8.1 Standardization of Bhunimbadi churna - An Ayurvedic Polyherbal Formulation


STANDARDIZATION OF ‘BHUNIMBADI CHURNA’ - AN AYURVEDIC POLYHERBAL FORMULATION

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Keywords: Standardization, Bhunimbadi churna, Phytochemical studies, HPTLC

ABSTRACT: Standardization of herbal formulation is essential in order to assess the quality of drugs for therapeutic value. The present work is an attempt to standardize ‘Bhunimbadi Churna’, an Ayurvedic polyherbal formulation, used in fever and diabetes etc. The various parameters performed included organoleptic characteristics, physico-chemical parameter, physical characteristics of Churna, preliminary phytochemical, Heavy metal and Microbial analysis and high performance thin layer chromatography (HPTLC) analysis. The results obtained may be considered as tools for assistance to the regulatory authorities, scientific organizations and manufacturers for developing standard formulation of great efficacy.

INTRODUCTION: The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy.

India having a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. The development of these traditional systems of medicines with the perspectives of safety, efficacy, and quality will helps not only to preserve the traditional heritage but also to rationalize the use of natural products in healthcare.

In India around 20,000 medicinal plant species have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases.

Standardization of herbal formulations is an essential factor in order to assess the quality, purity, safety and efficacy of drugs based on the concentration of their active principles.

In the present research work, an attempt was made to standardize “Bhunimbadi Churna” an Ayurvedic polyherbal formulation made up of nine herbs (Table 1) used in the treatment of is a polyherbal ayurvedic medicine used as a fever, jaundice, anemia and Antidiabetic activity.

However, the work deals with the details of following latest standardization guidelines involving Good Manufacturing Practices (GMP) for preparation of Ayurvedic medicines. Standardization guidelines to be followed for herbal products provided by international bodies like World Health Organization (WHO), Ayurvedic pharmacopoeia of India (APi), European...
Materials and Methods:

Plant material: The crude drugs used in preparation of Bhunimbadi Churna were collected from local market of Vadodara and identified by Dr. M. S. Jangid, Department of Botany, Modasa, Gujarat. Bhunimbadi Churna was prepared, as per the procedure mentioned in Ayurvedic text “Brihat Nighantu Ratnakar” (Ayurved Sar Sangrah), published by ‘Shree, Baidyanath Ayurved Bhavan Ltd., Zhasni (U.P.), 1985. 596. All plant parts were then dried in shade, powdered and passed through sieve no. 85 # and lastly packed in a well closed container to protect them from moisture. Composition of Bhunimbadi churna was given in Table 1.

Table 1: Composition of Bhunimbadi Churna

<table>
<thead>
<tr>
<th>Plant Name in Sanskrit</th>
<th>Botanical Source</th>
<th>Part Used</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chvatya</td>
<td>Svera chvata</td>
<td>Whole plant</td>
<td>4%</td>
</tr>
<tr>
<td>Indrajv</td>
<td>Holarhena autysenterica</td>
<td>Seed</td>
<td>4%</td>
</tr>
<tr>
<td>Sunthi</td>
<td>Zingiber officinale</td>
<td>Rhizome</td>
<td>4%</td>
</tr>
<tr>
<td>Marica</td>
<td>Piper nigrum</td>
<td>Fruit</td>
<td>4%</td>
</tr>
<tr>
<td>Pippadi</td>
<td>Piper longum</td>
<td>Fruit</td>
<td>4%</td>
</tr>
<tr>
<td>Nagarmoth</td>
<td>Cypera rotundas</td>
<td>Rhizome</td>
<td>4%</td>
</tr>
<tr>
<td>Kutkri</td>
<td>Picrorrhiza kurroa</td>
<td>Rhizome</td>
<td>4%</td>
</tr>
<tr>
<td>Chatrak</td>
<td>Plumbago zeylanica</td>
<td>Root</td>
<td>8%</td>
</tr>
<tr>
<td>Kada chhal</td>
<td>Holarhena autysenterica</td>
<td>Stem bark</td>
<td>6%</td>
</tr>
</tbody>
</table>

Standardization Parameters: The various standardization parameters studied were botanical parameters, physico-chemical investigations, pH analysis, preliminary phytochemical analysis, determination of physical characteristics of powder formulation. Heavy metal analysis, Microbial analysis and HPTLC fingerprint.

Table 2: Botanical Parameters of Bhunimbadi Churna

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Appearance</th>
<th>Colour</th>
<th>Taste</th>
<th>Odour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhunimbadi Churna</td>
<td>Moderately fine powder</td>
<td>Greyish yellow</td>
<td>Bitter</td>
<td>Characteristic</td>
</tr>
</tbody>
</table>

Microscopic characters: For microscopical study, properly washed plant material was cut in to desirable size. A few mg of powder was washed with plain water, treated with iodine and potassium iodide, drop of glycerine was added and mounted (Figure 1).

Physico-chemical investigations: Physico-chemical investigations of formulations were carried out were the determination of Moisture content, extractive values and ash values (Table 3).

Determination of pH: 10% solution of Polyherbal formulation was prepared in distilled water and pH was determined using pH meter Orion digital pH meter (Table 3).

Table 3: Physico-Chemical Parameters of Bhunimbadi Churna

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Bhunimbadi Churna</th>
<th>Physicochemical Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>8.1±0.13%</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>2.35±0.11%w/w</td>
<td></td>
</tr>
<tr>
<td>Extractive value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water soluble</td>
<td>21.62±0.18%w/w</td>
<td></td>
</tr>
<tr>
<td>Alcohol soluble</td>
<td>12.42±0.13%w/w</td>
<td></td>
</tr>
<tr>
<td>Ash value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ash</td>
<td>5.26±0.02%w/w</td>
<td></td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>1.68±0.06%w/w</td>
<td></td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>0.49±0.01%w/w</td>
<td></td>
</tr>
</tbody>
</table>

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Determination of Physical Characteristics of Powder Formulation: Physical characteristics like bulk density, tap density, Hausner’s ratio, and Carr’s index were determined for different formulations. The term bulk density refers to packing of particles or granules.

The volume of packing can be determined in an apparatus consisting of a graduated cylinder mounted on a mechanical tapping device that has a specially cut rotating cam. 100 grams of weighed formulation powder was taken and carefully added to cylinder with the aid of a funnel.

The initial volume was noted and sample was then tapped until no further reduction in volume was noted. The initial volume gave the bulk density value and after tapping the volume reduced, it gives the value of tapped density. Hausner’s ratio is related to inter-particulate friction and as such can be used to predict the powder flow properties. Carr’s index is a method of measuring the powder flow from bulk density (Table 4).

### Table 4: Physical Characteristics of Bhunimbadi Churna

<table>
<thead>
<tr>
<th>Parameters</th>
<th>'Bhunimbadi Churna' Physicochemical Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density</td>
<td>0.3692 g/cm³</td>
</tr>
<tr>
<td>Tapped density</td>
<td>0.5641 g/cm³</td>
</tr>
<tr>
<td>Hausner’s ratio</td>
<td>1.58</td>
</tr>
<tr>
<td>Carr’s index</td>
<td>34.55</td>
</tr>
<tr>
<td>Angle of repose</td>
<td>46.17</td>
</tr>
</tbody>
</table>

Preliminary Phytochemical Analysis: Preliminary qualitative phytochemical analysis of all the extracts was carried out on water extract by employing standard conventional protocols (Table 5).

### Table 5: Phytochemical Tests for Bhunimbadi Churna

<table>
<thead>
<tr>
<th>Test</th>
<th>Bhunimbadi Churna</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids and Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Proteins and Amino acids</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: + indicates presence – indicates absence
Heavy Metal Analysis\(^9,10\):

Preparation of Samples by Acid Digestion Method: Accurately weighed 2 g of sample was taken in Kjeldahl flask. Acid mixture of HNO\(_3\); HClO\(_4\) (4:1) was added in the flask and heated continuously till the solution is colourless. The sample was then transferred in a 25 ml volumetric flask and the volume was made-up with distilled water. Reagent blank was synchronously prepared according to the above procedure. The standards of Lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) were prepared as per the protocol in the manual and the calibration curve was developed for each of them.

Detection: Then samples were analyzed for the presence of Pb, Cd, As and Hg using Atomic Absorbance Spectrophotometer (AAS) 6300 (by SHIMADZU) (Table 6).

<table>
<thead>
<tr>
<th>Heavy Metal</th>
<th>Limit</th>
<th>Bhunimbadi Churna</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead (Pb)</td>
<td>10 ppm</td>
<td>9.40 ppm</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>0.3 ppm</td>
<td>0.3 ppm</td>
</tr>
<tr>
<td>Arsenic (As)</td>
<td>10 ppm</td>
<td>2.15 ppm</td>
</tr>
<tr>
<td>Mercury (Hg)</td>
<td>1 ppm</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Microbial Analysis\(^11\): Microbial analysis was carried for determination of microbial contamination as per procedures of Ayurvedic pharmacopoeia of India and WHO Guideline. The test included total bacterial count, total yeast and mould count, *Escherichia coli* and *Salmonella typhi* (Table 7).

<table>
<thead>
<tr>
<th>Microbial Analysis</th>
<th>Limit</th>
<th>Bhunimbadi Churna</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aerobic viable count</td>
<td>10(^{7})/gm</td>
<td>410 cfu/gm</td>
</tr>
<tr>
<td>Total yeast and mould</td>
<td>10(^{7})/gm</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Salmonella ssp</em></td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

HPTLC Analysis\(^12,13\): The HPTLC finger print profile of Methanolic extract of Bhunimbadi Churna was taken on aluminium plate coated with silica gel 60 F\(_{254}\) of 0.2 mm thickness (E. Merck) as adsorbent and employing CAMAG Linomat IV applicator. The mobile phase used was Toluene: Ethyl acetate: Formic acid (5: 1.5: 0.5 v/v).

The plate was dried and visualised under UV 254 nm and 366 nm. The plate was spray with Anisaldehyde-Sulphuric acid reagent and heated at 105° C till the spots appeared.

![Figure 2: HPTLC of Bhunimbadi Churna](image)

**FIGURE 2: HPTLC OF BHUNIMBADI CHURNA**

![Figure 3: HPTLC Finger Printing Chromatogram of Bhunimbadi Churna](image)

**FIGURE 3: HPTLC FINGER PRINTING CHROMATOGRAM OF BHUNIMBADI CHURNA**

RESULTS: As a part of standardization procedure, Churna was tested for relevant physical and chemical parameters, and also subjected to microbial screening through quality control measures. Botanical parameters revealed that Churna was Greyish yellow, moderately fine powder, odour- Characteristic, taste- Bitter (Table 2) and Microscopic characters showed the characters like orange coloured Parenchymatous cells (*Swertia chirata*), rosette and prismatic crystals of calcium oxalate (*Holarrhena antidysenterica*), Starch grains and vessels with...
spiral thickening up to 85µ in length (Zingiber officinale), Epicarp and Perisperm cells (Piper nigrum), Fragments of Parenchyma (Piper longum), Epidermis cells (Cyperus rotundus), Cork cells (Picrohriza kurrooa), fibers (Plumbago zeylanica), Stone cells (5-7µ) (Holarrhena antidysenterica St. Bk) (Figure 1).

Results of quantitative analysis for Loss on drying at 105°C, pH. Total ash. Acid insoluble ash. Water soluble ash. Alcohol soluble extractives and Water soluble extractive were calculated and results were shown (Table 3). Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards. Percent weight loss on drying or moisture content was found to be 8.11% w/w. The less value of moisture content could prevent bacterial, fungal or yeast growth.

Physical properties of churna like Bulk density, Tapped density, Hausser ratio and Carr's index indicates very poor flow ability and Angle of repose indicates Poor-must agitate, vibrate flow property (Table 4). Phytochemical analysis revealed the presence alkaloid, glycoside, flavonoid, Steroids & Triterpenoids, tannins and Carbohydrate and absence of protein and amino acid (Table 5). Heavy metals may be present in crude drugs through atmospheric pollution and through the soil. Moreover minerals and metals are also used in preparing Ayurvedic formulations.

However, heavy metals have been associated with various adverse effects including status hepatotoxicity, epilepticus, fatal infant encephalopathy, congenital paralysis and deafness, and developmental delay. Many case studies have reported serious adverse conditions due to heavy metals in Ayurvedic and other herbal drugs. Hence, heavy metals need to be detected in such preparations. In this study, all the samples tested negative for the presence of heavy metals (Table 6).

For detection of such microorganisms, colonies obtained on specific media were subjected to suitable microbial tests along with pure strains to detect their presence or absence. The results obtained (Table 7) revealed the absence of these microorganisms thereby confirming the non-toxic nature of the formulations.

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8.2 In-vitro antioxidant activity of – An Ayurvedic Bhunimbadi churna.

IN-VITRO ANTIOXIDANT ACTIVITY OF - AN AYURVEDIC BHUNIMBADI CHURNA

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Abstract: Medicinal plants represent a rich source of antioxidant agents and used medicinally in different countries as source of many potent and powerful drugs. The present study was undertaken to evaluate DPPH radical scavenging assay and ferric-reducing antioxidant power assay. The antioxidant activity of the methanol extract increased in a concentration-dependent manner. It is concluded that these results suggest that the Methanolic extract of Bhunimbadi Churna possess antioxidant effect in DPPH radical scavenging assay and ferric-reducing antioxidant power assay.

Keywords: Antioxidant, DPPH radical scavenging assay, Churna, Medicinal plants

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INTRODUCTION

Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease. The uses of natural antioxidants from plant extracts have experienced growing interest due to some human health professionals and consumer's concern about the safety of synthetic antioxidants in foods. Antioxidant activities in plants have been identified by many researchers. The natural occurring antioxidant is focused more on edible plants, especially spices and herbs. Spices and herbs are an excellent source of phenolic compounds (flavonoids, phenolic acid and alcohols, stilbenes, tocopherols, tocoferol), ascorbic acid and carotenoids which have been reported to show good antioxidant activity.

However, the Himalayan plant, *Swertia chirata* Buch. Ham. Ex Wall resulted in the isolation of seven polyoxygenated xanthenes, fraction Sc-1 – Sc-7. Out of seven compounds extracted from this plant, five components (Sc-3 - Sc-7) exhibited antioxidant activity at different magnitude of potency. The paper deals with these facts. Effect of Solvent extraction on Phenolic content, Antioxidant and Amylase Inhibition activities of *Swertia chirata*. The methanolic and ethanolic extracts from ginger (*Zingiber officinale* L.) seed (ZOS) were investigated for their antioxidant and radical scavenging activities in eight different assays, namely, total antioxidant activity, reducing power, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, total flavonoid content, total phenolic compound and metal chelating activities. The black pepper containing oil, the antioxidant aspects will be evaluated by means of the determination of the total phenolic content by the Folin-Ciocalteau method and by the antioxidant activity by the DPPH, ABTS and β-carotene methods. *Cyperus rotundus* was extracted by using different extraction solvents and evaluated for their antioxidant activity using different in vitro antioxidant assays. The protective effect of *P. kurroa* might be ascribable to its membrane-stabilizing property and/or antioxidant nature. The isolation and spectral data for new flavonoid 2-(2, 4-Dihydroxyphenyl)-3, 6, 8-trihydroxy-chromen-4-one from the roots of *Plumbago zeylanica* were determined and the antioxidant activity were studied by free radical scavenging and superoxide radical scavenging assays. The present study was carried out to investigate the antioxidant effect of the methanolic extract, its alkaloidal and non-alkaloidal fractions along with petroleum ether soluble, ether soluble and ethyl acetate soluble sub-fractions of non-alkaloidal part of the bark of *Holarrhena pubescens*.

MATERIALS AND METHODS

Materials

‘Bhunimbadi Churna’ containing the plant parts and their powder of fresh plant *Swertia chirata* (Whole plant), *Holarrhena antidysenterica* (Seed), *Zingiber officinale* (Rhizome), *Piper nigrum*...
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(Fruit), Piper longum (Fruit), Cyperus rotundus (Rhizome), Picrorhiza kurroa (Rhizome), Plumbago zeylanica (Root), and Holarrhena antidysenterica (Stem bark) were collected from local market of Vadodara, India. Ascorbic acid, 1,1-diphenyl-2- picrylhydrazyl (DPPH) and Potassium ferricyanide, Ferric Chloride, Potassium hydrogen phosphate were purchased from Sd-fine chemicals limited, Mumbai, India.

**Determination of free radical scavenging using DPPH method**

Product extract and standard ascorbic acid solution (0.1 ml) of different concentrations viz. 10, 20, 40, 60, 80, 100µg/ml was added to 3 ml of a 0.004% methanol solution of DPPH. An equal amount of methanol and DPPH served as control. After 30 minutes incubation in the dark, absorbance was recorded at 517 nm using UV spectrophotometer (Shimadzu-UV-1601). Samples were measured in three replicates and the percentage inhibition activity was calculated from 

\[
\left[ \frac{(A_0-A_1)}{A_0} \right] \times 100,
\]

where 

\( A_0 \) is the absorbance of the control, and 

\( A_1 \) is the absorbance of the extract/standard.

**Determination of Ferric Reducing Power determination method**

Different concentrations of Churna extract and standard ascorbic acid solution viz. 10, 20, 40, 60, 80, 100 µg/ml in 1ml of methanol were mixed with phosphate buffer (2.5ml, 0.2M pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 ml, 1%). The mixture was incubated at 50oC for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 g (rpm) for 10 min at room temperature. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (FeCl3) (0.5 ml, 0.1%) and the absorbance of the reaction mixture indicated increased reducing power. The absorbance was measured at 700nm using UV spectrophotometer (Shimadzu-UV-1601). All the tests were performed in triplicate and the graph was plotted with the average of three observations.

**Statistical analysis**

Experiment data were analyzed using Excel (Microsoft Inc.) and SPSS version 17.0 software. Significant differences between samples were analyzed using analysis of variance (ANOVA) and Dunnett’s test using GraphPad Instat 3 software (P< 0.05). Data obtained were reported as mean ± standard deviation.

**RESULTS AND DISCUSSIONS**

**Antioxidant capacity**

DPPH radical was used as a stable free radical to determine antioxidant activity of natural compounds. 19 The antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. 20 Thus, the purple colour of 2,2-diphenyl-1- picryl hydrazyl (DPPH) will reduce to α, α-
diphenyl-β-picyrylhydrazine (yellow coloured). The scavenging of the stable radical (DPPH) is considered a valid and easy assay to evaluate scavenging activity of antioxidants.

Result indicated the significant decrease in the concentration of DPPH radicals due to the scavenging ability of Methanol extract of Churna and Ascorbic acid, as a reference standard. The IC50 values in DPPH radical scavenging model were 14.26 % and 62.80 % for Ascorbic acid and Methanol extract of Churna respectively shows in figure 1.

In this study, the antioxidant activity is also determined on the basis of the ability of antioxidant in this plant extracts to reduce ferric (III) iron to ferrous (II) iron in result shows figure 2 illustrates that Methanolic extract of the Churna had ferric reducing capacity and also comparable to Ascorbic acid.

![Figure 1: Chart of DPPH radicals scavenging activity](image)
CONCLUSION:

The results obtained demonstrated that Bhunimbadi Churna had show antioxidant activity in free radical scavenging using DPPH method Ferric Reducing Power determination method. The mixture of plants extracts had showed no synergism effect.

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8.3 A review on Antidiabetic and antioxidant activity of *Bhunimbadi churna*.

**A REVIEW ON ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF BHUNIMBADI CHURNA.**

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**Abstract:** The role of natural products as a source of remedies has been recognized since ancient times. In addition the overuse of synthetic drug which result in higher incidence of adverse drug reaction, has enthused the human to revert to nature for safer herbal medicines. The current review focus on Bhunimbadi Churna Herbal formulation and its potential in the treatment of diabetes mellitus, Diabetes is world’s largest endocrine disease involving metabolic disorders of carbohydrate, fat and protein and it affects nearly 1-3% of world population. The review describes various aspect of Bhunimbadi Churna like active phytoconstituents having hypoglycaemic action, pharmacological properties and mechanism of action of its ingredients. This review also focuses on the traditional therapeutic action of the Bhunimbadi Churna mention in Brhat Nighantu Ratnakar. In various pharmacological studies, done in few decades on the drugs of Bhunimbadi Churna, it has been proved that almost all the constituents of Bhunimbadi Churna, posses anti hyperglycaemic, hypolipidemic, antioxidant and other therapeutic properties.

**Keywords:** Antidiabetic, hypolipidemic, antioxidant property, Herbal

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INTRODUCTION

Diabetes mellitus is a major public health problem. According to WHO reports, more than 176 million patients suffer worldwide and it is estimated that in 2025, there will be about 300 million patients living with this condition. The increase is expected to be 42% in developed countries and 70% in developing countries. Although different types of hypoglycaemic agents such as thiazolidinediones, insulin, biguanides and sulphonylurea are available; there is growing interest in herbal remedies due to the side effects associated with these therapeutic agents beside their limitations in managing the disease effectively.1,2

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism and represents a heterogeneous group of disorders characterized by hyperglycemia, which may be due to impaired carbohydrate utilization resulting from a defective or deficient insulin secretion response. Diabetes mellitus is marked by sustained hyperglycemia and has deleterious effect on kidney. The characteristic symptoms of diabetes are polyuria, polydypsia, polyphagia and unexpected weight loss3. *Momordica charantia*, *Trigonella foenumgraecum*, *Enicostema littorae*, *Gymnema sylvestre*, *Azadirachta indica*, *Syzygium cumini*, *Zingiber officinale*, *Swertia chirayata*, are some of the most effective and the most commonly studied Indian plants in relation to diabetes.

Bhunimbadi Churna:

Bhunimbadi Churna is a group of nine drugs, which has been mentioned in Bhunimbadi Churna under Brhat Nighantu Ratnakar.4 The botanical name, family and therapeutic uses of Bhunimbadi Churna as mention in Ayurvedic literature are described in Table 1.

Table 1: Composition of Bhunimbadi Churna

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Sanskrit Name</th>
<th>Botanical Name</th>
<th>Family</th>
<th>Therapeutic uses as described in Ayurveda4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chirayata (Bhunimba)</td>
<td><em>Swertia chirata</em></td>
<td>Gentianaceae</td>
<td>Fever, Jaundice, Anemia, Antidiabetic</td>
</tr>
<tr>
<td>2</td>
<td>Indrajav</td>
<td><em>Holarrhenan antidysenterica</em></td>
<td>Apocynaceae</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sunthi</td>
<td><em>Zingiber officinale</em></td>
<td>Zingiberaceae</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Marica</td>
<td><em>Piper nigrum</em></td>
<td>Piperaceae</td>
<td></td>
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<td><em>Cyperus rotundus</em></td>
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Pharmacological studies done on the drugs of Bhunimbadi Churna:

To establish the traditional therapeutic effects on modern scientific parameters, various pharmacological studies have been done in last few decades on the drugs of Bhunimbadi Churna. Among these scientific researches only antidiabetic studies conducted on individual drugs of Bhunimbadi Churna are reviewed here. The Body weight, Blood glucose, Serum cholesterol, Serum triglyceride, Serum creatinine, Serum ALPH, Serum ALT, Serum AST, Serum bilirubin, Serum total protein, Serum albumin were observed to be elevated in diabetic patients. It is also observed that oxidative stress is one of the main contributory factors in the patho-physiology of many diseases, including type-2 diabetes mellitus. So hypolipidemic and antioxidant studies done on these drugs are also discussed here.

*Swertia chirata* Buch.Ham:

*Swertia chirata* belongs to the family Gentianaceae. The plant is a robust annual herb which grows upto 1.5 meters in height in the Himalayas, usually at an altitude of 4,000-10,000 feet but it can also be grown in sub-temperate regions, as well as in a variety of soil conditions. The compounds isolated from Swertia chirata include a large number of xanthenes, glycosides, alkaloids and other compounds like chiratin, ophelic acid, palmitic acid, oleic acid, stearic acid. The first isolated dimeric xanthone was chiratan. Other important phytoconstituents include swerchirin, swertiamarin, swertanone, mangiferin, amarogentin, gentiopicrin and chiralactol. Chireta stimulates the digestion and helps to normalize blood sugar, which makes it useful for diabetics. Studies with animals suggest that this herb reduces the sugar levels only when they are high, which lowers the risk of hypoglycemia. Also 95% ethanol extract and four fractions of *Swertia chirayita* were tested for blood sugar lowering activity in rats. The hexane fraction caused maximum lowering although the ethanol extract was clearly active. Swertiamarin is main constituents of plant given the anti-diabetic effect due to an active metabolite, gentianine, that upregulates PPAR-y gene expression in 3T3-L1 cells. A xanthone compound, mangiferin effect on the atherogenic potential of streptozotocin (STZ)-diabetes was investigated. *Swertia chirayita* possesses in vitro and in vivo antioxidant activity, the liver and kidney of CCL4-intoxicated animals exhibited decrease in superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) levels. Additionally, these organs exhibited increase in malondialdehyde (MDA) level. Out of seven compounds extracted from this plant, five components exhibited antioxidant activity at different magnitude of potency. Figure 1 *Swertia chirata* whole plant.

*Holarrhena antidysenterica* Wall:

Drug consists of dried seeds of *Holarrhena antidysenterica* Wall belonging to family Apocynaceae. In Sanskrit called Bhadra Yava and in Hindi Indraju. The seeds were also reported...
to possess steroidal type alkaloids. Seeds are very useful in case of colitis and bleeding problems, diarrhoea and dysentery.\textsuperscript{17} It also used as antioxidant, antihyperglycemic, anti-malarial, spasmodytic and spasmodogenic properties.\textsuperscript{18} The hypoglycemic effect of ethanolic extract of its seeds in streptozotocin – induced diabetic rats and its effect on serum cholesterol, triglyceride, aspartate transaminase (AST), alanine transaminase (ALT), alkaline transferase (ALP), total protein, urea, creatinine and uric acid and indicated of its potent antidiabetic effect.\textsuperscript{19} Also studied the present experiment was conducted to search out the effect of hydro-methanolic extract of seed of \textit{Holarrhena antidysenterica} on intestinal α-glucosidase activity in dose dependent manner and on the management of postprandial hyperglycemia in starch loaded rats.\textsuperscript{20} The \textit{Holarrhena antidysenterica} seeds crude methanolic extract and ethyl acetate fraction indicated significant antioxidant activities in doze dependant manner and revealed maximum scavenging activity 68% and 80% at concentration of 250μg/mL.\textsuperscript{21} Figure 2 \textit{Holarrhena antidysenterica} seed.

\textbf{Zingiber officinale} Roxb:

The rhizome of \textit{Zingiber officinale}, is one of the most widely used species of the ginger family (Zingiberaceae) and is a common condiment for various foods and beverages. Ginger has a long history of medicinal use dating back 2,500 years in China and India for conditions such as headaches, nausea, rheumatism, and colds.\textsuperscript{22} Ginger is a rich source of volatile oil. Zingiberene, zingerberol, β-sesquiphellandrene, β-bisabolene, α-farnesene, ar-curcumene and smaller amounts of camphene, β-phellandrene, cineole, geraniol, curcumene, citral, terpineol, borneol important constituents of the plant.\textsuperscript{23} A methanolic extract of dried rhizomes of ginger produced a significant reduction in fructose-induced elevation of lipid levels, be achieved with a dietary supplement of either ginger or its extract containing aldose reductase inhibitors.\textsuperscript{24} Antidiabetic potential of \textit{Zingiber officinale} was mainly through inhibition of the glucose diffusion and to a limited extent by reducing the glycation.\textsuperscript{25} The antioxidant properties of [6]-gingerol which is very effective agent for anticipation of ultra violet B (UVB)-induced reactive oxygen species production and COX-2 idiomy, and a promising therapeutic agent against UVB induced skin disorders, has been studied both \textit{in-vitro} & \textit{in-vivo}. It also has a protective role to toxicity and lethality against some agent like carbon-tetra chloride, cisplatin etc.\textsuperscript{26} Ginger oil might act as a scavenger of oxygen radical and might be used as an antioxidant.\textsuperscript{27} The effect of (S)-[6]-gingerol increased glucose uptake in L6 skeletal muscle cells by activating AMPK. (S)-[6]-gingerol, a major component of \textit{Zingiber officinale}, may have potential for development as an antidiabetic agent.\textsuperscript{28} Anti-hyperglycaemic, lipid lowering and anti-oxidant properties of [6]-gingerol in db/db mice.\textsuperscript{29} Figure 3 \textit{Zingiber officinale} rhizome.

\textit{Piper nigrum} Linn:

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Piper Nigrum Linn (Black pepper) is a vine perennial plant producing berry-like and aromatic pungent fruits. It is locally known as “pamienta” or “paminta” Which belongs to family Piperaceae. Although black pepper is cultivated in many tropical regions, it is native to Kerala State in India where it still occurs wild in the mountains. Piperine the major active principle of black pepper, is closely related in structure to the known natural carcinogens-safrrole, estragole and methylenegol which are also widely distributed in spices and plant oils. The plants contains mainly alkaloids, amides, propanophenols, lignans, neolignans, terpenes, steroid, piperalides, chalcones, dihydrochalcones, brachyamide, piperamide, piparamine, pipereutine, pipericide, piperine, piperonide, trichostachine, sarmentine, sarmentosine, tricholein, retrofractamide. The effect of piperine on blood glucose level in alloxan-induced diabetic mice in acute and subacute study models. Oxidative stress plays a key role in diabetes, and treatment with P. nigrum and V. rosea are useful in controlling not only the glucose and lipid levels but these components may also be helpful in strengthening the antioxidants potential. The subacute administration of piperine has statistically significant antihyperglycemic activity while acutely it raises blood glucose at high doses. Figure 4 piper nigrum fruits.

Piper longum Linn:

Piper longum L. (Piperaceae), commonly known as “long pepper”, is widely distributed in the tropical and subtropical regions of the world. Piperine is the major and active constituent of long pepper. The fruits gave positive tests for the presence of volatile oil, starch, protein and alkaloids, saponins, carbohydrates, and amygdalin and negative test for tannins. The antihyperglycemic and antilipidperoxidative effects of ethanolic extract of Piper longum dried fruits in alloxan-induced diabetic rats were studied. The blood glucose level, carbohydrate metabolizing enzymes and the status of lipid peroxidation and antioxidants were assayed using specific colorimetric methods. Oral administration of dried fruits has shown significant antihyperglycemic, antilipidperoxidative and antioxidant effects in diabetic rats comparable to that of the standard reference drug glibenclamide. The antidiabetic and antihyperlipidemic potential of oil from Piper longum and piperine was investigated with their possible mechanism using α-glucosidase, aldose reductase (AR), and pancreatic lipase inhibitory activity. Figure 5 piper longum fruits.

Cyperus rotundus Linn:

Cyperus rotundus Linn belong to the family Cyperaceae, also known as purple nut sedge or nutgrass, is a common perennial weed with slender, scaly creeping rhizomes, bulbous at the base and arising singly from the tubers which are about 1-3 cm long. The tubers are externally blackish in colour and reddish white inside, with a characteristic odour. Different phytochemical
studies on C. rotundus revealed the presence of alkaloids, flavonoids, tannins, starch, glycosides, furocoumarins, monoterpenes, sesquiterpenes, sitosterol, fatty oil containing a neutral waxy substance, glycerol, linolenic, myristic and stearic acids.\(^4^0\) Oral daily administration of 500 mg/Kg of the extract (Once a day for seven consecutive days) significantly lowered the blood glucose levels in rats with alloxan induced diabetes.\(^4^3\) The scientists concluded that this antihyperglycemic activity can be attributed to its antioxidant activity as C. rotundus showed a strong 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging action in-Vitro. These results are convergent with C. rotundus potential to suppresses AGE formation and protein oxidation in a model of fructose-mediated protein glycoxidation.\(^4^2\) Amrita Bindu, a salt-spice-herbal mixture containing C. rotundus Linn exerts a promising antioxidant potential against the free radical 2, 2’-azinobis (3-ethylbenzolone-6 sulphonacid).\(^4^3\) Figure 6 cyperus royundus rhizome.

**Picrorhiza kurroa** Royle ex Benth:

**Picrorhiza kurroa** is a small perennial herb from the Scrophulariaceae family, found in the Himalayan region growing at elevations of 3,000-5,000 meters. Picrorhiza kurroa has a long, creeping rootstock that is bitter in taste, and grows in rock crevices and moist, sandy soil. Kutkin is the active principal of Picrorhiza kurroa and is comprised of kutkioside and the iridoid glycoside picrosides I, II, and III.\(^4^4\)\(^4^5\) **P. kurroa** extracts are able to ameliorate biochemical damages induced by alloxan in diabetic rats.\(^4^6\) Also the standardized extract of Picrorhiza kurroa possess significant antidiabetic activity in streptozotocin-nicotinamide induced type-2 diabetes mellitus in rats.\(^4^7\) All the extracts of picrorhiza kurroa and isolated compounds were evaluated for its antioxidant activity using two assays, 2,2-diphenyl-1-picrylhydrazyl radical and 2,2’-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) assay.\(^4^8\) The ethanol extract of rhizome of Picrorhiza kurroa, at the dose of 20mg/kg body weight, accelerated the healing of stomach wall of indomethacin induced gastric ulcerated rats by an in vivo free radical scavenging action.\(^4^9\) The antioxidant effect and the therapeutic dose and time window of picroside II by orthogonal test in cerebral ischemic injury in rats. The forebrain ischemia models were established by bilateral common carotid artery occlusion (BCCAO) methods.\(^5^0\) The oral administration of aqueous and methanol extracts of P. kurroa rhizomes (250 and 500 mg / kg body weight / day) for 15 days significantly reduced blood glucose, glycosylated haemoglobin and increased total hemoglobin, plasma insulin in alloxan-induced diabetes in albino rats. The treatment also showed significant correction in the level of nitric oxide radicals, superoxide radicals, peroxynitrite radical, lipid peroxidation, glutathione, glutathione reductase, glutathione-S-transferase, glutathione peroxidase, superoxide dismutase and catalase in the pancreas of alloxan diabetic rats.\(^5^1\) Also study shows that picroliv, picroside-I and kutkioside possess the properties of antioxidants which appear to be mediated through activity like that of
Superoxide dismutase, metal ion chelators and xanthine oxidase inhibitors. Figure 7 Picrorhiza kurroa rhizome.

**Plumbago zeylanica** Linn:

Chitrak consists of dried mature root of *Plumbago zeylanica* Linn. Belong to family Plumbaginaceae, a large perennial sub-candent shrub, found throughout India in wild state and occasionally cultivated in gardens. Naphthaquinones, alkaloids, glycosides, steroids, triterpenoids, tannins, phenolic compounds, flavanoids, saponins, coumarins, carbohydrates, fixed oil and fats and proteins of all the chemical constituents plumbagin is the principle active compound. Pharmacological studies carried out have indicated that *P. Zeylanica* has antihyperglycemic effect on diabetic induced animals. The ethanol extract of *P. Zeylanica* root on key enzymes of glycolysis and muscle hexokinase, phosphofructokinase, pyruvate kinase lactate dehydrogenase activities were diminished in diabetic rats. Also the evaluate the antidiabetic effects of plumbagin isolated from *P. zeylanica* L. root and its effect on GLUT4 translocation in STZ-induced diabetic rats. Also shows that oral administration of ethanolic root extract of *P. Zeylanica* (100 mg, 200 mg/kg/p.o), tolbutamide (250 mg/kg/p.o) increased the activity of hexokinase and decreased the activity of glucose-6-phosphatase (P < 0.001) in streptozotocin treated diabetic rats. In-vitro antioxidant and Total Phenolic Content (TPC) assay conducted on methanolic extract of the roots indicated *P. Zeynalica* to be a potent radical scavenger. The inhibition percentage by DPPH method was seen to be 88.45 % compared to ascorbic acid (96.5 %). *P. Indica* roots are indeed rich in phytochemicals and had substantial antioxidant activities, implying that *P. Indica* roots can be used as a potential source of natural antioxidant. The isolation and spectral data for new flavonoid 2-(2,4-Dihydroxy-phenyl)-3,6,8 trihydroxy chromen-4-one from the roots of *P. Zeylanica* were determined the antioxidant activity was studied by free radical scavenging and superoxide radical scavenging methods.

**Holarrhena antidysenterica** (Roth) A. DC:

*Holarrhena antidysenterica* (Linn.) Wall is a genus of trees or shrubs found in the tropics and subtropics of the old world. It comprises seven or eight species, which are distributed in Asia, tropical areas of Africa, Madagascar, India, Philippines and Malayan Peninsula. The plant is well known as ‘Khurti’. The bark is thick, brown and rough, with abundant milky white latex. The bark and seeds are bitter, constipating, astringent, acrid, refrigerant, anthelmintic, antiperiodic, aphrodisiac, carminative, expectorant, frebrifuge and tonic. They are useful in amoebic dysentery, diarrhoea, asthma, hepatopathy. *Holarrhena antidysenterica* is also a rich source of other steroidal alkaloids such as kurchine, kurchimine, conessidine, holarrime, conessidine, conkurchicine and regholarrhime. Bark powder of *H. antidysenterica* was subjected to hot

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continuous extraction (soxhlet) with various solvents like Alcohol, butanol, chloroform, aqueous and butanone showed significant antidiabetic activity in acute as well as prolonged treatment compared to control. Methanolic extract of bark possesses antihyperglycemic activity with antihyperlipidemic and antioxidant potential which may prove beneficial in cardiovascular complications associated with diabetes mellitus. Holarrhena antidysenterica also show in-vitro antioxidant potential according to FTC assay method. Holarrhena antidysenterica bark has both antihyperglycemic and antioxidant potential but no acute toxic effect. Figure 9 Holarrhena antidysenterica stem bark.

CONCLUSION:

Diabetes mellitus is a metabolic disorder caused due to relative or absolute deficiency of insulin or insulin resistance at the cellular level. This review article has presented the Antidiabetic action of Bhunimbadi churna, a group of nine plants which has been mentioned in Brhat Nighantu Ratnakar. It showed that these plants have varying degree of hypoglycemic activity along with antioxidant property. The Antidiabetic activity of these plants are attributed to the presence of polyphenols, terpenoids, alkaloids, flavonoids, glycosides and other active constituents, which shows reduction in blood glucose level. Numerous mechanisms of action have been predicted for these plant extracts. Some herbal drugs have effects on the activity of pancreatic β-cells (insulin release, β-cell regeneration) or some drugs enhance the insulin sensitivity and some of the plant extracts exhibit insulin-like activity. Other mechanism may involve improved glucose homeostasis (increase of peripheral utilization of glucose, increase or decrease of glycogenolysis), inhibition of intestinal glucose absorption, reduction of glycemic index of carbohydrates, reduction the effect of glutathione. All the actions may be responsible for the reduction and abolition of diabetic complications. Thus there is need for more investigation to evaluate the mechanism of action of these medicinal plants of Antidiabetic effect. Future it required for the Antidiabetic effect of these drug in clinical setting with appropriate parameters.

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