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Preparation and evolution of poly herbal formulation For Arthritis

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Abstract
Arthritis is a chronic, inflammatory, systemic autoimmune disease, affecting the joints with varying severity among patients. The risk factors include age, gender, genetics, and environmental exposure. The objective of present study was to evaluate the anti-inflammatory activity of selected herbs by in-vitro protein denaturation. All the herbs present in the study are being used for the treatment of Arthritis since thousands of years and documented in many scientific studies. Selected herbs have different medicinal value and also having a nutritional value and due to that they will be fights against the arthritis by different mechanism. Herbal formulation shows the good therapeutic effect with minimum side effects as compared to the allopathic medication. The Hydro-alcoholic extracts in different concentration like 100 µg/ml, 200 µg/ml, 400 µg/ml, and 800 µg/ml of the selected herbs namely *Adansonia digitata, Moringa oleifera, Rubia cordifolia,* and *Trapa natans* incubated with bovine serum albumin for complete protein denaturation and the result was assess spectrophotometrically at 660nm against the standard diclofenac sodium. The percentage inhibition of protein denaturation for individual herb extract at 800 µg/ml showed higher activity so further the mixture of herb extract in equal proportion was prepared and evaluated at 800 µg/ml and it showed the more potent and significant anti-inflammatory activity. As the result of in-vitro study shows significant difference in protein denaturation, on the basis of that poly-herbal syrup was prepared and evaluated for its physiological parameters.

Keywords: Arthritis, inflammation, bovine serum albumin, protein denaturation, *adansonia digitata*, *moringa oleifera*, *rubia cordifolia*, *trapa natans*, diclofenac sodium, poly-herbal syrup

Introduction
Arthritis is a chronic autoimmune disease. Arthritis often affects pairs of joints and can affect more than one joint, including the small joints in the wrists and hands. In which the addition to joint pain and stiffness, people with arthritis may also have symptoms such as weight loss, low-grade fever, and fatigue. It causes the joints to swell and can result in pain and progressive loss of function. Over time, other joints could be affected such as shoulders, elbows, knees, feet, and ankles.

Most common symptoms of a person with arthritis may experience are stiffness in the morning and pain and swelling of joints-in the same joint on both sides of the body. Some possible causes including:

- **Genetics:** People with family members who have arthritis may be more likely to get it
- **Hormones:** Female hormones also play role in diseases
- **Viruses or bacteria:** Arthritis may be related to viruses or bacteria that you come in contact with during your life

Arthritis occurs when the system attack the synovial the lining of the membranes that surround the joints the resulting is inflammation thickness the synovial, which can eventually destroy the cartilage and bone within the joint. The tendons and ligaments are hold the joint together weaken and stretch. In case of the joint losses its shape and ligament.

Arthritis is a musculoskeletal system disorder following mechanical and biological events that can be destabilize normal coupling between degradation and synthesis within articular cartilage. In witch the Arthritis can affected individuals of any age but is more predominant in the age range of 25 and 50 years with a peak in the age range of 40-50 years. 100 types of Arthritis which of the most commonly occurring include in arthritis, systemic lupus erythematous and juvenile arthritis. Main tow type of arthritis that is osteo-arthritis and rheumatoid arthritis in which the Indian population 22-39% and 5%, respectively. The worsening condition of the arthritis is requires proper therapy for arthritis along with the economical consideration for chronic treatment.

In witch the systematic drug for the arthritis is also available in the market there is use is limited due to serious side effect upon chronic use.

Traditionally herbal plant used both externally as well as internally for treating inflammatory conditions. In which the positive influence of herbal drug in modifying pathophysiology of the arthritis that case the result is substantial increase in their use as a treatment of arthritis. While the numbers of drugs are available to effectively reduce chronic joint inflammation in case of arthritis there should be prove their therapeutic effects through basic scientific research. This paper deals with the review on herbs showing potential for the treatment of arthritis.

Adansonia digitata

Various part of the plant are used to treat all diseases. *Adansonia digitata* leaves used for the control of inflammation and Plant fruit pulp is also reported to control the arthritis and this plant is also used for the nutritional purpose. [4] Chemical constituent present in seed are Carbohydrates, lipids, proteins and vitamins, fruit and leaves contains quercetin, 7 o-xylopyranoside and ß-sitosterol etc. It gives the main anti-inflammatory effect on arthritic condition.

***Moringa oleifera*:** Aurantiamide acetate and 1, 3-dibenzyl urea, isolated from roots shows this anti- inflammatory activity. Leaves, seeds and flowers of *Moringa oleifera* also give anti-inflammatory activity.

Rubia cordifolia: root extract used for arthritis condition. Anti-arthritic property is established regarding its anthraquinone reach fraction. It can be useful in all inflammatory conditions and increases the immunity. It is also used as a blood purifier, anti-oxidant and anti-inflammatory activity. Chemical constituent present are Purpurin, Munjistin, Xanthopurpurin.

Trapa natans*:* It can be used rheumatoid arthritis and other inflammatory disease will result in decreased blood vessel formation in cartilage, specifically joins, resulting in increased mobility and flexibility in this region. Chemical constituent Carbohydrate, phytosterols, saponins, fixed oil and fat in seeds extracts and pericarp extract of fruits.

Herbal formulation

An herbal formula consists of a selective combination of individual herbal ingredients those are formulated for a specific ailment or group of disease-conditions. Then the herbs are combined together, they become more potent and effective within the body than single herb due to their activating or catalyzing influence upon one another. These combinations acts as powerful catalysts in which order to activate over own individual healing energies which permeate the entire organism and reside in each and every cell in our bodies.

Review of literature

Review of Current and Future Scenario of Herbs and Herbal Products

The WHO has recently defined traditional medicine as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine it still use in today herbal preparation. Traditional preparations comprise medicinal plants, minerals and organic matter etc. Herbal drugs constitute only primarily use medicinal plant preparations for therapy. The classical Indian texts included the Rigveda, Atharvaveda, Charak Samhita and Sushruta Samhita.

World Health Organization has set specific guidelines for the assessment of the safety, efficacy, and quality of herbal medicines. WHO estimates that 80% of the world populations presently use herbal medicine for primary health care? Exceptionally, in some countries herbal medicines may also contain by tradition, natural organic or inorganic active ingredients which are not of plant origin. Herbal medicine is a major component in traditional medicine and a common element in ayurvedic, homeopathic, naturopathic and other medicine systems.

Ayurveda is a medical system primarily practiced in India that has been known form early 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment. Ayurveda which literally means knowledge (Veda) of life (Ayur) had its beginning in Atharvaveda. Charak Samhita and Sushruta Samhita are the two most famous treatises of Ayurveda several others were compiled over the centuries such as Bella Samhita, Kashyap Samhita, Agnivesh Tantra, Vagbhata's Ashtang hridaya, Madhava Nidan.

Herbals are traditionally considered as harmless since they belong to natural sources. Herbal formulations have reached widespread acceptability as therapeutic agents like antimicrobial, anti-diabetic, anti-fertility, anti-aging, anti-arthritic, sedative, antidepressant, anti-anxiety, antispasmodic, analgesic, anti-inflammatory, anti-HIV, vasodilator, and hepatoprotective, treatment of cirrhosis, asthma, acne, impotence, menopause, migraine, gall stones, chronic fatigue, Alzheimer's disease and memory enhancing activities. The use of herbal medicine due to toxicity and side effects of allopathic medicines, has led to sudden increase in the number of herbal drug manufacturers. For the past few decades, herbal medicines have been increasingly -consumed by the people without prescription. Seeds, leaves, stems, bark, roots, flowers, and extracts of all of these have been used in herbal medicine over the millennia of their use. Many herbs have undergone changes in their uses. Studies conducted on the herbs and their effects keep changing their potential uses.

Herbal Medicine Today

Chemists have analyzed the components of herbs, then isolated and extracted the healing properties. The chemical moiety responsible for efficacy was synthesized in modern laboratories so they can be incorporated into to the modern medicines. Several herbal preparations are made in to pills, tablets and capsules, but still have the same benefits derived from natural herbs.

Recently, herbal medicines or herb used for various purpose like Nutraceutical which prevent disease or optimum nutrition, Cosmeceuticals which includes use of herbs in cosmetics as they give better effect and least side effect and bio-pesticides which are biologically or herbs used widely as having no side effect or toxic effect on consumers health a brief introduction about Nutraceutical, Cosmeceuticals and pesticides given below including classification Serbs and marketed preparation.

Nutraceuticals

Is a broad umbrella term that is used to describe any product derived from food sources with extra health benefits in addition to the basic nutritional value found in foods? Nutraceutical, a portmanteau of the words "nutrition" and

"pharmaceutical", is a food or food product that reportedly provides health and medical benefits, including the prevention and treatment of disease. A product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic disease.

Traditional nutraceutical are simply natural, whole foods with new information about their potential health qualities. There has been no change to the actual foods, other than the way the consumer perceives them. Example includes lycopene in tomatoes, omega-3 fatty acid in salmon.

Non Traditional Nutraceuticals, are foods resulting from agricultural breeding or added nutrients and/or ingredients, to boost their nutritional values. Examples include β carotene-enriched rice, and soybeans, orange juice fortified with calcium, cereals with added vitamins or minerals.

Prebiotics are "good" bacteria that help keep your digestive system healthy by controlling growth of harmful bacteria. 'Probiotics' mean 'for life' and are defined as live microorganisms, which when consumed in adequate amounts, confer a health effect on the host. They are friendly bacteria that promote healthy digestion and absorption of some nutrients.

Soluble fiber, which dissolves in water, is readily fermented in the colon into gases and physiologically active byproducts, and can be prebiotic and viscous.

Dietary fiber or roughage is the indigestible portion of food derived from plants. It has two main components:

- Soluble fiber, which dissolves in water, is readily fermented in the colon into gases and physiologically active byproducts, and can be prebiotic and viscous.
- Insoluble fiber, which does not dissolve in water, is metabolically inert and provides bulking. Bulking fibers absorb water as they move through the digestive system, easing defecation.

Cosmeceuticals

Cosmetology is defined as the science of alteration in the appearance. Cosmeceuticals are cosmetic products with biologically active ingredients purporting to have medical or drug-like benefits. Herbal Cosmetics, referred as Products, are formulated, using various permissible cosmetic ingredients to form the base in which one or more herbal ingredients are used to provide defined cosmetic benefits only, shall be called as "Herbal Cosmetics".

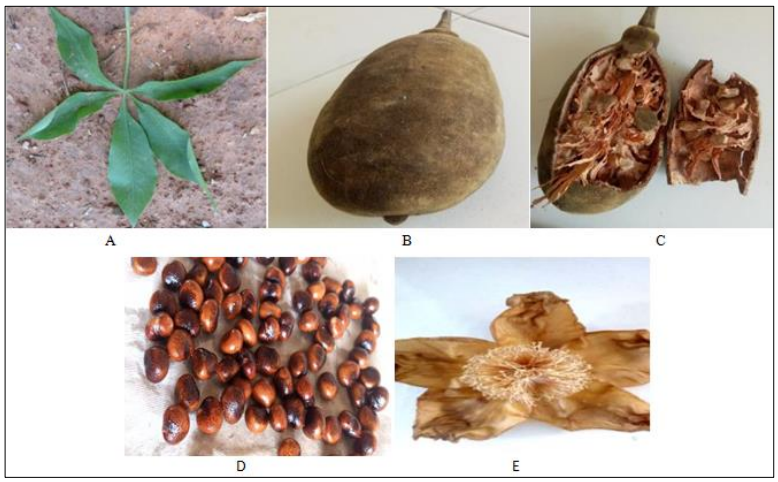

Fig 1: Parts of the plant in *Adansonia digitata*

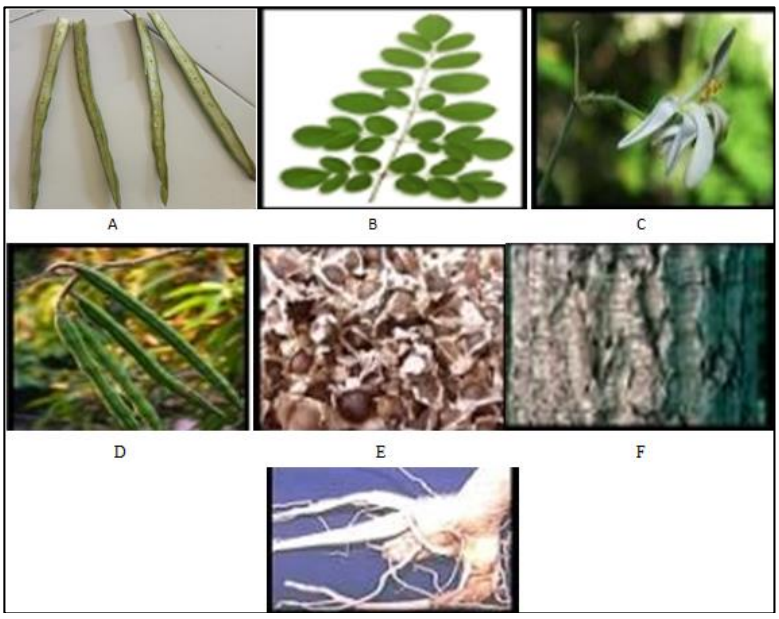

Fig 2: Parts of the plant in *Moringa oleifera.*

Fig 3: Parts of the plant in *Rubia cordifolia*.

Fig 4: Parts of the plant in *Trapa natans*.

Material and Method

Collection of plant material

The sample of *Adansonia digitata* has been collected from Sir Prbhasankar Patani institute of science collage, Bhavnagar *viz*. *Moringa oleifera* and *Trapa natans* has been collected from regular market. *Rubia cordifolia* is collected from lallu Vrajlal Gandhi & Sons Gandhinagar.

Authentication

The procured samples of individual herbs is authenticated by comparing morphological and microscopical characters. Morphology of the plant material has been done by evaluation of drugs by colour, odour, taste, size, shape and features like touch, texture, etc.

Microscopical of the plant material

It is used for qualitative evaluation of crude drugs in entire and powdered forms. The effective results, various reagents or stains could be used to distinguish cellular structure. Small quantities of drugs in powder from or a section of the drug is taken and a drop of phloroglucinol and concentrated hydrochloric acid give red stain with lignin. A study uses very thin section of drugs. The characteristic of cell wall, cell contents, starch grains, calcium oxalate crystals, trichomes, fibers, vessels, etc. has been measured.

Evaluation Parameters

Physicochemical parameters

Ash values

Ash values give approximate idea about purity and identity of the crude drugs. It is the measure of inorganic content of the crude drug present as physiological material or as adulteration.

Procedures

Total ash: Place about 2-4g of the ground air-dried material, accurately weighed, in a previously ignited and tarred crucible (usually of platinum or silica). Spread the material in an even layer and ignite it by gradually increasing the temperature up to 500-600 ^{0}c until it was white, indicating the absence of carbon, cool in a desiccator and weigh. If carbon free ash cannot be obtained in the manner, cool the crucible and mowasten the residue with about 2ml of water or a saturated solution of ammonium nitrate. Dry on water bath, then on a hot plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes, and then weight. Allow the residue to cool in a suitable desiccator for 30 minutes, and then weigh without delay. Calculate the content of total ash in mg/g of air-dried material.

$$\text{Total cash value} = \frac{\text{Weight of residue obtained}}{\text{Weight of the sample taken}} \times 100$$

Acid insoluble ash: The total ash was boiled with 25 ml of dilute hydrochloric acid for 5 minutes, insoluble matter was collected on an ashless filter paper, washed with hot water and ignited, cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated.

$$\text{Acid insoluble ash} = \frac{\text{Weight of residue obtained}}{\text{Weight of the sample taken}} \times 100$$

Water soluble ash: The water soluble ash obtained from the total ash was boiled for 5 minutes with 25 ml of distilled water insoluble matter was collected in an ash less filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450 ^{0}c. The percentage of water soluble ash was calculated.

$$\text{Water soluble ash} = \frac{\text{Weight of residue obtained}}{\text{Weight of the sample taken}} \times 100$$

Extractive Values

The extractive values obtained by exhausting the crude drugs with solvent are indicative of the approximate measure of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents used for determination of extractive values.

Water soluble extractive value: 5 g of the air dried coarsely powdered drug was macerated with 50 ml of methanol (95%) in a closed flask for 24 hours, shaking frequently (or in a mechanical shaker) during 6 hours and allowed to stand for 18 hours. Rapidly filtered, taking precautions against loss of solvent, evaporated 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish and dried at 105oc to constant weight and weighed. The percentage of water soluble extractive was calculated.

$$\text{Water soluble extractive value} = \frac{\text{Weight of the dried extract}}{\text{Weight of the sample taken}} \times 100$$

Alcohol soluble extractive value: Procedure for water soluble extractive was followed for the determination of

alcohol soluble extractive but the solvent used is 90% ethanol instead of alcohol.

$$\text{Water soluble extractive value} = \frac{\text{Weight of the dried extract}}{\text{Weight of the sample taken}} \times 100$$

Preliminary phytochemical screening
The crude raw material is subjected to preliminary phytochemical tests as per Phytochemical Methods.

Alkaloids
To the extract, added few drops of bismuth iodide solution (dragendorff's reagent), reddish brown color has been observed, which indicates the presence of alkaloids.

Carbohydrates
In a test tube containing ethanolic extract of powdered drug, added 2 ml of distilled water and 2 drops of freshly prepared 20% alcoholic solution of alpha napthol. Mixed well and added 2 ml of concentrated sulfuric acid along the side of the test tube. Formation of red violet ring is observed at the junction of two layers, which disappears on addition of excess alkali solution, which confirms the presence of carbohydrates.

Glycosides
Extracted 200 mg of drug with 5 ml dilute sulfuric acid by warming on a water bath filtered it and neutralized the acid extract with 5% solution of sodium hydroxide. Added 1 ml of Fehlings's solution A and B until it became alkaline and heated on a water bath for 2 minutes. Formation of red precipitate has been observed, which indicates the presence of Glycosides.

Phenol
Dissolved a small quantity of ethanolic extract of the drug with 2 ml of distilled water, added a few drops 10% aqueous ferric chloride solution. A blue or green color has been produced, which indicates presence of phenol.

Proteins (Biuret's test)
To 1 ml of ethanolic extract of the drug, 5 to 8 drops of copper sulphate solution (10%) has been added. Formation of violet color has been observed, which indicates the presence of proteins.

Saponins
To 5 ml of ethanolic extract of the drug, added a few drops of sodium bicarbonates solution. Shake the mixture vigorously and left for 3 minutes. Honey comb like froth developed, which indicates the presence of saponins.

Tannin
The substance has been mixed with basic lead acetate solution formation of white precipitate has been observed, which indicate presence of tannin.

Steroids
Treated the extract with few drops of acetic anhydride, boiled and cooled, and added concentrated sulfuric acid from the side of the test tube. A brown ring has been formed at the junction two layers and upper layer turns green, which shows presence of steroids.

Flavones (Shinoda test)
To the extract in alcohol few magnesium turnings and few drops of concentrated hydrochloric acid has been added and boiled for 5 minutes and red coloration has been observed, which shows for the presence of Flavones.

Triterpenoids
Treated the extract with few drops of concentrated sulfuric acid, formation of yellow color has been observed, which shows the presence of Triterpenoids.

Quantitative Estimation of Phytochemical
Quantitative Estimation of Total Alkaloids
5 g of the powder were weighed into a conical flask and 25 ml of alcohol, covered and allowed to stand for 4 h. This was filtered and take 10 ml extract in separating funnel add the 10 ml water, 10 ml 0.1 N H_2SO_4, 10 ml Chloroform. Transfer the organic layer in other separating funnel. Wash the layer with 10 ml 0.1 N H_2SO_4 .shake and allow to separate. Recheck the chloroform layer and acid wash give to mother liquor. Basify acidic solution with ammonia till blue colure with litmus paper. Extract with 10+10+10 ml of chloroform till complete extraction achieve. Than test with Mayers reagent. Wash the chloroform extract with water (10 ml) and transfer into distillation flask to remove the chloroform. Dissolve residue 5 ml of neutral alcohol. Evaporating the solution to dryness on boiling water bath. Distilled residue in 2 ml chloroform and 0.02 N H_2SO_4 (20 ml). Heat on boiling water bath to remove chloroform. Cool and back titrate excess of with 0.02 N NAOH using 3.4 drops of methyl red as an indicator. Colour change red to orange to yellow. Calculate the % of drug.

Quantitative Estimation of Total Saponins
2 g powders were weighed and 10 ml of alcohol added. Then the sample was heated and then over a hot water bath for 4 hours with continuous stirring at about 55º C. Mixture was filtered and the residue re-extracted with another 20 ml of alcohol. The combined extract was reduced to 40 ml over water bath at about 90º C. Concentrate was treated with 2 ml of diethyl ether and the aqueous layer was recovered while the ether layer was discarded. This process of purification was repeated three times and then 6 ml of n- Butanol was added and extracted. Than the n-Butanol extract obtained was then washed two times with 1 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath for evaporating the solvent. After evaporation the samples were dried in the oven to a constant weight and the Saponins content was calculated as percentage.

Quantitative estimation of Total Tannin
The tannin were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin- Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. Mixture was shaken and kept at room temperature for 30 min. a set of reference standard solutions of tannic acid (20, 40, 60, 80,100 µg/ ml) were prepared. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/ Visible spectrophotometer. The estimation of the tannin content was carried out in triplicate. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample.

Quantitative estimation of Total Flavonoids

The aluminum chloride method was used for the determination of the total flavonoid content of the sample extracts. Aliquots of extract solutions were taken and made up the volume 3ml with methanol. Then 0.1ml AlCl3 (10%), 0.1ml Na-K tartarate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30 minutes of incubation. Quercetin was used as a standard compound in the range of 2-12 mg/ml concentration to construct a standard curve.

Quantitative estimation of Total Phenolic
Preparation of standard solution
The total phenolic content was determined using Folin Ciocalteu reagent. A standard calibration curve was prepared and the absorbance against concentration of tannins at 765nm. Gallic acid was used as a standard and the total phenolic content was expressed as µg/ml gallic acid equivalents. Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in alcohol. 2ml each take and adding the 10ml distilled water and 1.5ml foline Ciocalteu reagent [1:2]. Then add the 4ml 20% Na_2Co_3. Up to 25 ml with distilled water. Rest for 40 minutes.

Preparation of Test solution
1. 5 g powders were weighed and 10-15ml of alcohol is added. Reflux for 1 hour filter drug solution and evaporate to dryness. 1000 µg/ml prepared the stock solution 2 ml of pipette out make up to 10 ml [200 µg/ml] than 1ml pipette out make up to 10 ml [20 µg/ml] than adding the 2ml each take and adding the 10ml distilled water and 1.5ml foline Ciocalteu reagent [1:2]. Then add the 4ml 20% Na_2Co_3. Up to 25 ml with distilled water. Rest for 40 minutes.

Inhibition of protein Denaturation
Chemicals and Instruments
Bovine serum, Diclofenac sodium, Phosphate buffer, UV Spectrophotometer.

Anti-inflammatory activity (Protein denaturation) using Bovine serum albumin
- **0.5% Bovine Serum Albumin (BSA):** Dissolved 500mg of BSA in 100 ml of water.
- **Phosphate Buffer Saline PH 6.3:** Dissolved 8 g of sodium chloride (Na-Cl), 0.2 g of potassium chloride, 1.44 g of disodium hydrogen phosphate, 0.24 g of potassium dihydrogen phosphate in 800 ml distilled water. The pH has been adjusted to 6.3 using 1N HCl and make up the volume to 1000 ml with distilled water.
- **Test solution** (0.5 ml) consists of 0.45ml of Bovine serum albumin (0.5% w/v aqueous solution) and 0.05 ml of test solution of various concentrations.
- **Standard solution** (0.5 ml) consists of 0.45ml of Bovine serum albumin (0.5% w/v aqueous solution) and 0.05 ml of Diclofenac sodium of various concentrations.

Procedure
- The reaction mixture (5ml) consisted of 0.2ml of bovine serum albumin 2.8ml phosphate buffered saline (6.4 pH) 2 ml of varying concentration of extract.
- Final concentration becomes 100 µg/l, 200 µg/l, 400 µg/l, 800 µg/l.
- Mixture were incubated at 37±2 ^{0}C in a BOD incubator for 15 min heated at 70 ^{0}C for 5 min.
- After cooling absorbance was measured at 560 nm vehicle as blank.
- In control 0.05 ml distilled water has been used instead of test extract and diclofenac sodium has been used as the standard.

$$\text{Percentage inhibition} = \frac{(\text{Abs Control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Abs control = Absorbance of control.

4.7 Preformulation studies of herbs powder for syrup preparation
Prior to the development of the major dosage forms, it is essential that fundamental Physical and chemical properties of the drug molecule and other derived properties of the drug Powders are determined. This information decides many of the subsequent events and approaches in formulation development. This first learning phase is known as Preformulation.

Definition
Preformulation involves the application of bio pharmaceutical principles to the Physicochemical parameters of drug substance are characterized with the goal of designing Optimum drug delivery system. Before beginning the Preformulation programs the Preformulation scientist must consider the following factors:
- The amount of drug available.
- The Physicochemical properties of the drug already known.
- Therapeutic category and anticipated dose of compound.
- The nature of information, a formulation should have or would like to have.

4.7.1 Determination of the herbs powder parameters:
- Bulk density
- Tapped density
- Compressibility index
- Hausner's ratio
- Angle of repose
-

Bulk density (ρb)
It is determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder or through a volume measuring apparatus into a cup. It is expressed in g/ml and is given by,

$\rho b = M/Vo$

Where
M- Is the mass of powder?
Vo- Is the bulk volume of the powder.

The inter particle interactions that influence the bulking properties of a powder are also the interactions that interfere with powder flow, a comparison of the bulk and tapped densities can give a measure of the relative importance of these interactions in a given powder. Such a comparison is often used as an index of the ability of the powder to flow.

Tapped density (ρt)
It is achieved by mechanically tapping a measuring cylinder containing a powder sample. After observing the initial

volume, the cylinder is mechanically tapped and volume readings are taken until little further volume change is observed.
The mechanical tapping is achieved by raising the cylinder and allowing it to drop under its own weight at a specific distance.
The tapped volume was measured by tapping the powder to constant volume. It is expressed in g/ml and is given

$\rho t = M/Vt$

Where
M - Mass of powder and
V_t- Tapped volume of the powder.

Compressibility index: (CI)
Compressibility is the ability of powder to decrease in volume under pressure. Compressibility is a measure that obtained from density determination. Weighed quantity of granules has been transferred to 50 ml graduated cylinder, volume occupied by granules has been noted down. Then cylinder has been subjected to 500/ 750 and 1250 taps. The difference between two tabs should be less than 2%. The percentage Compressibility Index is calculated by using formula.

$$\mathbf{CI} = \frac{\text{Vo-Vi}}{\text{Vo}} \times 100$$

Where, Vo- Untapped density; Vi- Tapped density

Hausner's Ratio
It is measurement of frictional resistance of the granular material. The Ideal range should be 1.2 -1.5, it has been determined by the ratio of tapped density and bulk density.

Hausner's Ratio = Vi / Vo

Where
Vo -Untapped density
Vi -Tapped density

Angle of repose
The tangent of angle of repose is equal to the coefficient of friction between the particles. Hence the rougher and more irregular the surface of particles, the greater will be angle of repose. For determination of angle of repose (Ɵ), the blends have been poured through the walls of a funnel which has been fixed at a position such that its lower tip has been at a height of exactly 2.0 cm above a hard surface. The drug or the blends have been poured till the time when upper tip of the pile surface touched the lower tip of the funnel. Angle of repose was calculated using following equation.
The angle of repose Ɵ was calculated by the formula,

Tan Ɵ = h/r,
Ɵ = tan-1 (h/r)

Where
Ɵ - Angle of repose
h- Height in cm and
r- Radius in cm.
Based on the Angle of repose, Compressibility index and Hausner's ratio, the flow property of the granules can be characterized.

4.8 Preparation of syrup
The crude drugs, dried fruit of *Adansonia digitata*, dried roots of *rubia cordifolia*, pods of *Moringa oleifera*, dried fruit of *Trapa natans*, were *Moringa oleifera and Trapa natans* has been collected from the local market and *Rubia cordifolia* has been collected from lallu Vrajlal Gandhi & Sons Gandhinagar. *Adansonia digitata* has been collected from Sir Prbhasankar Patani institute of science collage, Bhavnagar *viz*. Their identity has been confirmed by correlating their morphological and microscopical characters with those given in literature.

Development of herbal syrup
1. Local formulation
Adansonia digitata: 2gm
Rubia cordifolia: 0.6gm
Moringa oleifera: 2gm
Trapa natans: 0.01gm

2. Method of preparation of decoction
5 g of the prepared formulation has been taken and mixed with 40 ml of water. The mixture was boiled until total volume become one fourth of the initial volume. Then the decoction was cooled and filtered. Filtrate has been taken to prepare final herbal syrup.

3. Method of preparation of simple syrup
66.67 g of Sucrose has been weighed and added to purified water and heated until it dissolved with occasional stirring. Sufficient boiling water was added to produce 100 ml.

4. Method of preparation of final herbal syrup
One part of decoction was mixed with five parts of simple syrup (1:5). Required quantity of Sodium benzoate (0.2%) was added as preservative to the above mixture. Solubility was checked by observing the clarity of solution visually. The final herbal syrup was then subjected for evaluation.

Physicochemical parameters
The herbal syrup was evaluated for various physicochemical parameters such as physical appearance (Colour, odour, taste), pH, Wt/ml and Specific Gravity.

1. **Color examination:** Five ml final syrup was taken into watch glasses and placed against white back ground in white tube light. It was observed for its color by naked eye.
2. **Odor examination:** Two ml of final syrup was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.
3. **Taste examination:** A pinch of final syrup was taken and examined for its taste on taste buds of the tongue.
4. **Determination of pH:** Placed an accurately measured amount 5 ml of the final syrup in a 100 ml volumetric flask and made up the volume up to 100 ml with distilled water. The solution was sonicated for about 10 minutes pH was measured with the help of digital pH meter.

Stability testing
Stability testing of the prepared poly herbal syrup was performed on keeping the samples at accelerated temperature conditions. Nine portions of the final syrup (1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B and 3C), were taken in amber colored glass bottles and were kept at accelerated temperature at 4 ^{0}C, Room temperature and 47 ^{0}C respectively. The samples were tested for all the physicochemical parameters, turbidity and

homogeneity at the interval of 24hr, 48hr and 72hr to observe any change.

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