

1. *Moringa oleifera* Lam. Fam: Moringaceae
2. *Achyranthes aspera* Linn. Fam: Amaranthaceae

*Moringa oleifera* (*M. oleifera*) is a medium sized tree of about 10m high, found wild in the sub-Himalayan tract. It is also cultivated throughout India. It is called Sigru or Sobhanjana in Sanskrit, Soanjna in Hindi, Saragavo in Gujarati, and Drumstick or Horse-radish tree in English. The plant is reported to elicit good clinical response in children suffering from upper respiratory tract infection and skin infection. It has been reported that alkaloid from the plant closely resembles ephedrine in action and useful in treatment of asthma. Alkaloid *Moringine* relaxes bronchioles.
Achyranthes aspera (A. aspera) is an annual, stiff erect herb, about 0.3 to 0.9m high and found commonly as a weed throughout India. It is called Apamarga in Sanskrit, Chirchira, Chirchitta or Latjira in Hindi, Aghedo in Gujarati and Prickly chaff flower in English. It is used for asthmatic cough, snakebite, hydrophobia, urinary calculi, rabies, influenza, piles, bronchitis, diarrhea, renal dropsy, gonorrhea and abdominal pain. A powder of dried leaf mixed with honey is useful in early stages of asthma. The Siddha drug Naayuruvi kuzhi thailum in which A. aspera is a primary constituent reported to be quite effective in the management of asthma. Salt obtained from A. aspera is also reported to be effective in bronchial asthma.

Thus, looking to the pathophysiology of asthma and possible usefulness of these herbs, objectives of our present investigation was to evaluate the clinical efficacy and safety of M. oleifera and A. aspera in asthmatic patients and to evaluate mechanism of action of plant extracts of M. oleifera and A. aspera on various animal models of asthma. In herbal research, it is essential to authenticate the plant and to establish phytochemical standardization. Before undertaking clinical and pharmacological work, we also carried out pharmacognostic and phytochemical standardization of M. oleifera and A. aspera crude extracts.

Seed kernels of M. oleifera were purchased from the local market of Ahmedabad and were identified and authenticated by Dept.of pharmacognosy, L.M. College of Pharmacy, Ahmedabad, India. A voucher specimen was deposited at the Dept. of pharmacognosy, Ahmedabad. The whole herb of A. aspera was uprooted from the L. M. College of Pharmacy campus Ahmedabad, in the month of September-October at the end of flowering season. The plant was identified by comparing it morphologically and microscopically with description given in different standard texts and floras. The plant was further identified and authenticated at the Dept. of pharmacognosy, Gujarat Ayurved University, Jamnagar, India and a voucher specimen was deposited. The plant material of
both the drugs were cleaned and dried in shade. It was powdered, passed through 40# and stored at 25 °C.

Seed kernels of *M. oleifera* and whole plant of *A. aspera* were evaluated for complete pharmacognostical parameters such as macroscopy, microscopy, ash value and extractive values. Seed kernels were buff coloured ovoid and somewhat triangular in shape with one end acutely pointed. Its surface was smooth and showing three prominent longitudinally running furrows. It has no odour and taste is bitter. The T.S. of seed kernel showed outer reticulated parenchymatous cells followed by cotyledon. Cotyledon consisted of epidermis, one layer of palisade cells followed by parenchymatous cells filled with starch grains and oil globules. Procambium was found as thin parenchymatous elongated cells throughout the endosperm. Phloem was found to be developing from this tissue.

*A. aspera* used in the study was an erect herb or under shrub up to 1 m high. Roots were cylindrical taproot, slightly ribbed, yellowish brown in colour, odour, not distinct. Stems were yellowish brown, erect, branched, cylindrical, hairy, solid, hollow when dry. Leaves were simple, subsessile, extipulate, opposite, decussate, wavy margin, obovate, slightly acuminate and pubescent due to the presence of thick coat of long simple hairs. Flowers were greenish white, numerous, in a small dense axillary heads or spikes. Bracts and bracteoles were persisting, ending in a spine. Seeds were sub-cylindric, truncate at the apex, round at the base, black and shining.

The T.S. of young stem showed 6-10 prominent ridges and collenchyma was present under each ridge. The epidermis was single layered, covered with thick cuticle. Trichomes arising from the epidermis were simple, covering, multicellular straight or somewhat spirally running, highly warty. The cortex was composed of 6 to 8 layers of parenchymatous cells containing cluster and rosette crystals of Ca-oxalate. Xylem was composed of annular, spiral and pitted vessel, tracheids,
fibres and parenchyma. The diagrammatic T.S. of the young root showed a layer of epiblema with long unicellular hairs. Cortex was 5-6 layered, parenchymatous and narrow. The stelar region showed anomalous growth. Upper epidermal cells of leaf were more or less straight walled while the lower ones were wavy walled. Both the upper and lower epidermal cells were traversed with anomocytic and few anisocytic stomata. Trichomes were simple, covering, uniseriate, multicellular and many, arising from the lower epidermis. Rosette crystals of Ca-oxalate measuring 20-45 μm diameters were embedded throughout the parenchymatous cells of the mesophylls and the ground tissue of the mid rib.

Powder study of *A. aspera* showed plenty of simple multicellular spiral or straight walled warty trichomes from leaf and stem, fragments of the leaf with wavy epidermal cells and anomocytic and few anisocytic stomata in surface view, rosette, cluster of few prismatic and microsphenoidal crystals of Calcium oxalate, longitudinally cut fragments of xylem showing lignified spiral, pitted, scalariform and annular thickened vessels and parianth in surface view showing parallelly running thin walled narrow elongated parenchymatous cells.

Quantitative limit tests like ash and extractive values were used to standardize both the herbal drugs. *M. oleifera* seed kernels contained 2.04 % of foreign matter, 4.128 % of total ash, 0.52% of acid-insoluble ash, 48.4 % of ethanol soluble extractive and 31.2 % of water soluble extractive. *A. aspera* showed 1.1 % of foreign matter, 12.66 % of total ash, 2.53% of acid-insoluble ash, 8 % of ethanol soluble extractive, and 19.2 % of water soluble extractive. Further, total ash obtained from *A. aspera* was subjected to estimation of Sodium (Na) and Potassium (K) by Flame Photometer. It was found to contain 6% Sodium and 41% K.

Alcoholic extract of *M. oleifera* was prepared by defatting the coarse powder of seed kernels with petrol ether and then extracting with ethanol. In preliminary phytochemical screening, alcoholic extract of *M. oleifera* showed presence of
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alkaloids, flavanoids, glycosides, tannins and terpenoids. Alcoholic extract of *A. aspera* was prepared by extracting the powder of *A. aspera* with alcohol. Phytochemical analysis of alcoholic extract of *A. aspera* showed presence of alkaloids, flavanoids and saponins.

The clinical study was carried out on patients of either sex, in age range of 15-75 years and visiting Out Patient Department of Govt. Ayurvedic Hospital, Ahmedabad, India. The protocol for carrying out the clinical study was approved by Director, Dept of Ayurveda and homeopathic medicine, Govt. of Gujarat, India and also by the institutional ethics committee for the clinical study. Informed consent was obtained from all patients enrolled in the study. Patients having mild to moderate bronchial asthma as diagnosed by their clinical history, commonly observed symptoms of bronchial asthma (Dyspnea, wheezing, tightness in chest, cough etc.) and physical examination were enrolled in the study. Patients having breathlessness due to cardiovascular disorders, having very severe bronchial asthma (PEFR<20%, FEV1<20% of predicted value) or having pulmonary tuberculosis (confirmed by chest screening), cardiovascular disorders etc. were excluded from the study. Of the patients satisfying the inclusion and exclusion criteria, baseline characteristic were measured and clinical and family history was recorded. Details of duration of bronchial asthma and other diseases if present were recorded.

Patients were given finely powdered dried seed kernels of *M. oleifera* in the dose of 3 gm bid or four Ghanvatis of *A. aspera* bid for 3 weeks and were advised to take it with water. Ghanvatis of *A. aspera* were prepared by extracting dried powder of the whole plant of *A. aspera* with water and concentrating the filtered aqueous extract. Tablets of this mass was prepared by mixing small quantity of dry powder of *A. aspera*.

Baseline characteristics like age, height, weight, pulse rate, blood pressure etc. of patients were recorded. Hematological examination and PEFR or spirometric
measurements were carried out before the start of the treatment with *M. oleifera* or *A. aspera* and subsequently at the end of 3 weeks treatment. General physical examination was carried out every week after start of the treatment. The assessment was made at weekly intervals and the results were analyzed in terms of reduction or disappearance of symptoms. Student’s paired t test was applied on the results to evaluate their statistical significance.

Preliminary clinical study of *M. oleifera* was carried out on 25 patients by measuring the Peak expiratory flow rate (PEFR) and recorded with the help of mini Wright’s Peak flow meter. Response to asthma treatment is usually accompanied by an increase in PEFR and a decrease in its variability. Patients enrolled were 16 male and 9 female, with an average of 31.3 ± 2.78 years. Average duration of asthma was found to be 6.66 ± 1.55 years. 32% of patients were found to have extrinsic (allergic) asthma and 68% were found to have intrinsic (nonallergic) type of asthma. Habit of smoking was found to be prevalent to the extent of 71% among asthmatic male patients. The family history of patients for asthma revealed that 20% had such background. The significant increase in PEFR values (42.70 ± 5.51%) were observed between the baseline and after 3 weeks of treatment with *M. oleifera*.

Further, detailed clinical study using spirometry was conducted on 20 patients of either sex satisfying inclusion and exclusion criteria. Patients enrolled were in the age range of 17-70 years. Average duration of asthma was found to be 5.26 ± 1.35 years with a range of 3 months to 20 years. 20% of patients were found to have extrinsic (allergic) asthma and 80% were found to have intrinsic (nonallergic) type of asthma. Also 25% of patients were found to have family history of asthma. Habit of smoking was found to be prevalent to the extent of 86% among asthmatic male patients. In the present study, *M. oleifera* was found to significantly reduced symptom score of the commonly observed symptoms of bronchial asthma like dyspnoea, wheezing, chest tightness and cough.
Pulmonary-function tests are useful in diagnosing and measuring the effect of drug in the treatment of pulmonary diseases. Spirometry tests before and after 3 weeks treatment with *M. oleifera* revealed highly significant increase in Forced Vital Capacity (FVC). FVC before treatment was 1.942 ± 0.241 lit. After treatment, it was found to be 2.365 ± 0.220 lit with a mean % increase was 32.97 ± 6.03%. Out of total 20 patients, 25% of patients were having predicted values of FVC above 80% before treatment with *M. oleifera*. After treatment, 40% of patients were found to have predicted values of FVC above 80%. Out of total 20 patients, 45% of patients were having FVC less than 60% of predicted values before treatment with *M. oleifera*. After treatment, only 30% of patients were found to have FVC less than 60% of predicted values. *M. oleifera* also caused highly significant increase in Forced Expiratory Volume in 1 sec (FEV1) with a mean % increase of 30.05 ± 8.12%. Out of total 20 patients, 30% of patients were having predicted values of FEV1 above 80% before treatment with *M. oleifera*. After treatment with *M. oleifera*, 40% of patients were found to have predicted values of FEV1 above 80%. Out of total 20 patients, 50% of patients were having predicted values of FEV1 below 60% before treatment which was reduced to 35% by treatment with *M. oleifera*. Reduction in both FVC and FEV1 suggests the usefulness of *M. oleifera* in reducing asthma severity. However, no significant change was observed in the ratio of FEV1 and FVC (FEV1/FVC %).

Like preliminary clinical study, significant increase in PEFR were observed in detailed clinical study with *M. oleifera* (32.09 ± 11.75%). Out of total 20 patients, 40% of patients were having PEFR in range of 20-40% of predicted values before treatment with *M. oleifera*. After treatment, this was decreased to 20% of patients having PEFR less than 40 % of predicted values. Only 5% of patients were having PEFR above 80% of predicted values before treatment with *M. oleifera*. After treatment, this was increased to 15% of patients having PEFR above 80% of predicted values. Forced Expiratory Flow between 25 and 75% (FEF25-75%) was significantly increased with a mean % increase was found to be 20.04 ± 10.64. Out of total 20 patients, 45% of patients were having FEF25-75%
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less than 60 % of predicted values before treatment with *M. oleifera*. After treatment, this was decreased to 40% of patients having FEF\(_{25-75}\%\) less than 60 % of predicted values. Maximum ventilatory volume (MVV) was also significantly increased by *M. oleifera*. Mean % increase in MVV was found to be 34.95 ± 8.44. Further, the treatment with *M. oleifera* in both studies did not produce any change in general physical parameters like temperature, heart rate and blood pressure after 3 weeks of treatment. Hematological parameters showed no significant change by treatment with *M. oleifera* except Hb, and ESR. Majority of patients showed significant increase in hemoglobin values. ESR was significantly reduced by treatment with *M. oleifera*.

Statistical significant increase in lung volumes (FVC and FEV1) and lung flow rates (PEFR, FEF\(_{25-75}\%\) and MVV) and reducing the symptoms of bronchial asthma suggests the usefulness of *M. oleifera* in the treatment of bronchial asthma. No change in general physical parameters and hematological parameters and absence of any untoward effect during the course of the study suggests the safety of this drug in the dose used. Thus, the present study suggests the usefulness of *M. oleifera* in the treatment of bronchial asthma.

Similar clinical study was carried out to evaluate the clinical efficacy and safety of *A. aspera* in the treatment of bronchial asthma. 20 patients of either sex satisfying inclusion and exclusion criteria were enrolled in the study. Patients enrolled were in the age range of 14-67 years with an average age of 42.45 ± 2.97 years. Patients enrolled were 16 male and 9 female. Average duration of asthma was found to be 7.32 ± 2.87 years with a range of 3 months to 35 years. 30% of patients were found to have extrinsic (allergic) asthma and 70% were found to have intrinsic (nonallergic) type of asthma. Habit of smoking was found to be prevalent to the extent of 43 % among asthmatic male patients. Also 60% of patients were found to have family history of asthma. General physical parameters like temperature, heart rate, and blood pressure were recorded before, every week and at the end of 3 weeks of treatment with *A. aspera*. No
significant change was observed in any of these parameters by treatment with *A. aspera*. Hematological examination was carried out before start of the treatment with *A. aspera* and subsequently at the end of 3 weeks treatment. Hematological parameters were not changed significantly by treatment with *A. aspera*. In the present study, *A. aspera* was found to significantly improve symptom score of the commonly observed symptoms of bronchial asthma like dyspnoea, wheezing, chest tightness and cough.

Spirometry tests before and after 3 weeks treatment with *A. aspera* revealed significant increase in FVC and FEV₁. FVC values were increased from 1.681 ± 0.21 lit. to 2.131 ± 0.24 lit. with a mean % increase was found to be 30.66 ± 8.07%. FEV₁ values were increased from 1.164 ± 0.112 lit. to 1.442 ± 0.127 lit. with a mean % increase was found to be 27.89 ± 9.89%. Out of total 20 patients, 25% of patients were having predicted values of FVC above 80% before treatment with *A. aspera*. After treatment, 30% of patients were found to have predicted values of FVC above 80%. 45% of patients were having predicted values of FVC in 20-40% before treatment with *A. aspera*. After treatment, 30% of patients were found to have predicted values of FVC below 40%. Out of total 20 patients, 50% of patients were having predicted values of FEV₁ above 60% before treatment which was increased to 60% by treatment with *A. aspera*. However, no significant change was observed in ratio of FEV₁ and FVC (FEV₁/FVC %) by treatment with *A. aspera*.

*A. aspera* also significantly increase PEFR and MVV. PEFR values were increased from 2.37 ± 0.17 lit/sec to 2.93 ± 0.16 lit/sec with a mean % increase was found to be 28.64 ± 6.92%. MVV values were increased from 29.87 ± 2.52 lit/min to 37.07 ± 2.47 lit/min with a mean % increase was found to be 32.35 ± 10.98%. Out of total 20 patients, 40% of patients were having PEFR in range of 20-40% of predicted values before treatment with *A. aspera*. After treatment, this was decreased to 20% of patients having PEFR less than 40% of predicted values. Only 15% of patients were having PEFR above 80% of predicted values.
before treatment with *A. aspera*. After treatment, this was increased to 40% of patients having PEFR above 80% of predicted values. 30% of patients were having MVV in range of 40-60% of predicted values before treatment with *A. aspera*. After treatment, this was decreased to 20% of patients having MVV less than 40% of predicted values. However, Forced Expiratory Flow between 25 and 75% (FEF\textsubscript{25-75}) was not significantly increased by treatment with *A. aspera*.

Statistical significant increase in lung volumes (FVC and FEV1) and lung flow rates (PEFR and MVV) and reducing the symptoms of bronchial asthma suggests the usefulness of *A. aspera* in the treatment of bronchial asthma. No change in general physical parameters and hematological parameters and absence of any untoward effect during the course of the study suggests the safety of this drug in the dose used. Thus the present study suggests the usefulness of *A. aspera* in the treatment of bronchial asthma.

Since *M. oleifera* and *A. aspera* were found to be effective in reducing the symptoms of bronchial asthma and improving the lung function parameters of asthmatic subjects, several experimental studies using animal models were done on the alcoholic extracts of *M. oleifera* and *A. aspera* to reveal the possible mechanism of action of anti-asthmatic activity. Weighted quantities of the extracts were dissolved in water and subjected to various studies. All animals were housed under well controlled conditions of temperature (22±1°C), humidity (55±5%) and 12h/12h light dark cycle. Animals had access to standard pallet diet and water given ad libitum. The protocol of the experiment was approved by the institutional animal ethical committee as per the guidance of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Bronchial asthma is characterized by increased airway reactivity to spasmogens. An initial event in asthma appears to be the release of inflammatory mediators (e.g. Histamine, Tryptase, Leukotrienes and prostaglandins). Some of these
mediators directly cause acute bronchoconstriction. In present study, the bronchodilating effect of *M. oleifera* and *A. aspera* was evaluated by observing the effect of their alcoholic extract on acetylcholine and histamine aerosol induced bronchoconstriction in guinea pigs.

Significant increase in pre-convulsion time was observed due to pretreatment with *M. oleifera* (100mg/kg and 200mg/kg) when the guinea pigs were exposed to either Acetylcholine (0.5%) or Histamine (0.25%) aerosol. Similarly, *A. aspera* (150mg/kg and 300mg/kg) also significantly and dose dependently delayed the onset of convolution in guinea pigs. The bronchodilating effect of *M. oleifera* and *A. aspera* was comparable to ketotifen.

Spasmolytic effect of *M. oleifera* and *A. aspera* were also evaluated by observing the effect of varying doses of their alcoholic extract on contractile responses of ileum to histamine, Ach, 5-HT and BaCl₂. The responses to drugs were recorded on a student physiograph (BioDevices) using isotonic transducer. In the present study, *M. oleifera* was found to be dose dependently inhibited ileal contractions induced by histamine (3.84 X 10⁻⁴ mM), Ach (4.12 X 10⁻⁵ mM), 5-HT (5.67X10⁻⁵ mM) and BaCl₂ (2.4 X 10⁻³ mM). These indicate that *M. oleifera* has non-specific spasmolytic activity on smooth muscle.

In the same way, alcoholic extract of *A. aspera* also dose dependently inhibited ileal contractions induced by Histamine (3.84 X 10⁻⁴ mM) and Ach (4.12 X 10⁻⁵ mM). However contractile responses to 5-HT and BaCl₂ were not blocked. The possible mechanism of action may be blockade of H₁ and Ach receptors leading to inability of smooth muscle to respond to histamine and Ach induced spasm leading to inhibition of bronchoconstriction.

These effects of *M. oleifera* and *A. aspera* support the improvement in the symptoms and lung function parameters of asthmatic subjects.
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In addition to bronchodilators, a significant number of therapeutic approaches for bronchial asthma have been designed based on antagonising specific mediators released from mast cells. Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. Compound 48/80 is one of the most potent mast cell degranulator, which causes liberation of mediators of inflammation such as histamine, leukotrienes, platelet activating factors, chemotactic factors for eosinophils neutrophils etc. from mast cells. In the present study, mast cell stabilizing activity of *M. oleifera* and *A. aspera* were evaluated by observing the effect of their alcoholic extract on rat peritoneal mast cell degranulation induced by compound 48/80 and egg albumin.

Compound 48/80 (10μg/ml) was found to induce mast cell degranulation to the extent of 74.82%. Ketotifen (10μg/ml), a reference standard produces an inhibition of 77.14%. *M. oleifera* (0.5 - 2.0mg/ml) and *A. aspera* (10 - 40mg/ml) produced dose dependent inhibition of mast cell degranulation. Similarly, egg albumin (1·mg/ml) was found to induce mast cell degranulation to the extent of 79.56%. Ketotifen (10μg/ml) produces an inhibition of 79.81%. *M. oleifera* (0.5 - 2.0 mg/ml) and *A. aspera* (10 - 40 mg/ml) produce dose dependent inhibition of mast cell degranulation.

A significant protection of rat peritoneal mast cells from disruption by antigen and compound 48/80 by alcoholic extract of *M. oleifera* and aqueous extract of *A. aspera* points towards its ability to interfere the release and/or synthesis of mediators of inflammation, indicating its mast cell stabilizing activity.

Airway inflammation has been demonstrated in all forms of asthma. Even in mild asthma, there is an inflammatory response involving infiltration, particularly with activated eosinophils and lymphocytes and also with neutrophils and mast cells. Anti-inflammatory drugs suppress the inflammatory response by inhibiting infiltration and activation of inflammatory cells as well as their synthesis, or release of mediators and the effects of inflammatory mediators. The carrageenan
induced paw edema model in rats is known to be sensitive to cycloxygenase inhibitors.

Alcoholic extract of *M. oleifera* at 400mg/kg dose possesses anti-inflammatory activity (76.87% reduction in edema volume), which is comparable to that of standard Diclofenac Sodium, 20mg/kg (83.43% reduction in edema volume). Also, alcoholic extract of *A. aspera* at 500mg/kg dose possesses anti-inflammatory activity (55.9% reduction in edema volume), which is comparable to that of standard Diclofenac Sodium 20mg/kg (83.43% reduction in edema volume).

Since, serotonin, histamine and prostaglandins are the common mediators of both bronchial asthma and inflammation the beneficial effect of alcoholic extract of *M. oleifera* and *A. aspera* could be due to inhibition of their release possibly due to inhibition of the enzyme cycloxygenase leading to inhibition of prostaglandin synthesis.

Acute exacerbations associated with production of purulent sputum usually are due to infection with virus, bacteria and fungi. It is no unusual to accept that infection may lead to bronchial hypersecretion and hence the congestion. In allopathy, multidrug approach is there, where patients receive bronchodilators, corticosteroids along with antibiotics. Use of chemical components of medicinal plants can substitute antibiotics to treat associated infection.

Antibacterial activity of *M. oleifera* and *A. aspera* was tested against organisms like *E-coli*, *P.aeruginosa*, and *S.aureus* and Minimum inhibitory concentration (MIC) was found out. MIC for cold-water extract of *M. oleifera* was found to be 20, 20 and 10 mg/ml respectively. MIC for hot water extract was found to be 100 and 50 mg/ml respectively and MIC for alcoholic extract was found to be 10 and 50 mg/ml respectively. Hot water extract and alcoholic extract were ineffective against *P.aeruginosa*. Cold-water extract of *M. oleifera* was found to be more
active against Gram-positive bacteria, while alcoholic extract was found to be active against Gram-negative bacteria. Boiling of seeds for a short time in water diminishes anti microbial activity. Same way, MIC for water extract of *A. aspera* was found to be 10, 20 and 10 mg/ml respectively and MIC for alcoholic extract was found to be 15, 30 and 10 mg/ml respectively. Water extract was found to be more active as compared to alcoholic extract.

As a part of developing fingerprinting of the active extract responsible for anti-asthmatic activity from *M. oleifera*, marker compound, which was a major compound resolving at Rf 0.55 under the conditions of EtoAc/MeOH/H₂O (95:4:1) as mobile phase and silica gel as the stationery phase using by column chromatography was isolated. The fractions were chromatographed on TLC plate using the same solvent. The fractions giving single spot were trapped and subjected to the structure characterization using HPTLC, UV, IR, LC-Mass and NMR. The possible structure found to be derivative of Benzyl isothiocyanate.

In conclusion, our data suggest that *M. oleifera* and *A. aspera* possess prophylactic and therapeutic potential for anti-asthmatic activity. None of the patients showed change in any general parameters or any adverse effect suggest safety of drug in dose used. *M. oleifera* in addition can be used for enhancement in the Hb level. The possible mechanism of anti-asthmatic action of both the drugs may be bronchodilator, mast cell stabilizing and anti-inflammatory activity. Pharmacognostic parameters developed for *M. oleifera* seed kernels can be taken as one of the tool for the standardization. The marker compound isolated from *M. oleifera* was found to be a derivative of benzyl isothiocyanate.