

Study on the Medicinal Prospects of Plant Extracts from *Morus Alba* And *Tinospora Cordifolia* for Controlling Asthma

Abstract

With the increase in number of patients of asthma there has been an attention drawn towards this growing misery. There is no dispute regarding the effectiveness of chemical drugs but a cheap; which causes no side effect and eco friendly alternative is badly needed. The chemicals derived from plants have been projected as weapon to cure asthma. They act as an herbal medicine which in long run shows sign of positive result. The plant products have many advantages over synthetic chemicals, as natural products have little or no side effects on mammalian toxicity and thus cause no health hazards. Plants have evolved a variety of secondary metabolism compounds, some of them for providing protection against respiratory disorder. A plant extract is an active substance with desired properties isolated from plant's tissues to be used for a particular purpose. In the present piece of research work it was observed that the dose of combined extract of both the plants i.e., *Morus alba* and *Tinospora cordifolia* can be given in place of chemical drugs Dexamethasone. It proved to be a very effective and eco friendly alternative to synthetic drugs. The findings suggest that the plant extracted bio-product may play a major role in designing an integrated control program in near future.

Keywords: Asthma, *Morus Alba*, *Tinospora Cordifolia*, Alternative To Synthetic Drugs.

Sheela Kumar

Assistant Professor,
Deptt.of Botany,
Government Girls College,
Shivpuri, Madhya Pradesh

Introduction

Asthma is a long lasting respiratory disorder and inflammatory disease of air tracts. This disease is growing rapidly throughout the world. This respiratory disorder is often caused by allergens such as dust, pollen, animal fur or feathers, air pollution, particularly by Nitrogen-di-oxide and sulphur-di-oxide. These allergens may enhance the disease. According to Modern medicine Asthma is only a manageable disease, and it is incurable. The World Health Organization (WHO) and International Asthma Council (IAC) consultation report distributed in 1998 on usage of Asthma Guidelines, highlight that "Traditional Medicines" in Asthma care is the "Conventional Therapy" to cure asthma.

The subject i.e., "phytochemistry" has been developed as a distinct discipline. It can be understood as subject, which is developed in between the use of natural products and bio-chemistry. It is related to the various varieties of organic chemicals that are accumulated by plants in their metabolism. Throughout the ancient civilization India has known to be a rich store of medicinal plants. Forest contains plants which are crude materials for manufacturing of medications and perfumery items. Green plants synthesize and preserve a variety of biochemical products, many of which are extractable and can be used as chemical feed or as raw material for various scientific investigations. The plant products have many advantages over synthetic chemicals, as natural products have little or no side effects on mammalian toxicity and thus cause no health hazards.

Development in the field of ethnomedicine and ethnobotany is the last solution that would encompass the development of new antimicrobials drugs, (Fauci, 1998). Ethnobotany and Ethnomedical studies are today recognized as the most viable methods of identifying new medicinal plants for bioactive constituents, (Farnsworth, 1996).

Ayurveda, an ancient system of Indian medicine has recommended a number of herbal drugs that can be made from the plants

Manju Jain

Assistant Professor,
Deptt.of Botany,
Government Girls College,
Vidisha, Madhya Pradesh

found in India for the treatment of bronchial asthma and allergic disorders (Charak Samhita, 1949). Usually asthma involved constriction of airways, especially the broncho constriction and leave the individual gasping for breath. Typically, an attack of asthma is characterized by dyspnea and wheezing during while respirating.

Different plants are used in different areas depending on their availability. Ethnobotanical survey is required to find their importance for medicinal purposes. Ethnobotanical research enables the recording and documentation of traditional knowledge about the empirical use of plants. However, this knowledge orally passes from one generation to the next.

Objective of the Study

In the present piece of research work it was observed that the dose of *Morus alba* and *Tinospora Cordifolia* can be given in place of synthetic drugs. It proved to be a very effective and eco friendly alternative to synthetic drugs.

Material and Method

Collection of Plant material

In the present study two indigenous plants i.e., *Morus alba* of family Moraceae and *Tinospora Cordifolia* of family Menispermaceae were selected to obtain biologically active compound from it.

The collection was done and identification of the collected plants was carried out at the Govt. Motilal Vigyan Mahavidyalaya, Bhopal Madhya Pradesh. Out of total plants surveyed the two plants viz. *Morus alba* L., and *Tinospora cordifolia* (Willd.) Miers., are used for cough, cold and asthma by the habitants of that region, therefore these plants were selected for detailed phyto-pharmacological study.

The taxonomic position of the experimental plants is;

<i>Morus alba</i> Linn	<i>Tinospora cordifolia</i>
Kingdom: Plant Kingdom	(Willd.) Miers
Class :Dicotyledons	Kingdom:Plant
Sub-	Kingdom
class:Monochlamydeae	Class : Dicotyledons
Series:Unisexuales	Sub-class :
	Polypetalae
Family: Moraceae	Series: Thlamiflorae
Genus: <i>Morus</i>	Order :Ranales
Species: <i>alba</i> L	Family
	:Menispermaceae
	Genus : <i>Tinospora</i>
	Species: <i>Cordifolia</i>
	(Willd) Miers

Traditionally, the "mulberry fruit" has been utilized as a medical agent to nourish the blood, to benefits the kidneys and to treat weakness, fatigue, anemia and premature graying of hair. It is additionally used to treat urinary incontinence, tinnitus, dizziness and constipation in the elderly patient. *Tinospora cordifolia* used as general tonic because of its anti-inflammatory, anti-arthritis, anti-allergic, anti-malarial and immuno-modulatory properties, Kirtikar and Basu, (1975); Chopra *et al.*, (1982); Chopra *et al.*, (1956); Nadkarni *et al.*, (1976); Rawal *et al.*, (2009).

The present work was started from preparing dried powdered material of the plants. The first method for the extraction of organic constituent is Soxhletion where the solvent are Ethanol and Distilled water and second method is Cold percolation where the solvent are same. Percentage loss in weight after drying of the plant material was also recorded. Crude extracts obtained from the plant were subjected to purification process by Column Chromatography and Thin layer Chromatography. The extracts were further used for qualitative analysis.

The qualitative Phytochemical analysis of both plants were carried out to get bioactive constituent i.e., Alkaloids, Flavonoids, Cardiac Glycosides, Saponin Glycosides, Steroids and Triterpens.

Chemical Analysis and Identification of The Compound

The plants of *Morus alba* L. and *Tinospora cordifolia* were washed with 1% KMnO₄ solution and it was then dried in shade at room temperature. Weight of fresh material and shade dried material both were noted to know the loss in weight and eventually grounded to a fine powder in an electric grinder. The powders were then used for extraction which was done by Soxhlet extraction and Cold Percolation Method.

Extract so obtained was Separated and Purified through process of Column Chromatography and Thin layer Chromatography. The compound obtained after fractionation by chromatography was acid hydrolysed, methylated and tested by related chemical tests.

The collected purified fraction (10 ml.) was mixed with 2 ml. of methanolic HCL (10%) and was refluxed for four hours in vacuum evaporator at the temperature of 4⁰C. After which, the mixture was again diluted with 3 ml. of distilled water and then evaporated to remove methanol. The aqueous solution was extracted with CHCl₃. The aqueous layer was neutralized with 10% NaOH and concentrated under reduced pressure.

Methylation. Purified fractions were separately dissolved in MeOH. In this process compound was washed by MeOH until it got converted into crystal or powder form.

Test for Alkaloids

Dragendroff Reaction

When a drop of crude extract was mixed with Dragendroff reagent (potassium bismuth iodide solution), alkaloids gave reddish brown precipitate. This indicates the presence of alkaloids.

Mayer's Reagent

When the test solution was mixed with Mayer's reagent (potassium mercuric iodide solution) then the appearance of cream colored precipitate indicates the presence of alkaloids in the extract.

Test for Flavonoids

5 ml of dilute NH₃ were added to a portion of aqueous filtrate of plant extract followed by addition of concentrated H₂SO₄. A yellow coloration observed in extract indicates the presence of flavonoids and the yellow color disappeared on standing.

Test for Glycosides**Cardiac Glycosides (Keller- Killani Test)**

5 ml. of each extract was treated with 2 ml. of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered with 1ml. of con.H₂SO₄. A brown ring of the interface indicates a D-oxysugar characteristic of cardiac glycosides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

Test for Steroids and Triterpenes (Salkowski Test)

5 ml. of each extract mixed with 2 ml. of chloroform, and concentrated H₂SO₄ (3 ml.) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of steroids and Triterpenes.

Saponin Glycosides (Forth Formation Test)

Froth formation test was done with 2 ml. solution of crude extract and was mixed in 7ml of distilled water in a test tube and when shaken well, stable froth (foam) was formed, which showed the presence of saponin in the test sample.

For further characterization and structural elucidation of plant extract the purified samples of selected plants were sent to SAIF and CDRI, Lucknow for UV, IR, NMR and MASS spectral analysis.

UV spectrum

UV spectra of *Morus alba* represent maximum wave length at 425 and 417 nanometer, meaning the compound is colored and *Tinospora cordifolia*, the absorption spectra at 303, 342, 351,360 nanometer means the compound is colorless.

IR Spectrum

IR Spectra of isolated compound of *Morus alba* (M2) showed 10 peaks ranging between 721 and 2922 and IR spectra *Tinospora cordifolia* gave 7 peaks ranging between 1062 and 3417.26.

Mass Spectrum

The fragments obtain in case of *Morus alba* shows that the compounds contains the sugar unit and unsaturation and in case *Tinospora cordifolia* meta stable peak shows unit.

NMR

In the spectra of compound (*Morus alba*) the alcohol functional group give rise to a peak at 3.5, 4.0 and 4.5 δ . This is within the expected range for a saturated CH group with single bond oxygen. The spectrum of second compound (*Tinospora cordifolia*) singlet absorption peak around 3.56 was assigned to one hydroxy proton of compound. The doublet one 1H at δ 2.7- 2.84 and another doublet at δ 3.6 indicate the di-hydroxy furan was found.

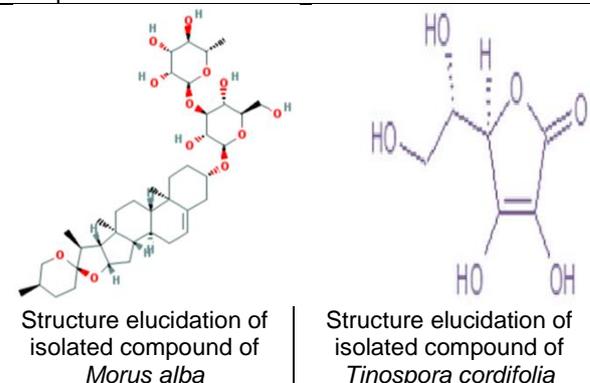
Structure Elucidation

A saponin was isolated from the leaves of *Morus alba*. The structure of this compound was determined as Spirostan-5- en -3 β - ol3 -O-[α - Lrhamnopyranosy -(1 \rightarrow 6)- β -D- glucopyranoside on the basis of UV, IR, NMR, mass spectral data and the compound 0.95 R_f was determined as flown when.

A flavonoid was isolated from the *Tinospora cordifolia* (T) plant extract, the structure of this compound was determined as (5R)-5-[(1S)-1, 2-

dihydroxyethyl]-3, 4- Dihydroxyfuran-2(5H) on the basis of UV, IR, NMR, mass spectral data and the

1.	2.
compound 0.94 R _f was determined as flown when.	

**Treatment: The sensitized mice were divided into seven groups**

Groups I was served as control and not treated with any extract and then sacrificed for the observation of mast cell. Extract of *Morus alba* and *Tinospora cordifolia* with two concentration i.e., 50mg/kg body weight of mice and 100mg/kg bodyweight of mice was administered orally. Group II was treated with *Morus alba* extract 50mg/kg body weight, which was orally administered to the mice. Group III was treated with *Morus alba* extract with 100mg/kg body weight concentration. To Group IV *Tinospora cordifolia* extract was given and to Group V another dose of 100mg/kg bodyweight of *Tinospora cordifolia*. Group VI was treated with *Morus alba* and *Tinospora cordifolia* with 100mg/kg bodyweight and to Group VII, standard drugs dexamethasone was given (10mg/kg bodyweight)

Result and Discussion

Following are the result obtained during the treatment using active fraction of herbal extract and standard drugs.

Groups I

Mast cell were found 14.50 \pm 2.5% intact and 87.20 \pm 2.2% disrupted.

Group II

The effect of the extract on mast cells degranulation was found to be 35.40 \pm 2.1% disrupted and 64.60 \pm 2.3% intact.

Group III

The effect of mast cells degranulation was 26.70 \pm 2.3% disrupted and 73.30 \pm 2.1% mast cells were intact.

Group IV

The disrupted of mast cells were found to be 28.70 \pm 2.5% and the intact mast cells were found to be 71.10 \pm 2.4% at 50mg/kg body weight.

Group V

This group showed disruption of mast cells as 24.60 \pm 2.6% and intact mast cell as 76.10 \pm 2.2%.

Group VI

It was noticed that 20.50 \pm 2.3% were disrupted and 82.20 \pm 2.4% were intact mast cells. They show synergistic effect when two plants comes together.

Group VII

It was observed that disruption of mast cells were found $18.40 \pm 2.7\%$, and intact cell is 83.50 ± 12.1 which was quite similar to Group VI.

In the control sensitized group, the number of mast cells was seen and which were larger in shape and size along with secretion of histamine around them as granules asthma was noticed but in the treated group, the mastcells were found to be disrupted but less in number and intact mastcell more in number.

All treated groups were compared with the standard groups VII of dexamethasone and was found quite significant with excellent stabilization show trans-granulation, which occurs between mastcell apparently transferring their granules to the cytoplasm of fibroblast.

When the mixture of plants was subjected it is about equal to standard drug dexamethasone means instead of chemical medicines plants extract were subjected for asthma treatment.

Conclusion

Plant extract was developed from the two selected plants i.e., *Morus alba* and *Tinospora cordifolia*, with the help of Soxhlet and Cold Percolation method and the effect was seen on experimental albino mice. Positive result was obtained when both plant extract were mixed and synergistic effect of the mixture is about equal to synthetic chemical drug- "dexamethasone". Herbal drugs are safe as compared to synthetic chemical drugs in terms of their side effects. Hence, it can be said that, this mixture of plant extract is a good medicine and can be used as a substitute for synthetic drug.

Recommendation

Medicinal value of plant should be propagated among the people in the tribal communities as well as to people outside communities. Plantation of *Morus alba* and *Tinospora cordifolia* should be done for utilization of these plants for medicinal use. These plants i.e., *Morus alba* and *Tinospora cordifolia* should be studied more from the perspective of finding their medicinal properties as they show great response in controlling asthma (as observed in the research).

And more and more research should be done on plant species that are found in rich forestry of India at large for finding out their medicinal properties for curing various diseases.

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