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Formulation and Evaluation of Churna Treatment of Hypertension

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Abstract: Hypertension is a chronic non communicable disease and often asymptomatic medical condition in which the pressure exerted by the blood on the wall of the artery is elevated. It is chiefly of unknown aetiology, but genetic factors play significant role for its development. Uncontrolled hypertension is a risk factors for various pathological conditions such as heart attack, heart failure, stroke, retinal haemorrhage and kidney disease. In Ayurveda a number of medicinal plants and Ayurveda compound formulation have been prescribed by Ayurveda doctors for the treatment of hypertension. The therapeutic efficacy of those plants has also being verified by using modern pharmacological experimental models. This various clinical and experimental studies conducted in the last few decades on the plants showing anti-hypertensive property.

Keywords: Ayurveda, hypertension, medicinal plants, heart failure, stroke, retinol haemorrhage, kidney diseases

I. INTRODUCTION

Churna is defined as a fine powder of drug or drugs in Ayurvedic system of medicine. Drugs mentioned in patha, are cleaned properly, dried thoroughly, pulverised and then sieved. The churna is free flowing and retains its potency for one year, if preserved in an airtight containers. Triphala churna, Trikatu churna, Drakeshadi churna and Sudharsana churna are some of examples. Churna formulation are similar to powder formulations in Allopathic system of medicine. In recent days churna is formulated into tablets in order to fix the dose easily. These forms of medicament are prescribed generally because of their particle size. Smaller the particle size greater is the absorption rate from g.i.t and hence the greater is bioavailability. It is prescribed by the Ayurvedic physician for treating conditions such as diabetes, indigestion, constipation etc. Indigestion is a common ailment affecting the general population and in allopathy system antacids are commonly prescribed.

Blood pressure is the force that the heart contracts with, applying pressure on the artery wall.1 Adults with hypertension often have a systolic blood pressure of 140 mm Hg or higher or a diastolic blood pressure of 90 mm Hg or higher, which indicates abnormally high arterial blood pressure. Hypertension is a lifestyle condition.2. Its primary cause is unknown, although it could be related to an underlying illness like an endocrine or renal problem, which usually causes the left ventricle to enlarge and the artery walls to thicken.3. Uncontrolled hypertension increases the chance of developing a number of diseases, including renal disease, retinal hemorrhage, heart attack, heart failure, and stroke. The condition has been aptly dubbed the "silent-killer" since hypertension typically has no symptoms and is ignored in the majority of sufferers.5. One issue with global health is hypertension. In 2000, hypertension affected about 26.4% of adults worldwide (26.6% of men and 26.1% of women), and by 2025, 29.2% of adults (29.0% of men and 29.5% of women) were expected to have the condition. In 2000, there were an estimated 972 million adults with hypertension worldwide; 333 million of those were in affluent nations and 639 million were in developing nations. There will be 1.56 billion persons worldwide with hypertension in 2025, an approximate 60% increase from the current estimate.

II. EVALUATION OF PHYSICAL PARAMETERS:

1) Determination of pH [13] :-

1% solution of the formulated churna was tested using a pH meter (Elico pH meter) to measure its acidity or alkalinity.

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2) Determination of Moisture content [13]:-

The moisture content of the churna was determined using a halogen moisture determining apparatus (Mettler Toledo) to ensure it meets specified standards.

3) Determination of Ash Values [13]

I. Total Ash Value

2 grams of churna were precisely weighed and heated in a silica crucible until all carbon was burned off, leaving only the mineral residue (ash). The weight of the ash was then calculated per gram of the original air-dried material.

II. Acid Insoluble Ash Value

After obtaining the total ash, hydrochloric acid (HCl) was added to dissolve the ash, leaving behind insoluble matter. This residue was filtered, washed, and ignited to constant weight. The weight of the acid-insoluble ash was then determined.

4) Determination of Extractive Values [13]

I. Water Soluble Extractive Value

5 grams of churna were soaked in a mixture of chloroform and water for 18 hours. The filtered extract was evaporated and dried, then weighed to determine the amount of water-soluble extract present.

II. Alcohol Soluble Extractive Values

Similar to the water soluble extractive value, except ethanol was used as the solvent. The procedure was otherwise identical.

5) Determination Of Crude Fibre Content [14]

Two grams of accurately weighed churna were placed in a round-bottom flask, to which 100 ml of 0.128 M sulfuric acid was added. The mixture was refluxed for one hour, then filtered through ashless filter paper. The residue was washed with water until the filtrate became neutral. The residue was then weighed (a), ignited to ash, and the final weight of the ash (b) was determined. The difference between weights a and b represented the crude fiber content, calculated on a dry weight basis.

6) Determination of Heavy Metal Contamination

I. Arsenic Content [14]

To prepare a standard solution of 10 PPM, 0.33 grams of arsenic trioxide were dissolved in 5 ml of 2M sodium hydroxide solution and then diluted to 250 ml with water. One volume of this solution was further diluted to 100 volumes with water

PREPARATION OF SAMPLE

Preparation of Churna solution

The churna solution was prepared by diluting 1 gram of churna to 100 ml with distilled water. This solution is used for conducting limit tests for iron and lead, as well as a qualitative test for mercury. To perform the test, 10 ml of the churna solution was pipetted into a flask, and approximately 10 ml of concentrated nitric acid was added. The mixture was evaporated to dryness on a water bath, and the residue was dried at 130°C for 30 minutes. About 10 ml of hydrazine molybdate reagent was then added, and the mixture was refluxed for 20 minutes. After cooling, the absorbance of both the standard and test solutions was measured at 800 nm using a Perkin Elmer UV spectrophotometer.

II. Limit test for Iron [14]

To prepare a standard solution of 20 PPM, one volume of a 0.1726% w/v solution of ferric amononium sulfate was diluted to ten volumes with 0.05 M sulfuric acid using distilled water.

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PROCEDURE

Limit test was performed in Nessler's cylinder. 2ml of test and standard solutions were taken in separate cylinders and then 2ml of 20% solution of citric acid and 0.1 ml thioglycollic acid were added. The solution was then mixed and made alkaline with iron free ammonia, diluted to 50ml with distilled water. It was then allowed to stand for 5minutes and colour obtained in sample was compared with that of standard colour. If the colour produced in test is more when compared to that of standard solution then the sample was said to fail the limit test and said to pass the test if vice versa occurs.

III. Limit Test For Lead [14]

Preparation of Standard (20 PPM) 0.4 gm of lead nitrate was dissolved in water containing 2ml of nitric acid and sufficient water to produce 250ml. About 1volume of above solution was diluted to 10 volume using distilled water.

PROCEDURE

Limit test was performed in Nessler's cylinder. 1ml of standard lead solution and test solution were taken in separate cylinders and were diluted to 25ml using distilled water and then pH was adjusted to value 3-4 by adding dilute acetic acid or dilute ammonia solution and then diluted to 35ml using distilled water. To both the solutions 10ml freshly prepared hydrogen sulphide solution was added, mixed and diluted with water to 50ml. It was then allowed to stand for 5minutes and viewed downwards over white surface. The colour produced in test solution should not be more intense than that of standard solution, if so then the sample is said to pass the limit test for lead.

IV. Test for MercurTo 10 drops of test solution 6M Hcl was added to get a white precipitate. The precipitate was then treated with 6M ammonia solution. If the colour of precipitate changes to grey or black colour then it indicates the presence of mercury.

7) DETERMINATION OF MICROBIAL CONTENT

1gm of churna was dissolved in lactose broth and volume adjusted to 100ml with the same medium. About 10ml of sample was transferred into 100ml of Macconkey broth and incubated for 18-24 hours at 43-45° C. A subculture was prepared on a plate with Macconkey agar and incubated at 43-45° C for 18-24 hours. The growth of red, generally non-mucoid colonies of gram negative rods appearing as reddish zones indicates the presence of E.coli if not then it indicates the absence of E.coli.

Determination of Digestive Property

Preparation of Extract

About 100mg of accurately weighed quantity of churna was extracted with 20% aqueous glycerol and phosphate buffer (pH7.8) in 1:4 ratio and filtered and the filtrate was used as enzyme source. [15,16]. The standard sample was prepared similar to the test sample.

i. Amylolytic activity

Extract (1ml) of churna and GASTRAP were incubated separately for 15minutes at 27°C and added to 1ml of the substrate (soluble starch1% in phosphate buffer). The enzyme reaction was interrupted by the addition of 2ml of DNS reagent and heated for 5minutes. The absorbance was measured at 520nm. [17, 18].

III. RESULTS

The results of the physical parameter evaluation such as heavy metals, moisture content, ash values including total ash value, acid insoluble ash value, extractive values such as water soluble & alcohol soluble extractive values and crude fibre content were given in table 1 and detection of heavy metals such as arsenic, iron, lead and mercury in table 2. Finally the result of microbial detection was given in table

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Physical Parameter:

1 Angle of repose:

The angle of repose is the maximum angle between the surface of a pile of granular material and the horizontal plane at which the material will remain in place without sliding. Beyond this angle, the material will start to slide or flow. $\theta = \arctan(rh)$

Where:

 θ \theta θ = Angle of repose hhh = Height of the pile rrr = Radius of the base of the pile

2. Tap Density:

The tap density of churna was determined using a tap density apparatus after subjecting it to 50 taps. The apparatus measures the tapped volume of churna, from which the tap density is calculated as the weight taken divided by the tapped volume.

3. Bulk density:

To determine the bulk density of churna, 10 grams of the powder sample were placed into a graduated measuring cylinder and tapped on a wooden surface. The bulk density was calculated using the tap density volumetric flask, into which the 10g powder sample was transferred. The bulk density was determined using the following formula:

Bulk Density = Weight taken Bulk volume

4. Carr' Index= <u>Bulk density (Tapped) – Bulk density (Untapped) × 100</u> Bulk density (Tapped)

5. Hausner's Ratio: The formula used to determine Hausner's ratio we use bulk density and tap density ratio. For the determination of Hausner'ratio following formula:-

Hausner's Ratio =Bulk density (Tapped)/ Bulk density (untapped)

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Sr no	Name	Physical Parameter Value		
1	Determination of pH	5.357		
2	Moisture content	10.8 % w/w		
3	Ash Values			
	I. Total ash	10% w/w		
	II. Acid insoluble ash	5% w/w		
4	Extractive value			
	I. Water soluble extractive value	0.12% w/w		
	II. Alcohol soluble extractive	2% w/w		
5	Crude fiber content	9.75% w/w		

TABLE 1 EVALUATION OF PHYSICAL PARAMETERS OF CHURNA

Observation of Physical parameter:

Sr no	Parameter	Observation
1	Angle of Repose	30 degree
2	Bulk Density	0.581
3	Tapped Density	0.5250
4	Carrs index(%)	27.46
5	Hausners ratio	1.15

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Organalatic Test:

Sr. No	Test	Results		
1	Colour	Brown		
2	Odour	characteristic		
3	Taste	Astringent		
4	Appearance	Powder		
Table no 1				

Table no 4

IV. MATERIAL AND METHOD

Method

The raw materials such as Sapagandha, Ashwagandha, Punarnava, Vacha, Jeera, Ajmoda, Arjun were used for the formulation. The raw material were purchased from market and authenticated in laboratory. The authentification carried out based on the microscopic characteristic of powder drug.

Sr no	Ingredient	Quantity taken	116.95
51 110	mgreutent	Qualitity takeli	uses
1.	Ajmoda	5gm	Normal digestion
2	Arjuna	10gm	obesity
3	Ashwagandha	12gm	Immune System
4	Jeera	5gm	Indigestion
5	Sarpagandha	16gm	High blood pressure
6	Vacha	10gm	Dental and oral problem
7	Puranava	12gm	Anti-helmintic

V. INFORMATION AND PROPERTIES OF SELECTED DRUGS :

A. Ajmoda

Biological Sources: Apiumleptophyllum

Family: Apiaceae

Chemical composition: Meethers of thymol, Carvacrol, 3nB and Thymoquinol.

Parts use: Seeds

Distribution: Ajmoda, also known as celery, is native to many places, including Italy, Algeria, Sweden, Ethiopia, Egypt, and India. In India, it was introduced to Amritsar, Punjab from France in 1930

Mechanism of action:

In one animal study, 3nB appears to lower blood pressure by functioning as both a diuretic and vasodilator. It impacts the production of prostaglandins and acts similarly to calcium-channel blockers. Another study administered 75 mg of celery seed extract per capsule, with a dose of one capsule taken twice daily by patients with mild to moderate hypertension. After three weeks, there was a significant decrease in systolic blood pressure (SBP) by 4.6 mmHg (P<0.005) and diastolic blood pressure (DBP) by 4.5 mmHg (P<0.005) compared to baseline. After six weeks, the decrease in SBP and DBP was 8.9 mmHg and 8.5 mmHg, respectively (P<0.005). This demonstrates a statistically significant effect of celery extract in managing hypertension.

Uses:

Digestion: Ajmoda can treat gastrointestinal issues like indigestion, bloating, flatulence, and abdominal cramps. It can also help regulate bowel movements.

Lung health: Ajmoda can improve lung health and treat respiratory infections.

Kapha conditions: Ajmoda has healing properties for Kapha conditions.







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Fig no 1

B. Arjun

Latin name: Terminalia arjuna (Roxb.) W & A)

Family: Combretaceae

Chemical composition: Tannins, Triterpenoid Saponins, Flavonoids, Gallic acid, Ellagic acid, OPCs, Phytosterols, Calcium, Magnesium, Zinc and Copper

Parts use: Bark

Distribution :The Terminalia arjuna tree, also known as the arjun tree, is native to central and southern India and Sri Lanka. It's also found in other parts of the Indian subcontinent, including Uttar Pradesh, Bihar, Maharashtra, Madhya Pradesh, West Bengal, and Odisha.

Mechanism of action:- Numerous studies have elucidated the effects of Terminalia arjuna on various cardiac disorders, including myocardial infarction, angina pectoris, hypertension, congestive heart failure, and coronary artery disease. In a study involving 15 stable and 5 unstable angina patients over three months, the impact of T. arjuna bark powder on blood pressure, anginal frequency, body mass index (BMI), blood sugar, cholesterol, and HDL cholesterol was examined. The results showed a significant reduction in systolic blood pressure and BMI (p<0.05), a slight increase in HDL cholesterol, and marginal improvement in left ventricular ejection fraction in stable angina patients.

Uses: which has been used as a cardiotonic in heart failure, ischemic, cardiomyopathy, atherosclerosis, myocardium necrosis and has been used for the treatment of different human diseases like blood diseases, anemia, venereal and viral disease



Fig no 2

C. Ashwagandha

Latin Name:- Withania somnifera Linn.

Family :- Solanaceae

Chemical composition :- Cuseohygrine, Anahygrine, Anaferine, Isopellertierine, Withanolides, Withaferins, Saponins.

Distribution:W. somnifera (Syn: ashwagandha, suranjan, winter cherry, Indian ginseng) is a xerophytic plant that nurtures abundantly in Africa, the Mediterranean, Sri Lanka, Pakistan, and India (5-9). **Part uses** :- Root

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Mechanism of action: A study was conducted to compare the antihypertensive effects of Ashwagandha root powder taken with milk versus water. The systolic blood pressure of Group I and Group II was measured before and after supplementation. Initially, the mean systolic blood pressure was 164 mmHg for Group I and 157 mmHg for Group II. After supplementation, the mean systolic blood pressure decreased to 142 mmHg for Group I and 138 mmHg for Group II. Similarly, the mean diastolic blood pressure initially was 100.5 mmHg for Group I and 101.2 mmHg for Group II, which decreased to 85 mmHg for Group I and 92 mmHg for Group II post-supplementation. The decrease in diastolic pressure was significant in both groups, with Ashwagandha in milk being more effective than in water for reducing blood pressure in hypertensive patients.

In another study, the effects of Ashwagandha on the cardiovascular and respiratory systems were examined in dogs and frogs. The study found that the alkaloids had a prolonged hypotensive, bradycardic, and respiratory-stimulant action in dogs. The hypotensive effect was primarily due to autonomic ganglion blocking action and a depressant effect on the higher cerebral centers.

Uses:

reducing stress. improving sleep. boosting athletic performance. improving memory. increasing male fertility. reducing inflammation. managing blood sugar.



Fig no 3

D. Jeera (Black Cumin):-

Latin Name :-Cuminum cyminum

Family :- Apiaceae

Chemical Composition :- Thymoquinone, Dithymoquinone, thymohydroquinone, Thymol 32, Carvacrol, t-anethole and 4-terpineol. Hypotensive action of nigella is mainly due to its volatile oils.

Part uses :- Seeds

Distribution:Black cumin (Nigella sativa) is native to southern Europe, northern Africa, and southwest Asia, but has become naturalized in many other areas, including parts of Europe and as far east as Myanmar.

Mechanism of action :- In an animal study, an oral dose of Nigella sativa extract (0.6 ml/kg/day) and furosemide (5 mg/kg/day) significantly increased diuresis by 16% and 30%, respectively, after 15 days of treatment. The urinary excretion of Cl-, Na+, K+, and urea also increased. Additionally, the mean arterial pressure decreased by 22% in the Nigella sativa-treated rats and by 18% in the nifedipine-treated rats (0.5 mg/kg/day). In conclusion, the diuretic activity observed in SHR rats treated with Nigella sativa seeds may contribute to its antihypertensive action. However, other pathways may also be involved in its cardiovascular effects.

Uses:

Antimicrobial Properties: Known for its antimicrobial and antifungal properties, making it useful in combating infections.

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Anti-inflammatory Effects: Has anti-inflammatory properties that may help in managing conditions like arthritis. Immune Support: Used to support immune function and promote overall well-being.

Antioxidant Activity: Contains compounds that act as antioxidants, protecting cells from damage caused by free radicals.



Fig no 4

E. Punarnava :-

Latin Name :- Boerhavia diffusa Linna.

Family :- Nyctaginaceae

Chemical Composition :- Lirridodendrin & Hypoxanthine.

Part uses :- Root

Distribution: Punarnava (Boerhavia diffusa) is a perennial herb that's commonly found throughout India, especially in warmer regions and up to 2,000 meters in the Himalayas.

Mechanisum of action :-

In a clinical trial, 250 mg of Punarnava extract was administered orally in a dose of 2 capsules twice a day with water for six weeks. The results showed a statistically significant reduction in both mean systolic and diastolic blood pressure. Before treatment, the mean systolic blood pressure (SBP) was 151.48 ± 5.75 mmHg, which decreased to 137.33 ± 5.23 mmHg. Similarly, the mean diastolic blood pressure (DBP) before treatment was 95.41 ± 2.06 mmHg, which reduced to 87.11 ± 4.75 mmHg. This antihypertensive effect is attributed to the active compounds liriodendrin, hypoxanthine, and boeravinones in Punarnava, which act as calcium channel antagonists. Additionally, Punarnava acts as a diuretic by increasing renal blood flow through the relaxation of the smooth muscles in the arterial walls.

Uses:

Anti-microbial: It possesses antimicrobial properties that may help in fighting infections.

Anti-oxidant: Punarnava contains antioxidants that can help in neutralizing free radicals in the body, potentially reducing oxidative stress.

Skin Disorders: It is sometimes used externally or internally to manage various skin conditions due to its antiinflammatory and detoxifying properties.

Respiratory Health: In Ayurveda, Punarnava is also used to support respiratory health, especially in conditions like asthma and bronchitis.





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Fig no 5

F. Vacha

Latin Name :- Acorus calamus L.

Family :- Acoraceae

Chemical composition :- Beta-Asarone, Beta-Gurjunene, Asarone. Sequesterpenes, Beta-Daucosterol, Xylose, D-Galacturonic Acid.

Distribution: Vacha, also known as Acorus calamus, is a semi-aquatic herb that grows in wet areas of India, including near rivers and rice fields, up to altitudes of 1,800 meters. It's also found in tropical and temperate regions of 42 countries worldwide.

Part uses :- Rhizome

Mechanism of action :-In normotensive rats under anesthesia, intravenous administration of crude extract of A. calamus caused a decrease in mean arterial pressure (MAP). The percent decrease in MAP at doses of 10, 30, and 50 mg/kg was $18.86\pm0.48\%$, $27.50\pm0.97\%$, and $42.25\pm1.0\%$, respectively, with statistically significant differences (P<0.05) observed at doses of 30 and 50 mg/kg compared to 10 mg/kg. This indicates that the crude extract has a combination of effects, likely mediated through calcium channel antagonism and a nitric oxide pathway. Calcium channel antagonists prevent the intracellular influx of calcium, resulting in smooth muscle relaxation and a decrease in heart rate, which contribute to lowering blood pressure. Similarly, nitric oxide induces vasodilation, which is essential for controlling blood pressure.

Uses:

Nervous System Support: Vacha is known for its ability to stimulate and rejuvenate the nervous system. It is believed to improve concentration, memory, and overall cognitive function.

Digestive Aid: It is used to treat digestive disorders such as indigestion, bloating, and flatulence. Vacha is thought to enhance digestion by stimulating the digestive fire (agni).

Respiratory Health: In Ayurveda, Vacha is used to treat respiratory conditions such as asthma, bronchitis, and cough. It is believed to have expectorant properties that help in clearing phlegm from the respiratory tract.



G. Sarpagandha :-Latin Name :- Rauwolfia serpentina Benth ex. Kurz

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Family :- Apocynaceae

Chemical composition :- Ajmalidine, ajmaline, ajmalinine, Ajmalicine, Rauwolfinine, Recanescine, Rescinnamine, Reserpiline, Reserpine, Reserpine, Sarpagine, Serpentine, Serpentinine, Thebaine, Vohimbinine. Part uses :- Root

Distribution: It is widely distributed in India, including the sub-Himalayan region, the lower hills of the Gangetic plains, the eastern and western Ghats, and some parts of central India

Mechanism of action :-

In a clinical trial evaluating Rauwolfia serpentina in essential hypertension, 50 patients with initial blood pressure greater than 160/95 mmHg were treated. Tablets containing dried root of R. serpentina were administered in optimal doses. Within one week of treatment, 77% of cases showed a reduction in systolic blood pressure ranging from 2 to 38 mmHg, with an average reduction of 13 mmHg. A decrease of 10 mmHg or more was observed in 40% of cases, with reductions ranging from 2 to 18 mmHg and an average of 6 mmHg. A diastolic response of 5 mmHg or more was noted in 35% of cases. After four weeks, 85% of cases showed a reduction in systolic blood pressure ranging from 2 to 54 mmHg, with an average reduction of 21 mmHg. A systolic reduction of 10 mmHg or more was noted in 74% of cases. Diastolic pressure showed a reduction of 4 to 34 mmHg in 81% of cases, with an average reduction of 11 mmHg. A diastolic reduction of 5 mmHg or more was observed in 72% of cases. Significant reductions in both systolic and diastolic pressure levels were seen in 62% of cases. The hypotensive effects of the drug persisted for up to 4 weeks after discontinuation, with 91% of patients still showing effects at 2 weeks and 75% at 4 weeks. No serious adverse side effects were reported.

Another study evaluated the effects of oral reserpine on a group of hypertensive patients in an outpatient clinic. Fifteen patients with initial blood pressure ranging from 160/98 to 240/150 mmHg received reserpine from CIBA Pharmaceuticals at a dosage of 20 mg twice a day. The results showed an average reduction in systolic blood pressure of 30.7 mmHg and an average reduction in diastolic blood pressure of 19 mmHg. Some patients reported transient side effects such as nausea, fainting, and dyspnea. The researchers concluded that reserpine was a potent and beneficial agent for some patients with both severe and mild hypertension.

Uses:

•Analgesic: It has mild analgesic properties and can be used to alleviate pain.

·Antispasmodic: Sarpagandha is used to relieve muscle spasms and cramps.

•Respiratory Disorders: In traditional medicine, it is used to treat respiratory disorders such as asthma and bronchitis.



Fig no 7

Procedure of Churna: The drugs are cleaned and dried properly. Make fine powder and seived. Weight accurately and all ingredient mixed together (seived 80) Fine powder has better therapetic value It should be stored in air tight container.

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Fig no 8

V. CONCLUSION

Hypertension is a leading global health issue and a major cause of death in both developed and developing countries. Despite the increasing number of patients suffering from hypertension, conventional medicine has not been entirely successful in its treatment. Over recent decades, Ayurvedic medicinal plants have shown effectiveness in lowering blood pressure and improving heart function. While some drugs have been experimentally proven effective, many others await discovery. This review article documents several medicinal plants and their modes of action that have been reported effective in managing hypertension within Ayurveda. Sarpagandha, Vacha, Arjuna, Ashwagandha, Jeera, Ajmoda, and Punarnava are among the most researched and frequently utilized plants for hypertension treatment. Scientific evidence supports the use of these plants in disease management. Numerous studies conducted worldwide indicate substantial potential for herbal medicine in controlling hypertension. This review aims to serve as a foundation for further scientific exploration into the use of various plants for treating health issues. Such studies will likely bolster the adoption of traditional remedies, supported by robust scientific evidence.

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