"DEVELOPMENT OF QUALITY CONTROL PARAMETERS OF HEPASAVE SYRUP"

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MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

BY

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CERTIFICATE

This is to certify that the dissertation work entitled "Development of quality control parameters of Hepasave syrup" submitted by Ms. Shikha .S. Jha with Regn. No. (14MPH306) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutical Analysis" is a bonafide research work carried out by the candidate at the Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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DECLARATION

I hereby declare that the dissertation entitled "Development of quality control parameters of Hepasave syrup", is based on the original work carried out by me under the guidance of Dr. Dipal Gandhi, Assistant Professor, Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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Dedication

MY DEDICATION IS TO LORD KRISHNA AND RADHA AND TO MY BELOVED FAMILY

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Abbreviations

WHO	World Health Organization
IUPAC	International Union of Pure & Applied Chemistry
RPM	Revolution per minute
PPM	Parts Per million
PDA	Photo-diode array
µV.Sec	Micro volt per second
AR grade	Analytical Research Grade
SOP	Standard operating procedure
OPA	Ortho-phosphric acid
ºC	Degree centigrade
λmax	Wavelength maxima
ml	Mililiter
mg	Miligram
µg	Microgram
cm	Centimeter
mm	Milimeter
µm	Micrometer
ml/min	Miliitere per minute
IU	International unit
Rf	Retention factor
Rt	Retention time
Sec.	Second
hr	Hour
Temp	Tempearture
v/v	Volume/volume
w/v	Weight by volume
Sr. no.	Serial number
Conc.	Concentration
Gm/ml	Gram per mililiter
TLC	Thin layer chromatography
RP	Reverse Phase
HPLC	High performance liquid chromatography
UV	Ultra Violet
N.D	Not detected
D.L	Detection Limit
K value	Rate constant
RP-HPLC	Reverse-Phase High Performance Liquid Chromatography
AAS	Atomic Absorption Spectroscopy
Fig.	Figure
Cht.no	Chapter number

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ABSTRACT

Development of quality control parameter of Hepasave syrup

Herbal medicines can be used one type of complementary and alternative medicine. People use herbal medicines to try to maintain or improve their health. Many people believe that products labelled "natural" are always safe and good for them. In recent era, there has been great demand for herbal products in developed countries. So it is necessary to perform evaluation of polyherbal formulation. The present research work aims for development of quality control parameters of HEPASAVE syrup, a polyherbal formulation. HEPASAVE syrup has been procured from Cadila Pharmaceuticals Ltd. It is basically used as hepatoprotective agent, bitter tonic and antioxidant. It is the lozenges solution which is made of combination of few medicinal plants, *Phyllanthus emblica, Terminalia chebula, Terminalia bellerica , Adhatoda vasica , Andrographis paniculata, Picrorrhiza kurroa*.

The work is carried for the total investigation of the formulation & it's biomarkers, various parameters like organoleptic character, physico chemical parameter, phytochemical screening, identification of biomarkers, fractionation of formulation were studied. Determination of bitterness value, extractive value were also studied. HPLC trials, forced degradation studies, microbiological studies, estimation of phytoconstituents & detection of heavy metals were also studied.

The results of physicochemical parameters, were found about 4.16,1.33gm/ml and 1560poise respectively of pH, density, viscosity. Detection of secondary metabolites like alkaloids, glycosides, etc by performing phytochemical screening. Estimation of total phenolic content, total flavonoid content and for starch & carbohydrates were 0.23,0.25,0.44 and 0.32%w/v respectively. Microbiological studies shows total viable count was found to be in limit in fungi and in bacteria as per WHO guidelines. The zone of inhibition was performed on E.Coli, B.Subtilus, and St.aureus and found to be more effective against st.aureus. Extractive value has performed taking 4 solvents and maximum extractive value was found in toluene which shows high presence of flavonoid. Bitterness value was performed in reference to quinine sulphate and the value was found to be in limit. The maximum wavelength of the gallic acid, tannic acid and vasicine were found to be 271, 280,260. Preliminary investigation of the biomarkers were performed using TLC and the Rf value were found 0.38 in gallic acid, 0.2875 in vasicine and 0.27 in andrographolide. RP-HPLC method was performed to detect presence of biomarkers like gallic acid, tannic acid using mobile phase acetonitrile :water (10:90)

maintaining pH 3 and Rt value was found to be near 4.5 and 6 minute. The Photolytic studies were performed on gallic acid and tannic acid which were found to be 31.31µg/ml & 60.48µg/ml respectively. In the pH degraradation study it was noted that as pH increases (alkaline condition) k value & %degradation decreases & concentration increases. The force degradation studies for the drug extract using stressor was also performed. The % degradation in acidic condition (3N HCl for 60& 90 minute) was 30 & 50% at 60& 90 minutes respectively for gallic acid & tannic acid was 22.22, 44.44, 26.46, 29.47 % respectively. In alkaline condition (0.1N NaOH for 60& 90 minute) was 33& 53.3 % at 60& 90 minutes. For gallic acid & tannic acid it was 10, 20 & 68.17%. The dry heat degradation was 65.66% for the extract & for the gallic acid & tannic acid was 4% & no change in tannic acid. The oxidative degeneration was 33% for the extract & for gallic acid & tannic acid was 26 &22 % respectively.

CHAPTER 1

INTRODUCTION TO LIVER DISORDERS

CHAPTER 1 - INTRODUCTION TO LIVER DISORDERS

1.1 Introduction to Liver

Liver is a vital organ of vertebrates. In the human, it is located in the upper right quadrant of the abdomen, below the diaphragm. It shows a wide range of functions, like detoxification of various metabolites, protein synthesis, and the production of biochemicals necessary for digestion.

It plays an important role in metabolism with numerous functions in the human body, including regulation of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It is accessory digestive gland and produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. Liver has highly specialized tissue consisting of mostly hepatocytes regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions.

Terminology related to the liver often starts in *hepat-* from the Greek word for liver

There is currently no way to compensate for the absence of liver function in the long term, although liver dialysis techniques can be used in the short term. Artificial livers are yet to be developed to promote long term replacement in the absence of the liver (Richard L 2006)

1.2 Introduction related to liver disorders

Liver disease (also called **hepatic disease**) is a type of damage to or disease of the liver.

Associated medical conditions

Types

There are more than a hundred kinds of liver disease, these are some of the most common:

- Fascioliasis, a parasitic infection of liver caused by a Liver fluke of the *Fasciola* genus, mostly the *Fasciola hepatica*.
- Hepatitis, also called as inflammation of the liver, is caused by various viruses.
- Alcoholic liver disease is a hepatic manifestation of alcohol overconsumption, including fatty liver disease, alcoholic hepatitis, and cirrhosis.

- Hereditary diseases that cause damage to the liver include hemochromatosis, showing accumulation of iron in the body, and Wilson's disease. Liver damage is also a clinical feature of alpha 1-antitrypsin deficiency and glycogen storage disease type II.
- Gilbert's syndrome, a genetic disorder of bilirubin metabolism found in a small percent of the population, can cause mild jaundice.
- Cirrhosis is the formation of fibrous tissue (fibrosis) in the place of liver cells that have died due to a variety of causes, including viral hepatitis, alcohol overconsumption, and other forms of liver toxicity. Cirrhosis causes chronic liver failure.
- Primary liver cancer most commonly manifests as hepatocellular carcinoma and/ or cholangiocarcinoma; rarer forms include angiosarcoma and hemangiosarcoma of the liver. (Many liver malignancies are secondary lesions that have metastasized from primary cancers in the gastrointestinal tract and other organs, such as the kidneys, lungs.
- Primary biliary cirrhosis is a serious autoimmune disease of the bile capillaries.
- Primary sclerosing cholangitis is a serious chronic inflammatory disease of the bile duct, which is believed to be autoimmune in origin.
- Budd–Chiari syndrome is the clinical picture caused by occlusion of the hepatic vein. (Richard L)

1.3 Introduction to Liver tonics / Hepatoprotectives

Beyond the treatment of liver disorders, everyday care of the liver lays a cornerstone for total body health. Naturopaths and others, who look beneath the symptoms of an illness to its underlying cause, often discover that the liver has had a role to play. More than 500 vital functions have been identified with the liver. The liver is important because a person’s nutritional level is not only determined by what he or she eats, but by what the liver processes. Unfortunately, it is extremely difficult to detect early warning symptoms specific to liver metabolic imbalances. People can suffer for a long time from a liver ailment without knowing of it. The incredible complexity of liver chemistry and its fundamental role in human physiology is so daunting to researchers that the thought that simple plant remedies might have something to offer is astonishing and incredible. (Tahmineh A et al.2015)

It leads for Liver tonics, for performing function of protecting the liver against toxins, poisons and pathogens, stimulates regeneration of liver cells, protects against inflammation. After

extensive search of the modern and traditional literature on this subject we are proud to present a range of safe herbal alternatives from India that can serve as an excellent hepatoprotectives if consumed as per directions.

1.4 Plants used in Hepato-protective formulation

The following are the plants present in Hepato-protective formulation (*Asija R et al 2014).* The details about plants in a hepato protective formulation is given in table 1.1

Table 1.1 Details about plants in a hepato protective formulation

Sr.no	**Botonical name**	**Family**	**Chemical constituent**	**Solvent**
1	*Amaranthus caudatus* Linn	Amaranthaceae	Flavonoids,saponins, glycosides	Methanol
2	*Anisochilus carnosus* Linn	Lamiaceae	Alkaloids, flavonoids, glycosides	Ethanol
3	*Asparagus racemosus* Linn	Asparagaceae	Phenols, coumarins	Ethanol
4	*Azima tetracantha*	Salvadoracaeae	Flavonoids, triterpenoids	Ethanol
5	*Cucumis trigonus* Roxb	Cucurbitaceae	Flavonoids	Pet ether, chloroform, alcohol, aqueous
6	*Ficus religiosa* Linn	Moraceae	Glycosides, steroids, tannins	Methanol
7	*Solanum nigram* Linn	Solanaceae	Flavonoids, terpenoids	Ethanol

1.5 Morphological Description of different species

1.5.1 *Amaranthus caudatus Linn*

Amaranthus, collectively known as amaranth, is a cosmopolitan genus of annual or short-lived perennial plants. The plant is astringent, anthelmintic and diuretic. It is used in the treatment of stranguary and is applied externally to scrofulous sores. (Kumar A et al *2011*).

1.5.2 *Anisochilus carnosus* Linn

The ethanolnolic extract of *A.carnosus* is effective hepatoprotective agent. Furthermore, it's leaves showes good analgesic and antipyretic activity. Such strong pharmacological activity of it is credited to the innumerable phytocompounds present in them. It also shows Stimulant, expectorant and diaphoretic properties. Juice of fresh leaves of it is used in urticaria and other allergic conditions; it also has a domestic remedy for coughs and cold. Alcoholic extract of the whole plant shows antibacterial property & it's essential oil shows antitubercular property. The oil exhibits antihistaminic property in vitro on smooth muscles of the uterus and the intestines. It also possesses muscle-relaxant action; bactericidal and fungicidal properties. *(S. Agrawal et al. 2010)*

1.5.3 *Asparagus racemosus* Linn

The roots are used in Ayurvedic medicine, following a regimen of processing and drying. It is generally used as a uterine tonic, as a galactogogue (to improve breast milk), in hyperacidity, and as a best general health tonic. (*Mishra J 2013.)*

1.5.4 *Azima tetracantha*

The pounded roots of *Azima tetracantha* are applied directly to snakebites and an infusion is taken orally as a treatment for them, mixture of roots and leaves is used for the same. The root decoction of it is used to treat stomach disorders. An infusion of the leaves is used to treat venereal diseases. The juice of the berries is applied directly into the ear to treat earache and the dried root is ground, put in cold water and given to cows to facilitate difficult parturition. The sap of the plant directly to treat toothache and bleeding gums after tooth extraction and also as a disinfectant. The root, root bark and leaves are added to food as a remedy for

rheumatism. The plant is considered diuretic and is also used to treat dropsy, dyspepsia, chronic diarrhea and as a stimulant tonic. Juice of the leaves is applied as eardrops against earache and crushed leaves are placed on painful teeth. (*Ekbote et al2010*)

1.5.5 *Cucumis trigonus* Roxb

The fruits of *Cucumis trigonus* Roxb are used in flatulence, leprosy, fever, jaundice, diabetes, cough, bronchitis, ascites, anaemia, constipation, other abdominal disorders and amentia. (Naik, V.R. *et al*., 1981). In addition, fruit pulp is bitter, acrid, thermogenic, anthelmintic, liver tonic, cardio tonic, appetizer, expectorant and intellect promoting (Kirtikar, K.R. *et al*., 2009).

1.5.6 *Ficus religiosa* Linn

Ficus religiosa is used in traditional medicine for about 50 types of disorders including asthma, diabetes, diarrhea, epilepsy, gastric problems, inflammatory disorders, infectious and sexual disorders (*Inder Kumar I.K. et al 2010*)

1.5.7 *Solanum nigram* Linn

It is anti-inflammatory, antihepatic, antioxidant and anti-hyperlipidemic. The herb is diuretic, diaphoretic, anodyne, expectorant alternative. It is useful for the treatment of measles. It is useful against cardiac pain. It is used as a blood purifier. It is helpful in enlargement of organs like liver and spleen. **(***Jani .Dilip J.D K.et al 2012*)

1.6 Formulations available in market for liver disorders

There are various formulations available in the market for liver disorders some of them are given below. Various marketed hepato-protective formulations is given in table 1.2

Table 1.2 Marketed hepato-protective formulations

Sr. no	Marketed formulation	Manufactured by	Active ingredient	Indications	Dose	References
1	Liv 52	Himalaya – Banglore	*Achillea millefolim, Capparis spinosa*	Ayurvedic medicine, protects liver against various hepatotoxins, & promote appetite and growth	2-3 teaspoon 2 to 3 times daily	C Girish, Bidhan Chandra konar, S.Jayanthi, K.Ramchandra Rao, B Rajesh Suresh Chandra Pradhan
2	Livergen	Standard Pharmaceuticals -West Bengal	*Andrographis paniculat, Apium graveolen,Asteracantha longifolia*	Ayurvedic medicine, gastrointestinal and hepatic regulator	2-4 teaspoon twice a day	C Girish, Bidhan Chandra konar, S.Jayanthi, K.Ramchandra Rao,B Rajesh Suresh Chandra Pradhan
3	Livokin	Herbo-med-Kolkata	*Andrographis paniculat,Apium graveolen, Berberis lycium, Carum copticum*	Ayurvedic medicine, for hepatic dysfunction	1-2 teaspoon 2 to 3 times daily	C Girish, Bidhan Chandra konar, S.Jayanthi, K.Ramchandra Rao,B Rajesh

						Suresh Chandra Pradhan
4	Octogen	Plethico Pharmaceuticals - Indore	Arogyavardhini rasa, *Phyllanthus niruri*	Ayurvedic medicine, highly potent hepato - protective	As directed by physician	C Girish, Bidhan Chandrakonar,S.Jayanthi, K.Ramchandra Rao,B Rajesh Suresh Chandra Pradhan
5	Stimuliv	Franco-Indians - Mumbai	*Andrographispaniculat, Eclipta alba,Phyllanthus niruri, Justicia procumbens*	Ayurvedic medicine, liver stimulant and tonic	1-2 teaspoon 2 to 3 times daily	C Girish, Bidhan Chandrakonar, S.Jayanthi, K.Ramchandra Rao,B Rajesh Suresh Chandra Pradhan
6	Tefroliv	TTK pharma Chennai	*Andrographis paniculata,Eclipta alba, Ocimum sanctum, Phyllanthus niruri, Picrrorhiza kurroa.*	Ayurvedic medicine, standardized liver formulation for effective hepatic regeneration	1teaspoon thrice a day or as directed by physician	C Girish, Bidhan Chandrakonar, S.Jayanthi, K.Ramchandra Rao,B Rajesh Suresh Chandra Pradhan

1.7 Quality control for herbal drugs

The quality control of herbal drugs may be defined as the status of a drug, which is determined either by identity, purity, content, and other chemical, physical or biological properties, or by the manufacturing process.

Quality control is based on three important pharmacopeial definitions:

- •Identity: Is the herb the one it should be?
- • Purity: Are there contaminants, e.g., in the form of other herbs which should not be there?
- • Content or assay: Is the content of active constituents within the defined limits?

So, from these objectives of Pharmacopeia standardisation parameters of a drug includes

1. Identification test :- This is through TLC, U.V spectroscopy, FTIR
2. Other test /Quality control parameters :- In this visual inspection, pH, density, viscosity, miscibility, solubility, melting & boiling point are checked.
3. Limit test :- In this test presence of heavy metals are checked.
4. Content/Assay :- This shows presence of active constituents in the drug.
5. Storage :- This shows how the drug has to be preserved.

All these parameters should be evaluated & validated for each &every herbal formulation and their results should come in the safe range so that any person can take that drug.

CHAPTER 2

INTRODUCTION TO FORMULATION-HEPASAVE SYRUP

CHAPTER 2 INTRODUCTION TO FORMULATION – HEPASAVE SYRUP

2.1 QUALITY CONTROL OF HERBAL DRUGS

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All these parameters should be evaluated & validated for each &every herbal formulation and their results should come in the safe range so that any person can take that drug.

Cadila Pharmaceuticals has developed a formulation which consists of many herbs, its detailed studies were performed

2.2 INTRODUCTION TO HEPASAVE

Hepasave is an Ayurvedic Proprietary Medicine. It is the lozenges solution which is made of combination of few plants. It is dark brownish in colour. It is stored at room temperature. It is Preservative Free and Alcohol free. It is natural powerful hepatoprotective agent, anti oxidant and bitter tonic. The dosage and storage of Hepasave is given below.

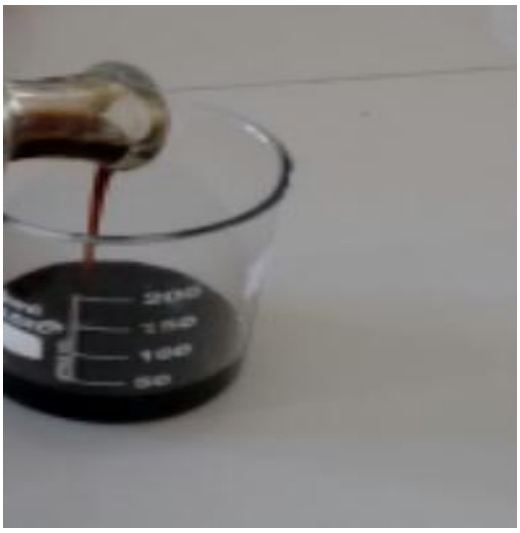
DOSAGE:

Adults: 10ml three times a day.

Children: 5ml three times a day.

STORAGE: Store below 30˚C.

Protect from Direct Sunlight.



(Fig. 2.1 Hepasave syrup)

2.3 Composition of Hepasave

The composition of Hepasave is *Amalaki fruit i.e. Phyllanthus emblica Ab., Haritaki fruit i.e., Terminalia chebula Ab., Bibhitaka fruit i.e., Terminalia bellerica AB., Vasa leaf i.e., Adhatoda vasica Ab., Bhunimba herb i.e., Andrographis paniculata, Katuka root i.e., Picrorrhiza kurroa, Nimba stem bark i.e., Azadirachta indica Ab, Amruta stem i.e., Tinospora cordifolia, Sarpankha herb i.e., Tephrosia purpurea Ab.*, and also a Flavored syrup base. The constituents under study for this project were Gallic acid (*Amalaki fruit i.e., Phyllanthus emblica Ab*.), Gallic acid (*Haritaki fruit i.e., Terminalia chebula Ab.*), Gallic acid (*Bibhitaka fruit i.e., Terminalia bellerica Ab*.), Vasicine (*Vasa leaf i.e., Adhatoda vasica Ab*.), Andrographolide (*Bhunimba herb i.e., Andrographis paniculata*), Kutkin (*Katuka root i.e., Picrorrhiza kurroa*). Composition of Hepasave syrup is given in table 2.1

Table 2.1 Composition of Hepasave Syrup

Sr. No.	Component	Biological Source	Chemical component	Quantity (in 10ml)
1.	Amalaki fruit	*Phyllanthus emblica*	Gallic acid, Tannic acid	130.0 mg
2.	Haritaki fruit	*Terminalia chebula AB*	Gallic acid, Tannic acid	130.0 mg
3	Bibhitaka fruit	*Terminalia bellerica AB*	Gallic acid, Tannic acid	130.0 mg
4	Vasa leaves	*Adhatoda vasica AB*	Vasicine	130.0 mg
5	Bhunimba herb	*Andrographis paniculata BHP*	Andrographolide	130.0 mg
6	Katuka root	*Picrorrhiza kurroa AB*	Kutkin	130.0 mg
7	Nimba stem bark	*Azadirachta indica AB*	Azadirachtin, nimbidin	130.0 mg
8	Amruta stem	*Tinospora cordifolia AB*	Tinosporic acid, Cordifoliside A to E, Berberine	130.0 mg
9	Sarpankh herb	*Tephrosia purpurea AB*	Pongamol, lanceolatin A, B	2.1 mg

2.4 Composition of Hepasave Syrup

2.4.1 Amalki fruit

Synonyms :- Emblica, Indian goose berry, Amalki

Botonical name :- *Emblica officinalis, Phyllanthus emblica* Linn.

Biological Source :- This consists of dried as well as fresh fruits of plant *Emblica officinalis* Gaerth (*Phyllanthus emblica* Linn.), belonging to family *Euphorbiaceae.* It contains not less than 1.0%w/w of gallic acid calculated on dry basis.

Family :- Euphorbiaceae

(a) Immature amalki (b) Mature amalki

(Fig. :- 2.2 Morphology of Amalki fruit)

Macroscopical characters

Colour :- Green changes to light yellow or brick red colour when matured

Odour **:-** None

Taste **:-** Sore & astringent

Size :- 1.5 to 2.5cm in diameter

Shape :- Globose

Extra features: Fruits are fleshy obscurely 4 lobbed with 6- trygonous seeds. They are very hard in appearance.

Part used:- Fruit, preferably fresh

Chemical constituents

The major chemical constituents of Amla are Phyllemblin, Ascorbic acid (Vitamin C), Gallic acid, Tannins, Pectin etc.

Medicinal Properties:

Emblica Officinalis is Cooling, Astringent, Refrigerant, Anabolic, Astringent, Antibacterial, Antidiarrhoeal, Antidysenteric, Expectorant, Antispasmodic, Antipyretic, Antioxidant, Antiviral, Antiemetic, Antihepatotoxic, Antianaemic, Antihaemorrhagic, Aphrodasiac, Bechic, Diuretic, Carminative, Laxative and Immunomoduater

Uses

The fruit is the richest source of Vitamin C and is a diuretic, aperient, Laxative and hair dye. It cures insomnia and is healthy for hair. It is used as the cardio protective, useful in hemorrhage, menprrhagia, leucorrhoea and discharge of blood from uterus. Amla power and oil are used traditionally in Ayurvedic applications for the treatment of scalp. Amla power improves immunity and gives physical strength. It improves complexion and removes wrinkles. Amla is also used to treat constipation and is used as a cooling agent to reduce the effects of sun strokes and sun burns. (C.K. Kokate)

2.4.2 Haritki fruit

Synonyms :- Chebulic myrobalan, Harade, Haritki

Botonical name :- *Terminila chebula*

Biological source :- It consists of dried ripe, and fully matured fruits of *Terminila chebula* Retzr belonging to family combretaceae. It contains not less than 5% of chebulagic acid and not less than 12.5% of chebulinic acid.

Family :- combretaceae

(Fig.2.3 Morphology of haritki fruit)

Macroscopical Characters

Colour :- Yellowish-brown

Odour :- Odourless

Taste:-Astringent, slightky bitter & sweetish at the end

Size :- 20-25mm long and 15-25mm wide

Shape :-Ovate & wrinkled longitudinally

Chemical constituents:- It contains Chebulagic, chebulinic,ellagic and gallic acid.

Medicinal uses :-

- Haritaki has laxative, rejuvenative, purgative, astringent and dry properties.
- The paste of its fruit is effective in reducing swelling, hastening the healing process and cleansing the wounds and ulcers.
- The paste gives relief to the eyelids, in case of conjunctivitis.
- Gargling with haritaki decoction helps in stomatitis, oral ulcers and sore throat.
- It serves as a good astringent for loose gums, bleeding and ulceration in gums.
- The herb is used in preparing 'Triphala' that is used for hair wash, brush teeth in pyorrhea and treat bleeding gums.
- Regular consumption of haritaki powder, fried in ghee, promotes longevity and boosts energy.
- It responds well to gastrointestinal ailments, tumors, ascites, piles, enlargement of liver-spleen, worms and colitis.
- Powdered haritaki, mixed with jaggery, works well in gout.
- Its powder, when mixed with honey and ghee, is an effective remedy for anemia.
- Haritaki is combined with sunthi powder and given with hot water to lighten asthma and hiccups.
- Its decoction, when taken along with honey, is of great help in hepatitis and obesity.
- The herb improves memory and is salutary in dysuria and urinary stones.
- Haritaki helps in improving appetite and helps in digestion.
- It is a good nervine and helps in nervous weakness and nervous irritability and promotes the receiving power of the five senses.
- Since it is anti-inflammatory and astringent, it is helpful in urethral discharges like spermatorrhea and vaginal discharges like leucorrhea. (C.K. Kokate)

2.4.3 Bibhikta fruit

Synonyms :- Belleric myrobalan, Baheda, Bibhitak

Botonical name :- *Terminaia belerica* Linn

Biological source :- It consists of dried ripe fruit of *Terminaia belerica* Linn belonging to family Combretaceae, and should contain not less than 0.3% of ellagic acid and 0.75% of gallic acid on dried basis.

Family :- Combretaceae

Images :-

(Fig.2.4 Morphology of Bibhikta fruit)

Macroscopical Characters

Colour:- Brown to black

Odour :- None

Taste :-Astringent

Size :- 1.3 to2 cm in length

Shape :- Globular & obscurely 5 angled

Chemical constituents :- It should contain about 20-30% of tannins and 40-45% water-soluble extractives. It contains gallic acid, ellagic acid, phyllemblin, ethyl gallate and galloyl glucose.

Medicinal Properties:

Fruits are asrtringent, acrid, sweet, thermogenic, anti-inflammatory, anodyne, styptic, narcotic, digestive, anthelmintic, aperient, expectorant, ophthalmic, antipyretic, antiemetic and rejuvenating. It has purgative, blood pressure depressant, antifungal, antihistaminic activity against viral hepatitis and vitiligo. Antiasthmatic, broncho-dilatory, antispasmodic,

antibacterial, CNS stimulant, amoebicidal, antistress, anti-atherogenic agent and endurance promoting activity. (C.K. Kokate)

Uses

Bibhitaki primarily supports the healthy formation of three bodily tissues-nutrients plasma (rasa dhatu), muscle (mamsa dhatu) and bone (asthi dhatu). It has been used both internally as well as externally.

2.4.4 Vasa leaves

Synonyms :- Adhatoda, Adusla, Malbar nut

Botonical name :- *Adhatoda vasica* Nees

Biological source :- It consists of dried, as well as, fresh leaves of the plant *Adhatoda vasica* Nees, belonging to family Acanthaceae, and contains not less than 0.6% of vasicine on dried basis.

Family :- Acanthaceae

Images

(Fig.2.5 Morphology of vasa leaves)

Macroscopical characters

Colour :- Green

Odour :- Characteristic

Taste :-Bitter

Size :- 10-30cm in length, 4-10cm in width

Shape :- Lanceolate

Margin :- Crenate with acuminate apex

Other characters :- Petiolate and extipulate. 8-10 lateral veins

Microscopical characters :- The epidemis shows caryphyllaceous stomata with sinous epidermal cells, and covering glandular trichomes. It is dorsiventral leaf with palisade having 2 layers of cells. 2-3 bicollateral vascular bundles are seen in the midrib. Mesophyll contains prismatic and acicular crystals of calcium oxalate. Stomatal index is from 10.8 to 18.2 and palisade ratio from 5 to 8.5.

Chemical constituents:- It contains quinazoline derivatives such as vasicine, vasicione and 6-hydroxy vasicine.

Medicinal uses :- It is used as an expectorant and bronchodilator. Also shows oxytocic property.

2.4.5 Bhunimba herb

Synonyms :- Andrographis, kirayat, Bhui-nimb.

Botonical name :- *Andrographis paniculata* Nees

Biological source :- It consists of dried leaves and tender shoots of the plant known as *Andrographis paniculata* Nees (Acanthaceae). It yields not less than 1.0% of andrographolide calculated on dry basis.

Family :- Acanthaceae

Images

(Fig.2.6 Morphoogy of bhunimb herb)

Macroscopical Characters

Colour :- Leaves are dark green, while flowers are rose coloured

Odour :- Odourless

Taste:- Intensely bitter

Size :- Leaves 7*25cm, Flower 1.8cm in length

Shape :-Leaves are lanceolate and petiolate with entire margin and acuminate apex

Chemical constituents :- It contains bicyclic diterpenoid lactone & kalmeghin(0.85- 2.5%)

Uses

It is used in Indigestion, Fatty Liver, Liver cirrhosis, Worm Infestation, haematological disorders, oedema, skin disorder, Malaria with Chilli Powder, general weakness, Chronic fever, Liver Disorder Like Jaundice (C.K. Kokate)

2.4.6 Katuka root

Synonyms :- Indian gentian, kutki

Botonical name :- *Picrorrhiza kurroa*

Biological source :- It consists of dried rhizomes of plant *Picrorrhiza kurroa* (Scrophulariaceae), cut in small pieces and freed from attached root-lets. It contains not less than 5.0% kutkin on dried basis

Family :- Scrophulariaceae

(Fig.2.7 Morphologyof katuka root)

Microscopical characters

Colour :- Rhizomes are deep greyish brown, externally white, blackish internally with whitish-wood.

Odour :- Slight & unpleasant

Taste :- Bitter

Size :- 3 to5 cm in length and 0.5 to 1cm in diameter.

Shape :- Cylindrical pieces with longitudinal wrinkles and annulations at the tip.

Chemical constituents:- D-Mannitol, Kutkiol, Kutkisterol,Apocyanin: phenol glucosides; androsim and picein iridoid glycosides; Kutkin, picroside I, ii & iii; Kutkoside, Minecoside, Picrorhizin,arvenin iii etc.

Medicinal uses :-

The roots of Katuka are used in medicines, usually, orally. Katuka works very well in fevers associated with burning sensation of the body. It is as effective as quinine in malarial fever. One gram of root powder is added to 30 ml hot water and the water is drunk after cooling. The patient gets his bowels opened and within three to four days, get cured. Katuka causes, sometimes, spasmodic pains in abdomen, hence are given along with sunthi. It also improves appetite when given with honey, in small dosage viz. 0.5 gm twice a day. In relapsing fever, it works well in slightly higher doses. As katuka alleviates kapha and pitta dosas and destroys ama, it is very beneficial in fevers due to vitiation of these doses.

Katuka combines well with yastimadhu with equal quantity of sugar as a therapy for heart diseases. It alleviates the edema in ascites and enlargement of liver. It has a marked effect of hepatobilliary system as a hapato-protective and its cholegouge action. The distinguished activity of katuka is extremely useful in the treatment of jaundice. Its decoction, in jaundice, is given with honey or its dry powder with sugar. Numerous market preparations in the treatment of jaundice are incorporated, chiefly, with katuka as the main ingredient. The most popular preparation designed by Nagarjuna, Arogyavardhini, contains more than 50% katuka. Ancient scriptures have recommended this divine formulation in number of diseases like hepatosplenomegaly, ascites, skin diseases, obesity, anaemia, hepatitis, fever, sinusitis, anorexia, alcoholism, chronic constipation etc. Aptly it is known as Arogyavardhini (health promoter). (C.K. Kokate)

2.4.7 Nimba stem bark

Synonyms :- Margosa

Botonical name :- *Azardirachata indica***,**

Biological source :- It consists of all aerial parts of plants known as *Azardirachata indica,* family Meliaceae

Family :- Meliaceae

(Fig.2.8 Morphology of neem)

Macroscopical characters

1. **Leaves**

   Colour :- Green

   Taste :- Bitter

   Size :- 20-25 cm

   Shape :- Lanceolate

   Other features :-Alternate, extipulate, imparipinnate leaflets, closely clustered towards the end of branches

2. **Bark :** Moderatey thick, rough, brown in colour longitudinally & obliquely furrowed. Internally starchy white, laminated with characteristic smell of neem and bitter taste.

   Chemical constituents:- Neem contains a bitter fixed oil, nimbidin, nimbin, nimbinin and nimbidol, tannin.

Medical Properties

The medical properties of Neem have been known to Indians since time immemorial. The earliest Sanskrit medical writings refer to the benefits of Neem's fruits, seeds, oil, leaves, roots and bark. Each has been used in the Indian Ayurvedic and Unani systems of medicines, and is now being used in the manufacture of modern day medicinals, cosmetics, toiletries and pharmaceuticals.

Neem fruits, seeds, oil, leaves, bark and roots have such uses as general antiseptics, antimicrobials, treatment of urinary disorders, diarrhoea, fever and bronchitis, skin diseases,

septic sores, infected burns, hypertension and inflammatory diseases. This is mainly due to the chemical constituents which enable Neem to protect itself from a multitude of pests by a substantial number of pesticidal ingredients. Its main chemical composition is a blend of 3 to 4 related compounds along with over 20 lesser ones, which are equally as active. The general class of these compounds is triterpenes and within this category, the most effective are the limonoids, which are abundant in Neem oil. At least nine limonoids are effective in inhibiting insect growth, especially some of the most deadly varieties found in human health and agriculture worldwide. Of these limonoids, azadirachitin has been found to be the main ingredient for fighting insects and pests, being up to 90% effective in most instances. It repels and disrupts the life cycle, however does not kill immediately, but is nonetheless one of the most effective growth and feeding deterrents ever examined. Meliantriol is another feeding inhibitor which prevents locusts chewing, and has therefore been in traditional use in India for crop protection. Nimbin and nimbidin, also found in Neem, have anti-viral properties and these have been effective in inhibiting fungal growth on humans and animals. Gedunin, a lesser limonoid, is effective in treating malaria through teas and infusion of the leaves.

2.4.8 Amruta stem

Synonyms :- Gulvel, Tinsopora, Gloe, Amrita

Botonical name :- *Tinosporia cordifolia*

Biological source :- It consists of dried leaves and stem pieces of woody climber *Tinosporia cordifolia,* Miers belonging to family Menispermaceae. It consists of not less than 0.02% of cordifolioside

Family :- Menispermaceae

(Fig.2.9 Morphology of amruta stem)

Macroscopical characters

Colour :- Grayish black

Odour: None

Taste: Bitter

Size :3-5 cm in length, 3-8cm in diameter

Shape : Cylindrical

Fracture : Fibrous

Chemical constituents:- It consists of tinosporic acid, tinosporal, gilonin, gilonin, berberine, cordifol, pinosporidine.

Medicinal uses :- Anti-dibaetic, anti-cancer,immunomodulatory. (C.K. Kokate)

2.4.9 Sarpankh herb

Synonyms :- *Tephrosia purpurea var. angustata Miq., Tephrosia purpurea var. angustissima*

Botonical name :- *Tephrosia purpurea*

Biological source :- The drug consists of the whole herb known as *Tephrosia purpurea* (Family: Fabaceae), found throughout India, widely distributed in tropical, sub-tropical and arid regions of the world.

Family :- Fabaceae

(Fig.2.10 Morphologyof Sarpankh herb)

Chemical constituents

The constituents of *Tephrosia purpurea* include alkaloids, saponins, glycosides, tannins, flavonoids etc. Some of the constituents may have direct activity and the other inert substances may increase bioavailability and reduces the toxicity. Roots contain tephrosin, dengulin, quercetin, isotephrosin and rotenone. In the roots and leaves 2.5% rutin is found. A new β-hydroxychalcopurpurnone,Isolonchocarpin, pongamol, Lanceolatin A, Lanceolatin B, Karanj in, Kanjone and β-sitosterolis isolated from roots.

Description

Flowers :- They are red or purple in leaf opposed racemes, bracteoles usually absent; pedicel 2-6 mm long; flower 4-8.5 mm long, purplish to white.

Leaves :- They are imparipinnate; stipules narrowly triangular, 1.5-9 mm x 0.1-1.5 mm; rachis up to 14.5 cm long, including the petiole of up to 1 cm.

Seeds :- They are rectangular to transversely ellipsoid, 2.5-5 mm x 1.8-3 mm, light to dark brown to black, sometimes mottled.

Root :-They are cylindrical, tapering, posses characteristic odour, brownish yellow in colour and has a complex bitter taste.

Fruits:- They are of *Tephrosia purpurea* are large and 2-12cm long, very densely villous or tomentose.

Uses

According to Ayurveda literature this plant has also given the name of "Sarwa Wranvishapaka" which means that it has the property of healing all types of wounds. It is an important component of some preparations such as Tephroli and Yakrifit used for liver disorders. In Ayurvedic system of medicine various parts of this plant are used as remedy for impotency,

asthma, diarrhoea, gonorrhoea, rheumatism, ulcer and urinary disorders. The plant has been claimed to cure diseases of kidney, liver spleen, heart and blood. The dried herb is effective as tonic laxative, diuretics and deobstruents. It is also used in the treatment of bronchitis, bilious febrile attack, boils, pimples and bleeding piles.

The roots and seeds are reported to have insecticidal and pesticidal properties and also used as vermifuge. The roots are also reported to be effective in leprous wound and their juice, in the eruption of skin. An extract of pods is effective for pain, inflammation and their decoction is used in vomiting. The aqueous extract of seeds has shown significant *in vivo* hypoglycemic activity in diabetic rabbits. The ethanolic extracts of *Tephrosia purpurea* possessed potential antibacterial activity. The flavanoids were found to have antimicrobial activity. The phytochemical investigations on *Tephrosia purpurea* have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, and sterols. (C.K. Kokate)

Pharmacological Activity

Root

Antiulcer Activity

Antiulcer activity shows aqueous extract of *Tephrosia purpurea studied by* Deshpande *et al.*, (2003)

Anti-carcinogenic and Anti-lipid Peroxidative studied by Kavitha *et al*., (2014) on ethanolic root extract of *Tephrosia purpurea*

Anti-Inflammatory and Analgesic

Krishan G *et al*., (2007) studied the ethanolic Extracts of the aerial and root parts of *Tephrosia purpurea* for antiinflammatory and analgesic activities.

In-Vitro Antioxidant

Shah R *et al*., (2014) performed the *in-vitro* antioxidant activity on hydroalcoholic extract of shade dried roots of *Tephrosia purpurea* .

Antimicrobial Activity

Rangama D *et al.*, (2009) screened for their antimicrobial activity of *Tephrosia purpurea.*

2.5 Active constitutents present in the formulation

There are 9 components present in the formulation from which all the components possess active constituents. There are 5active constituent present in the formulation they are as follows

1. Gallic acid
2. Tannic acid
3. Vasicine
4. Andrographolide
5. Kutkin

2.6 Drug profile of biomarkers

2.6.1 Gallic acid

Gallic acid is a trihydroxybenzoic acid, a type of phenolic acid, a type of organic acid, also known as 3,4,5-trihydroxybenzoic acid, found in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants.The chemical formula is $C_6H_2(OH)_3COOH$. Gallic acid is found both free and as part of hydrolyzable tannins. The gallic acid groups are usually bonded to form dimers such as ellagic acid. Hydrolysable tannins break down on hydrolysis to give gallic acid and glucose or ellagic acid and glucose, known as gallotannins and ellagitannins respectively.

Gallic acid forms intermolecular esters (depsides) such as digallic and trigallic acid, and cyclic ether-esters (depsidones).

Gallic acid is commonly used in the pharmaceutical industry. It is used as a standard for determining the phenol content of various analytes by the Folin-Ciocalteau assay; results are reported in *gallic acid equivalents*. Gallic acid can also be used as a starting material in the synthesis of the psychedelic alkaloid mescaline.

The name is derived from oak galls, which were historically used to prepare tannic acid. Despite the name, gallic acid does not contain gallium. Salts and esters of gallic acid are termed "gallates".

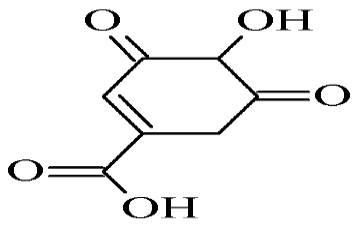


Fig. 2.11 sturucture of Gallic acid

General description about gallic acid. General description of gallic acid is in table 2.2

Table 2.2 General description of gallic acid

Sr.no	**Physical characters**	**Result**
1	Molecular formula	$C_7H_6O_5$
2	IUPAC name	3,4,5-trihydroxy benzoic acid
3	Molecular weight	170.120gm/mol
4	Category	Phytochemical organic solvent Pharmacological- Astringent, Antioxidant, Hepatoprotective
5	Apperance	White-yellowish white, or pale-fawn coloured crystals
6	Solubility	Soluble in alcohol, ether, glycerol, acetone. Negligible in benzene, chloroform, petroleum ether
7	Solubility in water	1.19g/100ml in anhydrous at 20º 1.5g/100ml in monohydrate at 20º
8	Nature	Acidic
9	Refractive Index	1.69
10	Melting point	260º
11	Density	1.694gcm-3 for anhydrous
12	Log P	0.70
13	pKa value	COOH:4.5, OH:10

2.6.2 Tannic acid

Tannic acid is a specific commercial form of tannin, a type of polyphenol. Its weak acidity (pK_a around 10) is due to the numerous phenol groups in the structure. The chemical formula for commercial tannic acid is often given as $C_{76}H_{52}O_{46}$, which corresponds with decagalloyl glucose, but in fact it is a mixture of polygalloyl glucoses or polygalloyl quinic acid esters with the number of galloyl moieties per molecule ranging from 2 up to 12 depending on the plant source used to extract the tannic acid. Commercial tannic

acid is usually extracted from any of the following plant part: Tara pods (*Caesalpinia spinosa*), gallnuts from *Rhus semialata* or *Quercus infectoria* or Sicilian Sumac leaves (*Rhus coriaria*). According to the definitions provided in external references such as international pharmacopoeia, Food Chemicals Codex and FAO-WHO tannic acid monograph only tannins sourced from the above mentioned plants can be considered as tannic acid. Sometimes extracts from chestnut or oak wood are also described as tannic acid but this is an incorrect use of the term. It is a yellow to light brown amorphous powder; 2850 grams dissolves in one litre of water (1.7 moles per liter).

While tannic acid is a specific type of tannin (plant polyphenol), the two terms are sometimes (incorrectly) used interchangeably. The long-standing misuse of the terms, and its inclusion in scholarly articles has compounded the confusion. This is particularly widespread in relation to green tea and black tea, both of which contain tannin but not tannic acid.

Tannic acid is not an appropriate standard for any type of tannin analysis because of its poorly defined composition. General description of Tannic acid is given in tabe 2.3

Table 2.3 General description of tannic acid

Sr.no	Physical characters	Result
1	Molecular formula	$C_{76}H_{52}O_{46}$
2	IUPAC name	3,5-dihydroxy-2-(3,4,5-trihydroxybenzoyl)oxy-6-[(3,4,5-trihydroxybenzoyl)oxymethyl]oxan3,4,5-trihydroxybenzoate]
3	Molecular weight	1701.198
4	Apperance	Yellowish-white to light brown
5	Solubility in water	2850g/L
6	Density	2.12 +- 0.1g/cm3
7	Melting point	210^{o}c
8	pKa value	10

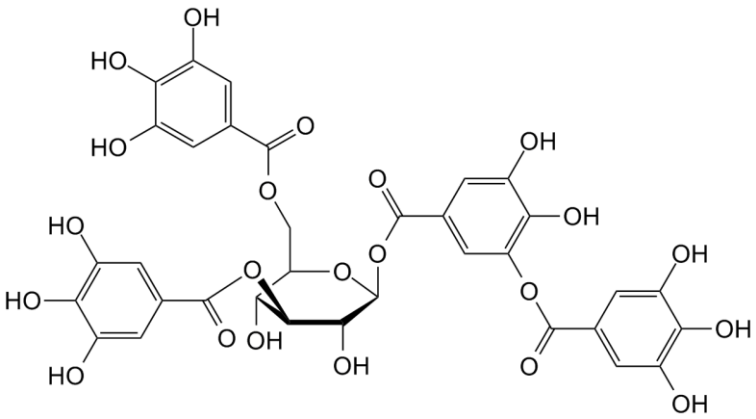


Fig. 2. 11 Structure of tannic acid

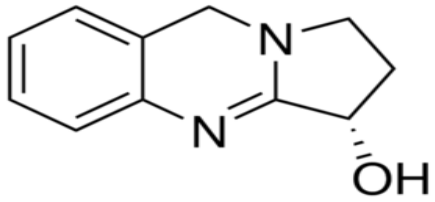
2.6.3Vasicine

Vasicine (**peganine**) is a quinazoline alkaloid. It is the active compound of *Justicia adhatoda*, after which the chemical is named.

Vasicine has been compared to theophylline both *in vitro* and *in vivo*. It has also been studied in combination with the related alkaloid vasicinone. Both the alkaloids in combination (1:1) showed pronounced bronchodilatory activity *in vivo* and *in vitro*. Both alkaloids are also respiratory stimulants. Vasicine has a cardiac–depressant effect, while vasicinone is a weak cardiac stimulant; the effect can be normalized by combining the alkaloids. Vasicine is reported to have a uterine stimulant effect. General description of vasicine is given in table 2.4.

Table 2.4 General description of vasicine

Sr.no	Physical characters	Result
1	Molecular formula	$C_{11}H_{12}N_2O$
2	IUPAC name	1,2,3,9-Tetrahydropyrrolo[2,1-b]quinazolin-3-ol
3	Molecular weight	188.23g/mol
4	Category	Phytochemical- Quinazoline alkaloid Pharmacological- Antioxidant, Anti-inflammatory
5	Apperance	White to yellow powder
6	Refractive Index	1.709
7	Melting point	209-211^{o}c



(Fig.2.12 shows image of vasicine)

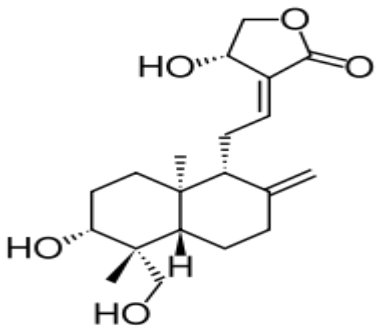
2.6.4 Andrographolide

Andrographolide is a labdane diterpenoid that has been isolated from the stem and leaves of *Andrographis paniculata*. Andrographolide is an extremely bitter substance.

Andrographolide has been studied for its effects on cell signaling, immunomodulation, and stroke. Study has shown that andrographlide may bind to a spectrum of protein targets including NF-κB and actin by covalent modification.General description of andrographolide is given in table 2.5

Table 2.5 General description of andrographolide.

Sr.no	Physical characters	Result
1	Molecular formula	$C_{20}H_{30}O_5$
2	IUPAC name	(3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a- dimethyl-2-methylene-1-napthalenyl]ethylidene]dihydro- 4-hydroxy-2(3H)-furanone)
3	Molecular weight	350.45 gm/mol
4	Category	Phytochemical- Labdane diterpenoid (Diterpene lactone) [29] .Pharmacological- Anti-inflammatory, Anti-protozoal [29]
5	Apperance	White crystalline powder
7	Refractive Index	1.69
8	Melting point	260ºC
9	Nature	Acidic



(Fig.2.13 shows image of Andrographolide)

2.6.5 Kutkin

Picrorhiza kurroa is one of the major income generating non-timber forest products found in the Nepalese Himalayas. It is one of the oldest medicinal plants traded from the Karnali zone. known as **Kutki** , it is a perennial herb and is used as a substitute for Indiangentian (*Gentiana kurroo*).

It is found in the Himalayan region from Kashmir to Sikkim at an elevation of 2700-4500m and in Nepal, found abundantly between 3500 and 4800m. It is found far away from the

community and takes from hours to days to walk to its growing habitat. It has been reported that Picrorhiza has been harvested to near extinction.

Description

Leaves: 5–15 cm long leaves, almost all at the base, often withered. Leaves are coarsely toothed, narrowed to a winged stalk.

Rhizomes of the plant are 15–25 cm long and woody.

Flowers: small, pale or purplish blue, borne in cylindric spikes, spikes borne on almost leafless erect stems.

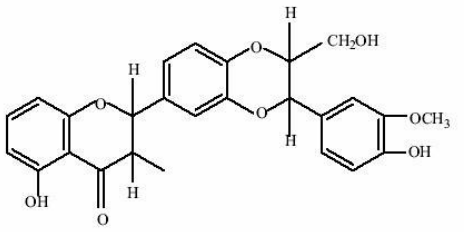
Flowers about 8 mm, 5-lobed to the middle, and with much longer stamens.

Fruits: 1.3 cm long.

Chemistry: Chemical composition of Picrorhiza kurroa include Kutkin, a bitter glycoside which contains two C-9 iridoid glycosides-Picroside I and Kutakoside. General description of kutkin is given in table 2.6

Table 2.6 General description of kutkin

Sr.no	**Physical characters**	**Result**
1	Molecular formula	$C_{22}H_{28}O_{12}$
2	IUPAC name	2-Methoxy-4-(1,1,2-trihydroxyethyl)phenyl beta-D-glucopyranoside 1-cinnamate
3	Molecular weight	484.153
4	Category	Phytochemical- Bitter glucoside. Pharmacological Hepatoprotective, Antioxidant, Anti-inflammatory
5	Apperance	Yellowish brown powder
6	Melting point	214-215ºc



(Fig.2.17 Structure of kutkin)

CHAPTER -3

LITERATURE REVIEW

CHAPTER 3 LITERATURE REVIEW

The literature review of this polyherbal formulation shows various methods for development of quality control parameters. The literature review shows various HPLC, TLC & U.V method development & validation of the biomarkers present in the given formulation.Details on literature review is given on table 3.1.

Table 3.1 Literature review

Sr. no	**Article name**	**Description**	**Author name**
1	Qualitative HPLC analysis of andrographolide in andrographis paniculata at 2 sifferent stages of life cycle of plant	This article HPLC development for determination of Andrographolide in *Andrographis paniculata.* Mobile phase was methanol: water (65:35), flow rate 1.5 mL/min. Retention time of pure Andrographolide was 2.871 minutes.	Meenu sharma and Sandeep tyagi
2	A validated stability-indicating HPLC method for analysis of glabridin prodrugs in hydrolysis studies	The article shows details about HPLC method development and validation for glabridin diacetate and dihexanoate. The chromatographic separation was achieved on a reverse phase C18 (Thermo Hypersil Keystone, 250 × 4.6 mm, 5 micron) column. Elution of the mobile phase was operated on isocratic (acetonitrile 76%: water 24%)for 9 min, followed by gradient (acetonitrile from76% to 90%) within 9 min and isocratic (acetonitrile90%: water 10%) for 12 min at 1 mL/min flow rate, detected at 280 nm.	Warunee Jirawattanapong1, Ekarin Saifah2, Chamnan Patarapanich1,
3	Journal of medicinal plants Pharmacognostic Study and Establishment of Quality	This article shows pharmacognostical analysis of vasicine	Arabind Kumar1, Vipin K Garg1, Ratendra Kumar1,

	Parameters of Leaves of *Adhatoda vasica*. Linn		Lubhan Singh1, Shivani Chauhan1, Sweety1
4	Unique Research Journal of Chemistry Antimicrobial activity of Andrographis Paniculata Nees & recuperative effect on mixing with antibiotics	This article gives details about the phytochemical screening of Andrographis *Paniculata Nees*	Shobana M1, Maheshwari R Syed Muzammil M
5	Development and validation of UV spectrophotometric method for the quantitative estimation of eugenol	This article gives details about U.V method development and validation of Eugenol as per ICH guidelines.	K. Pramod, Shahid H. Ansari, Javed Ali
6	Estimation of Adrographolide in A*ndrographis paniculata H*erb, Extracts and Dosage forms	This article shows method development and validation using HPLC and HPTLC of andrographolide.	Katta Vijaykumar, Papolu B.S.Murthy, Sukalak Kannababu, B. Syamasundar and Gottumukkala V. Subbaraju
7	Isolation and Identification of Flavonoid "Quercetin" from *Citrullus colocynthis (Linn.)* Schrad.	This article shows details about Flavonoid "quercetin" that was used for In-vivo, In-vitro studies which was purified and used for HPLC and IR studies	Mahesh Chand Meena and Vidya Patni
8	Pharmacognostical and Phytochemical Evaluation of *Adhatoda Vasica* Leaf	This article shows detailed information regarding pharmacognostical parameter of vasicine. It also shows its macroscopy.	Thakur Shilpa, Tadavi Daxa, Bhatt Mehul
9	Validated RP – HPLC method for the	This article shows RP-HPLC method development to carry out the	Kotagiri Ravikanth, Anil

	quantification of andrographolide in toxiroak premix, a polyherbal mycotoxin inhibitor	quantification of Andrographolide Phenomenex luna C18 column (250 mm x 4.6 mm, 5 µm) using isocratic mixture of Acetonitrile and 0.1 % ortho-phosphoric acid in a ratio of 40:60,v/v as a mobile phase, 1.0 ml/min as flow rate 226 nm as the wavelength for detection and quantification.	Kanaujia, Parveen Singh and Deepak Thakur
10	Development, Standardization and Evaluation of a Polyherbal Syrup	This article shows details about standardization of polyherbal formulation in general.	N.Thangarathinam, N.Jayshree, A.Vijay Metha, L. Ramanathan
11	Preformulation and Formulation Studies of the Poly Herbal Syrup of Hydroalcoholic Extracts of *Zingiber officinale* and *Piper nigrum*	The article shows preformulation studies of the extract and also microbiological studies.	Nirmala Korukola, Vinay Kumar Medisetti, Swathisri Sravanam, Kumar Raju Sanga
12	HPLC Method Development and Characterization of Bio-Active Molecule Isolated from Andrographis paniculata	The article shows details regarding extraction method and to isolate and characterize the compound obtained from the leaves of *Andrographis paniculata* (Burm. F.) Nees (Acanthaceae). This was studied through preliminary phytochemical screening of the various extracts of the leaves revealed the presence of flavones and terpenoids in them.	Chandana Majeet, B. K. Gupta, R. Mazumder, G. S. Chakraborthy
13	A review on standardisation of herbal formulation.	This article shows details on herbal medicines in general	Arun Rasheed Sravya Reddy B, Roja C
14	Determination of Gallic acid from their Methanolic Extract of Punica granatum by HPLC Method	This article shows analytical method for high performance liquid chromatographic (HPLC) procedure based on isocratic elution with UV detection has been developed for the determination of gallic acid	Nikita R. Sawant, Abhijit R. Chavan

		from their methanolic extract of punica granatum. Thes C-18 column with methanol: ethyl acetate:water (25:5:70) as isocratic elution mode with UV detection (λ=270 nm).	
15	Prospective process validation of polyherbal cough syrup formulation	This article shows details about validation studies of polyherbal cough syrup formulation in general	Lay Desai, Jignasa Oza, Kapil Khatri
16	Production, isolation & identification of flavonoids from aerial parts of *Hiptage benghalensis.*	The article shows details about *Hiptage benghalensis* (L) Kurz In the present study flavonoids have been extracted from dried and powdered samples of stem,leaves, and flowers of *Hiptage benghalensis* by well established method. Free (ether fraction) and bound (ethyl acetate) fraction of flavonoids were extracted from different parts of plants and were separately dried.	Shweta Yadav & Padma kumar
17	In vitro-study effect of herbal syrup of medicinal plant extract against ESBL producing *Klebsiella Pneumonia* causing UTI infection.	The article shows In vitro effect of extract of ESBL on herbal syrup for UTI infection	V. Singh, Sandip Patil, Shakti Pal and P. K. Chauhan
18	High Performance thin layer Chromatographic Analysis of 10-Gingerol in *Zingiber officinale* Extract and Ginger-Containing Dietary Supplements, Teas and Commercial Creams	The article shows details regarding *ginger rhizome powder.* HPTLC method has been developed to determine the quantity of 10-gingerol in Zingiber officinale extract it was done on aluminum-packed silica gel 60 F254 plates withn-hexane:ethyl acetate 50:50 (v/v) as mobile phase. The retention factor (Rf) of 10-gingerol was found to be 0.42 ± 0.02.	Hasan yusufoglu, Saleh I. alqasoumi
19	In vitro Antimicrobial Activity of the Extract of *Mitracarpus scaber* Leaves Formulated as Syrup	This article shows details about In vitro antimicrobial activity of crude extract of the leaves of Mitracarpus scarber "Zucc" as a syrup.	TA Abere, AO Onyekweli, and GC Ukoh

20	RP-HPLC Method Development and Validation of Gallic acid in Polyherbal Tablet Formulation	This article shows RP-HPLC method development & validation for the estimation of Gallic acid in bulk and pharmaceutical tablet dosage form. The chromatographic conditions used for the separation was Phenomenex Luna C18 (2) (4.6 x 250mm, 5µ), rheodyne manual injector with capacity of 20µL and mobile phase comprised of Water: Acetonitrile (80: 20%v/v) and pH is maintained at 3.00 using Ortho phosphoric acid (OPA). The flow rate was 1.0mL/min with detection at 272nm. The retention time was found to be 3.60min.	Kamal Kardani1, Nilesh Gurav, Bhavna Solanki, Prateek Patel, Bhavna Patel
21	Microbiological characteristics & phytochemical screening of some herbal teas in Nigera	The present study was undertaken to investigate the bacterial and fungal contamination of herbal teas	Omogbai, Barry Aigbodion, PhD Ikenebomeh, Marcel, PhD
22	HPTLC method development & validation for the estimation of 6-Gingerol in oral thin film	The article shows HPTLC method for assay of 6-Gingerol and determination from herbal oral thin film (OTF). The chromatographic condition were: Toluene: Ethyl acetate: methanol: Formic Acid (5: 4:1:1v/v/v/v) as mobile phase; wavelength of the detector was 520nm drying temperature after development was 60°C.	Safeena sheikh, Suhail asghar & Showkat 3
23	Development & validation of U.V spectrophotometric method for estimation 0of glycyrrhetinic acid in hydroalcoholic extract of glycyrrhiza glabara	The present article shows spectrophotometric method for estimation of Glycyrrhetinic acid in hydro-alcoholic extract of *Glycyrrhiza glabra L.* have been developed. Glycyrrhetinic acid show absorbance maximum at 254 nm when Phosphate Buffer (pH-6.8) Ethanol are used as solvent in 70:30 proportion, so	SK. Patil, VR. Salunkhe and SK. Mohite

		absorbance was measured at the same time.	
24	Variable response of 3 morphotypes of tecomella undulata seem towards human pathogenic bacteria	The article shows antimicrobial potential of the three morpho types of *Tecomella undulata* against human pathogenic bacteria to select suitable morphotype for medicinal use.	Mis sagg, Navdeep kaur, Arneet gill
25	Method development for simultaneous analysis of marker scopoletine, andrographolide, qucertin & lutenolin in antihypertensive formuation on Jamuformulation using RP-HPLC	The article shows simultaneous analysis of marker compounds in antihypertension jamu formulation using reverse phase high performance liquid chromatography (RP-HPLC). Analysis of marker compounds in anti hypertension jamu formulation using HPLC with reverse phase ODS C18 column (LiChroCART®, 125 x 4.6 mm, particle size of 5 µm) and gradient mobile phase of formic acid 1% and methanol could separate markers in 35 min at flow rate 2 mL/min.	Irda fidrianny, Ummi rukoyah, Komar ruslanw
26	The High-Resolution Reversed-Phase HPLC Separation of Licorice Root Extracts Using Long Rapid Resolution HT 1.8-µm Columns	The article shows HPLC analytical studies using licorice, a licorice hydrolysis product, and commercial licorice samples, showed that resolution and throughput using a ZORBAX 1.8-µm column greatly exceeded that obtained using the conventional 5.0-µm column.	Bernard Permar and Ronald E. Majors
27	Development & validation of GC method for estimation of eugenol in clove extract	The article shows GC method development & validation for estimation of eugenol in clove extract. Here nitrogen is used as gas.	B.Y.K Surthi, B.M. Gurupadayya venkata sairamK, T. narendra kumar
28	Identification and Quantification of Andrographolide from	The article shows HPLC method development of andrographolide with C18 column using a mixture of water and methanol (35:65) as mobile phase at a flow rate of 0.7 mL/min, and the	B. K. Sajeeb1, Uttom Kumar, Shimul Halder and Sitesh C. Bachar

	Andrographis paniculata (Burm. f.) Wall. ex Nees by RP-HPLC Method and Standardization of its Market Preparations	estimated concentration level of andrographolide.	
29	GC-MS Analysis of Essential Oil from Long Pepper Growing in Tepi, South-west Ethiopia	The article deals with GC method development for analysis of essential oil from Long-pepper	Bizuneh Adinew
30	The Voltammetric and Titrimetric Determination of Ascorbic Acid Levels in Tropical Fruit Samples	The article shows voltammetric method for determination of ascorbic acid levels in tropical fruit samples	W. Okiei, M. Ogunlesi, L. Azeez, V. Obakachi, M. Osunsanmi, G. Nkenchor
31	Study of antimicrobial activity of ayurvedic & unani medicine & their comparative analysis with commercial antibiotics	The article shows various forms of medicines like Ayurveda, unnani. All these forms are studied in this article.	Sajib chakrabotty & Zakaria ahmed
32	Quantitative analysis of glycyrrhizic acid in crude drug and its herbal formulation by UV Spectrophotometry	The article shows details about U.V method studies in herbal formulation.	M. Senthil Raja, Imran Khan, Perumal. P, Surya Rao Srikakolapu, Srujana Divya Gotteti
33	Antibacterial activity of seagrass species of *cymodocea serrulata* against chosen bacterial fish pathogens	The article shows details about the microbial studies that was made on *cymodocea serrulata* against bacterial fish pathogens	Sundaram Ravikumar, K. Nanthini devi, T.T. Ajith kumar and M. Ajmalkhan
34	Development and Validation of RP-HPLC Method for Simultaneous Analysis of Andrographolide, Phyllanthin, and	The article shows details about liquid chromatographic method with photodiode array detector that was developed for the determination of andrographolide, phyllanthin,	S.G. bhope, V.V kuber

	Hypophyllanthin from Herbal Hepatoprotective Formulation	and hypophyllanthin. The separation was carried out on a reverse-phas 250 mm × 4.6 mm,5µm symmetry C8 column (Waters). The gradient was prepared from 0.1% orthophosphoric acid (solvent A) and (1:1) acetonitrile:methanol (solvent B) as mobile phase delivered at a flow rate of 1 mL/min	
35	A study on the retention of pyrrolquinazoline alkaloid vasicine in nutraceutical formulations of vasa leaves	The article shows details about HPLC studies of different vasa leaves	K. Srinivasan and C. Sivasubramania n
36	Pathogenicity reduction of Rhizoctonla by phyllosphere modification of vasa leaves	This article shows pathogencity reduction of Rhizoctonla. Various vasa leaves were taken for study	Debojit Saha, Rishika Chakraborty, Arpan Majumdar, Joyce D. John and Kasturi Biswas, Arup Kumar Mitra
37	Standardization of Zymodyne syrup- a polyherbal formulation.	The article shows details about Zymodyne syrup whose all the parameters were checked. It was just like standardisation. And all it's value were found to be in limit.	Saurabh Parmar, Nihar Shah, Kinjal Shah2, Natubhai Patel
38	Optimization and validation of reverse phase HPLC and HPTLC method for simultaneous quantification of vasicine and vasicinone in *Sida* Species	The article shows details regarding HPLC&HPTLC method development for vasicine & vasicinone in Sida species using a mobile phase of acetonitrile - 0.1 M phosphate buffer - glacial acetic acid (15:85:1, *v/v/v*) with pH 4.0 on a C18-ODS-Hypersil column in isocratic mode. The retention times of vasicine and vasicinone were 5 and 8.7 min, respectively. In the HPTLC method,	Kamlesh Dhalwal, Vaibhav M. Shinde and Kakasaheb R. Mahadik

		mobile phase of ethyl acetate, methanol, ammonia (8:2: 0.2, *v/v*) was used on precoated plate of silica gel 60 F254.	
39	Evaluation of *Picrorhiza kurrooa* accessions for growth and quality in north western Himalayas	The article shows details about evaluation of *Picrorhiza kurrooa* Species.	Rakesh Kumar, Pamita Bhandari2, Bikram Singh1 and P. S. Ahuja1
40	Isolation of piperdine from *Piper nigrum* and its antiproliferative activity	The article shows details about isolation of piperdine from *Piper nigrum*	S. K. Reshmi, E. Sathya and P. Suganya Devi
41	Induction of Andrographolide, A Biologically Active Ingredient in Callus of *Andrographis Paniculata (*Burm.F) Wallich Ex. Nees	The article shows details about andrographolide	Alwar Vidyalakshmi, Subramanian Ananthi
42	Development and Validation of Rapid RPHPLC Method for Estimation of Piperine in *Piper nigrum* L.	The article shows details about HPLC method that was carried out by using (250x4 mm, 5 µ) C18column with a mobile phase consisting Acetonitrile, Water, Acetic acid (60:39.5:0.5). The flow rate was set to 1.0 ml/min with UV detection at 340 nm with run time 10 min and injection volume set at 20µl.	Vipul Upadhyay, Neeru Sharma, Himanshu M. Joshi, Amreesh Malik, Manoj Mishra, B.P Singh, Sanjeev Tripathi
43	Phytochemical analysis and antibacterial activity of Pepper (*Piper nigrum* L.) against some human pathogens	The article shows details about phytochemical analysis and antibacterial activity of *nigrum* L.	P. Ganesh, R. Suresh Kumar and P. Saranraj
44	Design, Development and Phytochemical Evaluation of a Poly Herbal Formulation Linkus Syrup	The article shows phytochemical and evalution of herbal formulation	Zeeshan Ahmed Sheikh, Aqib Zahoor, Saleha Suleman Khan, Khan Usmanghani,

45	Development and Validation of Improved RP-HPLC method for Identification and Estimation of Ellagic and Gallic acid in Triphala churna	The article shows details about estimation of ellagic acid and gallic acid in Triphala churna. RP-HPLC method for the separation and quantification determination of the Gallic acid and Ellagic acid from Triphala has been developed and validated. The use of an RP18 column with a gradient acidic mobile phase enabled the efficient separation of gallic acid and ellagic acid within a 30 min analysis.	Patel Madhavi G., Patel Vishal R., Patel Rakesh K.
46	Development and Validation of Visible Spectrophotometric Method for Determination of Andrographolide in Kalmegh Plant Extract	The article shows details about U.V method development of andrographolide kalmegh plant extract.	A. Suneetha and K.Manasa
47	Evaluation of in-process quality control parameters of Ayurvedic preparation kankasava	The article shows details about general Ayurvedic preparation kankasava.	Bharti Ahirwar
48	Quantitative estimation of gallic acid and tannic acid in bhuvnesvara vati by RP-HPLC	The article shows details about gallic acid and tannic acid in bhuvneswara vati through RP-HPLC.	Surya Prakash Gupta and Gopal Garg
49	Phytochemical screening & thrombolytic activity of the leaf extracts of Adhatoda vasica	The article shows details about leaf extract of vasicine. This article shows details about phytochemical screening.	Mohammad Shahriar
50	Development and Validation of HPTLC Method for Determination of Vasicine in Polyherbal Cough Syrup	The article shows details about TLC &HPTLC. TLC aluminium plates precoated with silica gel 60F-254 (0.2 mm thickness) were used. Mobile phase Ethyl acetate: methanol: ammonia (8.0 : 2.0 : 0.2 v/v/v) at 254 nm.	Unmesh Keshwar1, Soumit Pimplapure, Neeraja Sabnis, Dhurde, Shrikhande

51	High Performance Thin layer Chromatography: Application in Pharmaceutical Science	The article shows details about HPLC method application in pharmaceutical science.	Naveen Bimal and Bhupinder Singh Sekhon
52	HPLC determination of vasicine &vasicinone in *Adhatoda vasica* with photo diode array detection	The article shows HPLC determination of vasicine.The separation of 1 and 2 was performed with acetonitrile– phosphate buffer (pH maintained to 3.9 using glacial acetic acid) (15:85) using a Hibar Merck make C18 column.	Shaifali Srivastava, Ram K. Verma, Madan M. Gupta, Subhash C. Singh, and Sushil Kumar
53	HPLC method for the estimation of Andrographolide in rabbit serum	The article shows development of HPLC method for the estimation of andrographolide in biological fluids.A Neuclosil C18 octadecyl silane (ODS) column (5m, 250x4.6mm Hypersil) was used. The mobile phase used was methanol: Water in the ratio of 65:35 v/v at a flow rate of 1.0 ml/min. The elutes were monitored at 223 nm.	K. Senthil kumaran, P. Thirugnasam
54	Identification, evaluation and standardization of herbal drugs: A review	The article shows details about general identification, evalution & standardization of herbal drugs	Archana Gautam, Shiv Jee Kashyap, Pramod Kumar Sharma, Vipin Kumar Garg, Sharad Visht, Nitin Kumar
55	Various Biochemical Parameters of Protease Isolated From *Adhatoda Vasica*: A Medicinally Important Plant	The article shows details about vasicine's medicinal importance.	B Khurana, A Mishra, N Jabalia and N Chaudhary
56	Extraction of Glycyrrhizic Acid and Glabridin from Licorice	The article shows extraction and separation of glycyrrhizic acid and glabridin from licorice. By changing the different extraction solvents, procedures,	Minglei Tian, Hongyuan Yan and Kyung Ho Row

		times and temperature, the optimum extraction condition was established: the used of ethanol/water (30:70, v/v) as an extraction solvent, and 60 min dipping time under 50°C.The extracts of licorice were separated and determined by RP-HPLC with a methanol/water (70:30, v/v, containing 1% acetic acid) as the mobile phase.	
57	HPLC Profiles of Standard Phenolic Compounds Present in Medicinal Plants	The article shows HPLC study of medicinal plant. It also shows procedure for the HPLC Membrane Filter (0.45µm & 47mm diameter) before injecting 20 µl in Column: Symmetry C18 (5µm, 4.6*250mm) & flow rate 1.0 ml/min. UV-detection at 280 360 nm was performed using 515 HPLC pumps and 2489 UV/VIS detectors.	Gupta Mradu, Sasmal Saumyakanti, Majumdar Sohini, Mukherjee Arup
58	Improved Method for Quantifying Capsaicinoids in *Capsicum* Using Highperformance Liquid Chromatography	The article shows improved high-performance liquid chromatography (HPLC) method for analysis of capsaicinoids in dried *Capsicum* fruit powder. Extraction of *Capsicum* fruit powder using acetonitrile proved to be the best capsaicinoid extractor in the shortest time interval. Solvents used for HPLC separation and quantification of capsaicinoids include methanol and water at 1 mlmin–1 flow rate.	Margaret D. Collins, Loide Mayer Wasmund, and Paul W. Bosland
59	*In-vitro* Evaluation of Antimicrobial Branded Herbal Formulations for their Efficacy	The present article deals with various evalution parameters for herbal formulation. It also deals with in-vitro studies. The article also shows anti-microbial studies various micrrorganisms like E.coli was used.	Faheem Jan, Ifthekhar Hussain, Naveed Muhammad, Muhammad Adnan Khan, Mazhar Ali Khan, Muhammad Taj

			Akbar and Waqas Ahmad
60	HPLC-MS study of phytoestrogens from *Glycyrrhiza glabra*	The article deals with HPLC-MS study of Glycyrrhiza glabra	Ibrahim khalaf, Laurian vlase
61	Isolation, characterization and inhibition effect of vasicine on mild steel corrosion in 1N HCl	The article shows corrosion study of vasicine using 1N HCL	V. Thailan1, K. Kannan and S. Gnanave
62	Pharmacognostic Study of *Adhatoda vasica* Nees	The article shows details about pharmacognostical analysis of vasicine.	Kanthale P R and Panchal V H
63	HPLC, NMR and MALDI-TOF MS Analysis of Condensed Tannins from *Lithocarpus glaber* Leaves with Potent Free Radical Scavenging Activity	The article shows in general details about HPLC, NMR MALDI-TOF MSfor analysis of condensed tannins.	Liang Liang Zhang and Yi Ming Lin
64	Comparative pharmacokinetic profiles of picrosides I and II from kutkin, Picrorhiza kurroa extract and its formulation in rats.	The article shows details about comparative analysis of picrosides I & II of kutkin.	Dilawar Upadhyay, Ranjeet Prasad Dash , Sheetal Anandjiwala , Manish Nivsarkar
65	Quantitative HPLC Analysis of Ascorbic Acid and Gallic Acid in *Phyllanthus emblica*	The article shows simultaneous estimation of gallic acid & ascorbic acid in Phyllantus emblica. Here C18 column was used with a gradient elution of methanol and 0.1% (v/v) acetic acid in HPLC-grade water as mobile phase at a flow rate of 0.9mL min-1. UV detection was performed at 278 nm.	Laxman Sawant, Bala Prabhakar and Nancy Pandita
66	Content of gallic acid in selected plant extracts	The article shows details about gallic acid in various plants that are fractionated by a Sephadex LH-20 column chromatographic method with 95% (v/v) ethanol and acetone :water (1:1; v/v) as	Magdalena Karamaæ, Agnieszka Kosiñska, Ronald B. Pegg

		mobile phases: one fraction consisted of low molecular-weight phenolics and the other of tannins.	
67	Method development and validation of forced degradation studies of metformin hydrochloride by using U.V spectroscopy	This article shows force degradation studies of metformin tablet that was done on Stress degradation by acid,basic hydrolysis, oxidative degeneration, photolytic reduction, heat exposure.	Doredla Narasimha rao, M. Prasada rao, J. Naga Hussain, S. Lakshmi Sumanoja and V. Rajeswara rao
68	Hepatoprotective activity of 6 polyherbal in CCL4 induced liver toxicity in mice	This article gives details about various hepato-protective drugs and their animal study.	C Girish, Bidhan Chandra konar,S.Jayanthi, K.Ramchandra Rao,B Rajesh Suresh Chandra Pradhan
69	Hepatoprotective Activity of *Asparagus racemosus* Root against Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats	This article shows details about in-vitro studies in rats for checking hepatoprotective activity.	Fasalu Rahiman O.M, Mohthash Musambil, Shejina M
70	Plants used in the hepaoprotective remedies in traditional medicines	This article gives details about traditional medicines in hepatoprotective activity.	Suruchi Singh, Maryam Bincy Thomas, Sharada Pal Singh, D.Bhowmik
71	Herbal Induced Hepatoprotection and Hepatotoxicity : A Critical Review	This article gives details about hepatoprotective activity &toxicity in herbal drugs	Onkar Bedi1, Krishna Reddy V. Bijjem, Puneet Kumar and Vinod Gauttam

CHAPTER 4

AIM AND OBJECTIVE

CHAPTER 4 AIM AND OBJECTIVE

4.1 Aim of present work

"DEVELOPMENT OF QUALITY CONTROL PARAMETERS OF HEPASAVE SYRUP".

4.2 Objectives of present work

Development of Quality control parameters by ….

1. Identifying marker compounds present in the syrup using UV visible spectroscopic method.
2. Fractionation of the biomarkers present in formulation by TLC.
3. Evaluating of Physico-chemical parameters of Syrup by checking its appearance, density, viscosity etc.
4. Phytochemical screening of the formulation for secondary metabolites.
5. Estimation of phytoconstituents in the given formulation.
   Investigation of bitterness value, extractive value of the given formulation.
6. Evaluating stability of syrup by checking microbial contamination total viable count and yeast and mould count.

7. RP-HPLC method development and stability study of different biomarkers of the given formulation.
8. Evaluation of heavy metal screening using AAS.
9. Forced degradation study of the biomarkers using U.V spectroscopy.

CHAPTER 5

QUALITY CONTROL PARAMETER OF HEPASAVE SYRUP

Chapter 5 Quality control parameters of Hepasave syrup

Quality control parameters are defined as the quality control of phytopharmaceuticals may be defined as the status of a drug, which is determined either by identity, purity, content, and other chemical, physical or biological properties, or by the manufacturing process.

Quality control is based on three important pharmacopeial definitions:

- **Identity**: Is the herb the one it should be?
- **Purity**: Are there contaminants, e.g., in the form of other herbs which should not be there?
- **Content or assay:** Is the content of active constituents within the defined limits?

5.1 Experimental work

5.1.1 Materials and reagent

5.1.1.1 Procurement of Formulation

The formulation, Hepasave syrup (100ml), was received from Cadila Pharmaceuticals Limited, Trasad Road, Dholka-387810, Dist. Ahmedabad (Gujarat), India.

5.1.1.2 Solvents used in the experiment

Ethyl Acetate, Methanol, Petroleum Ether, Toluene, Chloroform, Acetonitrile were used of AR grade (S.D.fine chemicals, Mumbai, India & Merck solutions, Mumbai ,India) and Distilled water

5.1.2 INSTRUMENTS AND EQUIPMENTS

Following is the list of equipments and instruments used throughout the project work.

All the instruments were if required was calibrated periodically as per in house SOP of Institute of Pharmacy, Nirma University.

- **Analytical balance** CITIZEN Scale CX-220, USA, having weighing capacity of 10 mg to 220 mg.
- **Water bath,** EIE instrument Pvt. Ltd., Ahmedabad, India.
- **U.V Spectrophotometer-**Shimadzu UV-Visible double beam spectrophotometer, model - 2450 PC, (Shimadzu,Japan).
- **Sonicator** Model: TRANS-O-SONIC; D-compact., Capacity: 2 lit, EIE Instrument Pvt. Ltd. (Ahmedabad, Gujarat, India)

- **Vaccum pump**: Vaccum pump of Millipore Pvt. Ltd was used to filter the different solvents for analysis.
- **HPLC (High Performance Liquid Chromatography)**
  JASCO 200 Series, with borwin software, Jasco Inc, (Japan).

5.1.3 Preparation of extracts

Take 20ml of the sample in a separating funnel add 20ml of solvent in the separating funnel shake the funnel for 20 minutes such that sample layer and solvent layer gets separated. Solvent layer is collected. This procedure is to be repeated 3 times. A clean dry porcelain dish is taken. It is weighed and "tared". The solvent layer is added in it after that this layer is to be evaporated to dryness. The residue left in the porcelain dish is to be dissolved with sufficient amount of diluent and then it is made volume of 50ml with diluent.

5.1.4 Preliminary Thin Layer chromatography

All the extract was qualitatively evaluated by chemical tests, TLC studies, estimation, for the presence of various phytoconstituents like alkaloids, Steroids, saponins and tannins.

5.1.5 Organoleptic characters

This was done by checking the formulation by visual inspection as per WHO guidelines.The formulation was observed through eye, texture in between thumb and finger, odour through nose and taste through tongue were checked.

5.1.6 Physicochemical parameters

Physicochemical parameters of the formulation were studied as per WHO guidelines. The physicochemical properties of the formulation are pH, density, viscosity, misicibility and physical stability. This was performed using pH meter for pH, specific gravity bottle for density, Brooke-field viscometer for viscosity, dissolving formulation in different organic solvent for miscibility and keeping the sample in extreme condition for physical stability.

5.1.7 Phytochemical screening test for presence of secondary metabolite

5.1.7.1 Test for Alkaloid

The following tests are performed for the alkaloids

5.1.7.1(A) Mayer's reagent

Alkaloids gives cream colour precipitate using Mayer's reagent (Potassium Mercuric Iodide solution).

5.1.7.1(B) Wagner's reagent

Alkaloids gives reddish brown precipitate using Wagner's reagent (Iodine-Potassium Iodine solution).

5.1.7.1(C) Hager's reagent

Alkaloids gives yellow precipitate using Hager's reagent (Saturated solution of picric acid).

5.1.7.1(D) Tannic acid test

Alkaloids gives buff colour precipitate using Tannic acid solution.

5.1.7.2 Test for Carbohydrates

The following tests were performed for carbohydrates

5.1.7.2 (A) Molisch's test

To the test solution add few drops of alcoholic α-napthol, then add few drops of con. Sulphuric acid through sides of test-tubes, purple to violet colour ring appears at the junction.

5.1.7.2 (B) Barfoed's test

1ml of test solution is heated with 1m of Barfoed's reagent on water bath, if red cupric oxide is formed, monosaccharide is present. Disaccharides on prolong heating (about 10minutes) may also cause reduction, owing to partial hydrolysis to monosaccharides.

5.1.7.2(C) Selivanoff's test (Test for ketones)

To the test solution add crystals of resorcinol and equal volume of concentrated hydrochloric acid and heat on a water bath, rose colour is produced.

5.1.7.2 (D) Test for pentoses

To the test solution add equal volume of hydrochloric acid containing small amount of phloroglucinol and heat, red colour is produced.

5.1.7.3 (E) Osazone formation test

Heat the test solution with solution of phenyl hydrazine hydrochloride, sodium acetate and acetic acid. Examine the yellow crystals formed under microscope. These crystals are characteristic shape for particular sugar.

5.1.7.3 Test for Flavanoids

The following tests were performed for flavonoids

5.1.7.3 (A) Alkaline reagent test

To the test solution add few drops of sodium hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicate presence of flavonoids.

5.1.7.3 (B) Zinc hydrochloride test

To the test solution add a mixture of zinc dust and concentrated hydrochloride. It gives red colour after few minutes.

5.1.7.4 Test for Glycosides

5.1.7.4 General test

Test A

Extract 200mg of drug with 5ml of dilute sulphuric acid by warming on a water-bath. Filter it. Then neutralize the acid extract with 5% solution of sodium hydroxide. Add 0.1ml of Fehling A and B until it becomes alkaline (test with pH paper) and heat on a water for 2 minutes. Note the quantity of red precipitate formed.

Test B

Extract 200mg of drug with 5ml of water instead of dilute sulphuric acid. After boiling add equal amount of water as used for sodium hydroxide in the above test. Add 0.1ml of Fehling A and B until it becomes alkaline (test with pH paper) and heat on a water for 2 minutes. Note the quantity of red precipitate formed. Compare the quantity of precipitate formed in Test B with that of Test A. If the precipitate in Test A is greater than in Test B than glycoside may be present. Since Test B represents the amount of free reducing sugar already present in the crude drug, whereas Test A represents free reducing sugar plus those related on acid hydrolysis of any glycoside in the crude drug.

5.1.7.5 Chemical tests for specific glycosides

5.1.7.5 Anthraquinone glycosides:

5.1.7.5 (A) Borntrager's test

Boil the test material with 1ml of sulphuricacid in a test-tube for 5 minutes. Filter while hot. Cool the filtrate and shake with equal volume of chloroform. Separate the lower layer of chloroform and shake it with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammonical layer.

5.1.7.5 (B) Modified borntrager's test

Boil 200mg of test material with 2ml of dilute sulphuric acid. Treat it with 2ml of 5% aqueous ferric chloride (freshly prepared) for 5 minutes, shake it with equal volume of chloroform and continue the test as above. As some plants contain anthracene aglycone in reduced form, if ferric chloride is used during the extraction, oxidation to anthraquinone takes place, which shows response to borntrager's test.

5.1.7.5 (C)

Hydrolyzed ether extract with methanolic acetate gives pink colour in day light and greenish orange colour under UV light.

5.1.7.5 (D) Test for hydroxyl-anthraquinones

Treat the sample with potassium hydroxide solution red colour is produced.

5.1.7.6 Cardiac glycosides

5.1.7.6(A) Keller-killani test (test for deoxy sugars)

Extract the drug with chloroform and evaporate it to dryness. Add 0.4ml of glacial acetic acid containing trace amount of ferric chloride. Transfer to a small test tube, add 0.5ml of con. Sulphuric acid by the side of the test tube. Acetic acid layer shows blue colour.

5.1.7.6(B) Raymond's test

Treat the test solution with hot methanolic alkali, violet colour is produced.

5.1.7.6 (C)Legal's test

Treat the test solution with pyridine and add alkaline sodium nitroprusside solution, blood red colour appears.

5.1.7.7 (D) Baljet's test

Treat the test solution with picric acid, orange colour is formed.

5.1.7.8 Saponin glycosides

5.1.7.8 (A) Froth formation test

Place 2ml solution of drug in water in a test tube, shake well, stable froth (foam) is formed.

5.1.7.8 (B) Haemolysis test

Add 0.2ml of solution of saponin (prepared in 1% normal saline) to 0.2ml of blood in normal saline and mix well. Centrifuge and note the red supernatant. Compare with control tube containing 0.2ml of 10% blood in normal saline diluted with 0.2ml of normal saline.

5.1.7.9 Tannins (phenolic compounds)

5.1.7.9 (A) Ferric chloride test

Treat the extract with ferric chloride solution, bue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.

5.1.7.9(B) Gelatin test

To the test solution add 1% gelatin solution containing 10% sodium chloride. Precipitate is formed.

5.1.7.9(C) Test for catechin

Dip a matchstick in the test solution, dry it and lastly moisten with con. hydrochloric acid. Then warm the stick near flame. The colour of the wood changes to pink due to phloroglucinol.

5.1.7.10 Steroids and triterpenoids

5.1.7.10 (A) Libermann-Buchard test

Treat the extract with few drops of acetic anhydride, boil and cool. Then add con. sulphuric acid from the side of the test-tube, brown ring is formed at the junction 2 layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoids.

5.1.7.10 (B) Salkowsi test

Treat the extract with few drops of con. Sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

5.1.7.10 (C)Sulfur powder test

Add small amount of sulfur powder to the test solution, it sinks at the bottom.

5.1.8 Estimation of phytoconstituents from Hepasave liquid

5.1.8.1 Estimation of Phenolics

To 1 ml of the extract 10 ml of distilled water and 1.5 ml of diluted (1:2) Folin ciocalteu reagent were added and the mixture was kept aside for 5 min. After adding 4 ml of 20 %w/v Na_2CO_3 solution the final volume was adjusted to 25 ml using distilled water. The absorbance was measured at 765 nm at an interval of 30 min up to 2 h using distilled water as a blank. The total phenol content was measured using following formula of straight line.

5.1.8.2 Estimation of flavonoids

To 3 ml of the extract 3 ml of methanolic $AlCl_3$ was added. After 10 min, the absorbance was read at 430 nm. Results were expressed in g/100g of dry matter with respect to Qucertin serves as a standard.

5.1.8.3 Estimation of carbohydrate

5.1.8.3 (A) Estimation of Sugar

Prepared 10, 20, 30,.90 μg/ml concentration of standard glucose solution (100 μg/ml). Take nine test tubes, to each test tube 2 ml standard glucose solution of different concentrations, to this 1 ml of 5 %w/v phenol solution and mixed well in test tubes. Test sample was prepared in the same way by taking Ext A instead of standard glucose solution. Blanks were prepared with 1 ml of water instead of sugar solution. From a fast flowing pipette, 5 ml of 96 %v/v sulphuric acid was added to each tube, mixed well. After 10 min, the tubes were reshaken and placed in water bath at 25-30˚C for 20 min. The yellow orange color developed was measured at 482 nm. The amount of sugar was determined by reference to a standard curve previously prepared for glucose being assayed**.**

5.1.8.3 (B) Estimation of Starch

Prepared 12.5, 25, 37.5, 50, 62.5, 75, 87.5, and 100 μg/ml concentrations of standard glucose solution (500 μg/ml). Take nine test tubes, to each test tube added 3 ml standard glucose solution of different concentrations, then cooled in an ice bath and add 10 ml of fresh Anthrone reagent (0.2 %w/v in 95 %v/v cold H_2SO_4). The contents were mixed well and heated for 7.5 min at 100˚C. The tubes were rapidly cooled to 25˚C on an ice bath and the color intensity of the solution was read at 620 nm using spectrophotometer. A standard curve was prepared using different concentration of standard glucose solution to estimate starch content.

5.1.9 Heavy metal screening by atomic absorption spectroscopy. /Evaluation of Toxicity by performing heavy metal screening using AAS.

5.1.9.1 For arsenic

Remove the cap from pellet dispenser and load with sodium borohydride pellet. Place the dispenser cap. Raise the quartz tube and ignite the flame. Place the tube ori the flame. Set the inert gas (nitrogen) supply regulator to 1’4 kg/cm2 through the reaction vessel. Optimise the response of the instrument by the adjustment of burner height and flame adjustment. Specification for hydrochloric acid**.** The specification for nitric acid. Specification for sulphuric acid. Remove the

stopper assembly from reaction vessel and add 20 ml reagent blank in the reaction vessel. Replace the stopper assembly and switch on the stirrer. After 30 seconds turn the disperser knob through 180" to allow the pellet to drop. Turn the knob back and take the absorption reading. Similarly take reading with 5 working standards and plot the graph in concentration US absorbance. Take sample in a reaction vessel, proceed as above and take the absorbance. From the graph calculate the concentration of arsenic in ppm in the sample.

CALCULATION

$$\text{Arsenic, percent by mass} = \frac{C*V*100}{M*1000000}$$

Where

C= concentration of arsenic in µg/ml in the final solution,

V= Volume in ml in the final solution, and

M= mass in g of the sample in the final solution.

5.1.9.2 For lead

(a) Pre-treatment :- Homogenize product if necessary, using non contaminating equipment. Check for leaching metals if the apparatus consists of metal parts.

(b) Drying:- In a crucible, weigh 10–20 g test portion to nearest 0.01 g. Dry in a drying oven, on a water-bath, or a hot plate at 100◦c , if there is a risk of heavy boiling in the ashing step. Proceed according to type of furnace.

(c) Ash :-(1) Ashing in a programmable furnace :-Place dish in furnace at initial temperature not higher than 100◦c. Increase temperature at a maximum rate of 50◦c/h to 450◦c. Let dish stand for at least 8 h or overnight. Continue according to (d).

Preparation of Ash in a muffle furnace with thermostat following drying and pre-ashing in apparatus described in B(d)–(h). Place crucible with the test portion covered with the glass cover on the ceramic plate, and let purified air coming through a glass tube sweep over the product. Put

IR lamp down at the cover. Pre-ash product by increasing temperature slowly with IR lamp by gradually increasing temperature on hot plate to maximum. Final temperature on ceramic plate should then be about 300◦C. Time required for pre-ashing varies with product. Put crucible in muffle furnace at 200◦–250◦C and slowly raise tem per a ture to 450◦C at a rate of no more than 50◦C/h. Let stand for at least 8 h or overnight. Take crucible out of furnace and let cool.

(d) Solution:- Wet ash with 1–3 mL water and evaporate on water-bath or hot plate. Put crucible back in furnace at no more than 200◦C and raise tem perature (50◦–100◦C/h) to 45◦c. Proceed with ashing at 45◦c for 1–2 h or longer. Repeat procedure until product is completely ashed, i.e., ash should be white/grey or slightly colored. Number of repetitions necessary depending on type of product. Add 5 mL 6M HCl, C(b), to crucible ensuring that all ash comes into con tact with acid. Evap o rate acid on water-bath or hot plate. Dissolve residue in 10.0–30.0 mL, to the nearest 0.1 mL, of 0.1M HNO3, C(d). Swirl crucible with care so that all ash comes into con tact with acid. Cover with watch glass and let stand for 1–2 h. Then stir solution in crucible thoroughly with stir ring rod and transfer contents to plastic bottle. Treat blanks in the same way as products. Include 2 blanks with each analytical batch.

(e) Atomic absorption spectrophotometry :- Pb and Cd in foods generally require graphite furnace AAS for determination. Zn, Cu, and Fe can, in most foods, be determined by flame AAS. Wave length, gas mixture/temperature program, and other instrumental parameters that are most appropriate for each metal are found in the manual provided with the instrument. Back ground correction must always be used in flameless AAS and for flame applications at low concentrations. When results are outside of the linear range, dilute the test solutions with 0.1M HNO3, C(d).

(1) Flame technique :- Prepare calibration curves from a minimum of 3 standards.
(2) Graphite furnace (flameless) technique :-The method of addition should always be used. Measurements must be made in the linear range when method of addition is used. Measurements are preferably made with peak area rather than peak height.
E. Calculations and Evaluation of Results
Detection limit :- Calculate the detection limit, DL, for each metal as:
DL = 3 standard deviation of the mean of the blank determinations (n = 20)

Calculate the concentration, c, of metal in the test sample
according to the formula:

$$C = \frac{(a-b)*V}{m}$$

where c = concentration in the test sample (mg/kg); a = concentration in the test solutions (mg/L); b = mean concentration in the blank solutions (mg/L); V = volume of the test solution (mL); m = weight of the test portion (g).

5.1.10 Extractive values of the formulation

Take 10 ml of formulation with 10 ml of toluene in a separating funnel. Shake the funnel vigorously for 10 minutes. Collect the solvent layer. Repeat this for 3 times than collect the extract in the clean dry weighed and previously tarred porcelain dish. Now evaporate it to dryness and again weight the porcelain dish. Repeat this procedure for methanol, benzene and petroleum ether.

5.1.11 Determination of Bitterness value

Stock and diluted quinine hydrochloride solutions

Dissolve 0.100 g of quinine hydrochloride R in sufficient safe drinking-water to produce 100 ml. Further dilute 5 ml of this solution to 500 ml with safe drinking water. This stock solution of quinine hydrochloride (*S*q) contains 0.01 mg/ml.

Procedure for bitterness value

After rinsing the mouth with safe drinking-water, taste 10 ml of the most dilute solution swirling it in the mouth mainly near the base of the tongue for 30 seconds. If the bitter sensation is no longer felt in the mouth after 30 seconds, spit out the solution and wait for 1 minute to ascertain whether this is due to delayed sensitivity. Then rinse with safe drinking-water. The next highest concentration should not be tasted until at least 10 minutes have passed. The threshold bitter concentration is the lowest concentration at which a material continues to provoke a bitter sensation after 30 seconds. After the first series of tests, rinse the mouth thoroughly with safe drinking-water until no bitter sensation remains. Wait for at least 10 minutes before carrying out the second test. In order to save time in the second test, it is advisable to ascertain first whether the solution in tube no. 5 (containing 5 ml of *S*T in 10 ml) gives a bitter sensation. If so, find the

threshold bitter concentration of the material by tasting the dilutions in tubes 1−4. If the solution in tube no. 5 does not give a bitter sensation, find the threshold bitter concentration by tasting the dilutions in tubes 6−10. All solutions and the safe drinking-water for mouth washing should be at 20−25 °C.

Calculate the bitterness value in units per g using the following formula:

$$\frac{2000*C}{a*b}$$

where a = the concentration of the stock solution (*S*T) (mg/ml);

b = the volume of *S*T (in ml) in the tube with the threshold bitter concentration;

c = the quantity of quinine hydrochloride R (in mg) in the tube with the threshold bitter concentration.

5.1.12 Microbial studies

Microbial studies were performed as per WHO guidelines.

5.1.12.1 Test for specific microorganisms

In test for specific microorganisms the test was performed using borer's method. Three microorganisms were selected (*E. coli, B. subtilus and St. aureus*.) 3 pairs of agar patriplate were taken ("+") sign was made in the patriplate such that each part becomes a zone a borer was taken and boring was done in the add one zone with a concentration of drug with culture another with other concentration of drug with culture third with culture and forth with only drug. They were covered and zones were observed after 48 hours.

5.1.12.2 Total viable count

For bacteria use Petri dishes 9–10 cm in diameter. To one dish add a mixture of 1 ml of the formulation and about 15 ml of liquefied casein-soybean digest agar at a temperature not exceeding 45 °C. Alternatively, spread the formulation on the surface of the solidified medium in a Petri dish. If necessary, dilute the material to obtain an expected colony count of not more than 300. Prepare at least two dishes using the same dilution, invert them and incubate them at 30–35 °C for 48–72 hours, unless a more reliable count is obtained in a shorter period of time. Count the number of colonies formed and calculate the results using the plate with the largest number of colonies, up to a maximum of 300.

For fungi use Petri dishes 9–10 cm in diameter. To one dish add a mixture of 1 ml of the formulation and about 15 ml of liquefied *Sabouraud glucose agar with antibiotics* at a temperature not exceeding 45 °C. Alternatively, spread the formulation on the surface of the solidified medium in a Petri dish. If necessary, dilute the material as described above to obtain an expected colony count of not more than 100. Prepare at least two dishes using the same dilution and incubate them upright at 20–25 °C for 5 days, unless a more reliable count is obtained in a shorter period of time. Count the number of colonies formed and calculate the results using the dish with not more than 100 colonies.

5.1.13 Identification of biomarkers using U.V Spectroscopy

Preparation:-1 The formulation HEPASAVE solution was procured from Cadila Pharmaceuticals. The extract of the Hepasave syrup with ethyl acetate was prepared and U.V of it was taken.

Preparation:-2 The biomarkers Gallic acid, Tannic acid, and vasicine were procured from Cadila Pharmaceuticals. 10µg/ml & 25 µg/ml of solution were prepared in methanol.

CHAPTER 6

RP-HPLC METHOD DEVELOPMENT OF HEPASAVE SYRUP

CHAPTER -6 RP- HPLC METHOD DEVELOPMENT FOR HEPASAVE SYRUP

6.1 Materials

6.1.1 Procurement of the formulation

Hepasave syrup :- It was procured from Cadila Pharmaceuticals Limited, Trasad Road, Dholka-387810, Dist. Ahmedabad (Gujarat), India.

6.1.2 Standards used in the Experimental Work

Tannic acid :- It was procured from Cadila Pharmaceuticals Limited, Trasad Road, Dholka-387810, Dist. Ahmedabad (Gujarat), India.

Gallic acid :- It was procured from Cadila Pharmaceuticals Limited, Trasad Road, Dholka-387810, Dist. Ahmedabad (Gujarat), India.

Vasicine :- It was procured from Cadila Pharmaceuticals Limited, Trasad Road, Dholka-387810, Dist. Ahmedabad (Gujarat), India.

6.1.3 Solvents, chemicals and reagents used in the Experimental work

Acetonitrile:- AR Grade Acetonitrile was used of Nirma University.

Methanol:- AR Grade Methanol was used of Nirma University.

Ethyl Acetate:- AR Grade Ethyl Acetate was used of Nirma University.

Ortho-Phosphoric Acid:- OPA of Sigma- Aldrich was used.

6.2 INSTRUMENTS AND EQUIPMENTS

6.2.1 High Performance Liquid Chromatography [HPLC]

- Pump : Jasco PU-2080 PLUS
- Photo Diode Array Detector(PDA) : MD – 2015 PLUS - Multi wavelength detector
- Ultraviolet – Visible Detector (UV) : UV- 2075 PLUS – UV/Visible Detector
- Column used - Purosphere® STAR , Reversed Phase (C-18, 250mm × 4.6mm, 5 μm)

6.2.2 Analytical Balance- Citizen CX 220, Capacity 10 to 220 mg (Citizen Pvt. Ltd)

6.2.3 Sonicator- D-Compact, Capacity 2 L (Trans-o-sonic, Mumbai)

6.3 EXPERIMENTAL WORK

RP-HPLC method development and optimization Chromatographic conditions for RP-HPLC method

Following chromatographic conditions were optimized and were kept constant throughout the analysis.

Column: C18 PUROSPHERE STAR Hyber 250 × 4.5 mm i.d., with 5 μm particle size

Mobile phase: Acetonitrile : Water (10:90 v/v) maintaining pH3 using Ortho-Phosphoic Acid

Flow Rate: 1.0 mL/min

Detection wavelength: 254 nm

Injection volume: 20 μL,

Run time was 20 minutes

6.3.1 Preparation of extract

The formulation Hepasave syrup was procured from Cadila Pharmaceuticals. Take 20ml of the formulation in clean and dry separating funnel. Add 20ml of ethyl acetate in to the separating funnel. Shake the funnel vigorously such that solvent layer changes to yellowish. Remove the formulation from the funnel and then take the extract in the clean and dry and previously tared porcelain dish. Repeat this for 3 times and than combine all the extracts and than again weight the porcelain dish. Now evaporate the extract to dryness. Now again add the solvent in the porcelain dish such that the residue present in that gets dissolved. And in the suitable volumetric flask make the volume with solvent.

6.3.2 Preparation of standard solutions

The biomarkers Gallic acid, Tannic acid, and vasicine were procured from Cadila Pharmaceuticals. 10μg/ml of solution were prepared in methanol.

6.3.3Preparation of mobile phase

90ml of HPLC grade water was added to10ml of HPLC grade acetonitrile. The pH was checked. Ortho-Phosohoric acid was added to the solution for method development and pH was maintained 3 using it.

Trials in HPLC

1. Standard Gallic acid
2. Standard Tannic acid
3. Standard Vasicine
4. Extract Ethyl acetate

6.3.3.1 Standard Gallic acid

Preparation of Solution :- 10 PPM solution of Gallic acid was prepared in methanol.

Mobile Phase :- HPLC grade water: Acetonitrile is 90:10 maintaining pH 3 using orthophosphoric acid.

Stationary Phase :- Column: C18 PUROSPHERE STAR Hyber 250 × 4.5 mm i.d., with 5 µm particle size

Flow Rate: 1.0 mL/min

Detection wavelength: 276nm

Injection volume: 20 µL

Run time :-10 minutes

6.3.3.2 Standard Tannic Acid

Preparation of Solution :- 10 PPM solution of Tannic acid was prepared in methanol.

Mobile Phase :- HPLC grade water: Acetonitrile is 90:10 maintaining pH 3 using orthophosphoric acid.

Stationary Phase :- Column: C18 PUROSPHERE STAR Hyber 250 × 4.5 mm i.d., with 5 µm particle size

Flow Rate: 1.0 mL/min

Detection wavelength: 276nm

Injection volume: 20 µL

Run time :- 10 minutes

6.3.3.3 Standard Vasicine

Preparation of Solution :- 10 PPM solution of Vasicine was prepared in methanol.

Mobile Phase :- HPLC grade water: Acetonitrile is 90:10 maintaining pH 3 using orthophosphoric acid.

Stationary Phase :- Column: C18 PUROSPHERE STAR Hyber 250 × 4.5 mm i.d., with 5 μm particle size

Flow Rate: 1.0 mL/min

Detection wavelength: 276nm

Injection volume: 20 μL

Run time :-10 minutes

6.3.3.4 Ethyl Acetate Extract

Preparation of Solution :- Ethyl acetate extract was prepared by extracting the formulation with ethyl acetate and then evaporating the extract and the left residue is dissolved in ethyl acetate and making it upto the volume

Mobile Phase :- HPLC grade water: Acetonitrile is 90:10 maintaining pH 3 using orthophosphoric acid.

Stationary Phase :- Column: C18 PUROSPHERE STAR Hyber 250 × 4.5 mm i.d., with 5 μm particle size

Flow Rate: 1.0 mL/min

Detection wavelength: 276nm

Injection volume: 20 μL

Run time :- 10 minutes

CHAPTER 7

FORCED DEGRADATION STUDIES OF THE BIOMARKERS PRESENT IN THE HEPASAVE SYRUP

CHAPTER 7 FORCED DEGRADATION STUDIES OF THE BIOMARKERS OF THE FORMULATION

7.1 Materials

7.1.1 Procurement of Formulation

The biomarker Gallic acid and Tannic acid were received from Cadila Pharmaceuticals Limited, Trasad Road, Dholka-387810, Dist. Ahmedabad (Gujarat), India.

7.1.2 Solvents used in the experiment

- Methanol, and Distilled water of AR grade (S.D.fine chemicals, Mumbai, India & Merck solutions, Mumbai ,India).

7.1.3 Chemicals used in the experiment

- 3N Hcl, 0.1N NaOH, 30%H2O2, 2N HCl, 2N NaOH

7.2 INSTRUMENTS AND EQUIPMENTS

Following is the list of equipments and instruments used throughout the project work.

All the instruments were if required was calibrated periodically as per in house SOP of Institute of Pharmacy, Nirma University.

- **Analytical balance** CITIZEN Scale CX-220, USA, having weighing capacity of 10 mg to 220 mg.
- **U.V Spectrophotometer-**Shimadzu UV-Visible double beam spectrophotometer, model - 2450 PC, (Shimadzu,Japan).
- **Hot air oven :-** EIE 108, EIE Instrument Pvt. Ltd. (Ahmedabad, Gujarat, India)
- **Photostability chamber :-** HSK090DICI, Thermolab Scientific Equipments.
- **pH meter :-** pH Cal, Analab Scientific Instuments Pvt. Ltd. (Vadodara, Gujarat, India)

7.3 Experimental work

7.3(A) Photolytic degradation studies

Gallic acid and tannic acid were exposed to near ultraviolet lamp in photostablity chamber providing illumination of not less than 1.2 million lux hours. Ten milligrams sample was dissolved in methanol and volume made up to 100 ml. From this solution appropriate dilution (10µg/ml) was

made using methanol and taken in cuvette for the U.V analysis. This was done for 24 hours at interval of every 2hours.

7.3 (B) pH degradation studies

The pH effect on the drug was carried out by using 0.1N Hydrochloric acid, 2N Hydrochloric acid, 0.1N Sodium Hydroxide and 2N Sodium Hydroxide solution. The drug solutions (100µg/ml) from pH 0-14 were prepared and these were allowed to stands for 4 hours. Finally the absorbances were measured at 271nm for gallic acid and 280nm for tannic acid. The K value for 1st order kinetics was determined by using the formula:

$$K = (2.303/t) \log (Co/C)$$

Where,

K =1st order rate constant,

Co = initial drug concentration,

C = final drug concentration

7.3(C) Stress degradation by hydrolysis under acidic condition

To 3 ml of stock solution(1000µg/ml) of gallic acid and tannic acid 1 ml of 3 N HCl was added in 10 ml of volumetric flask and the volume was made up to the mark with methanol. Then, the volumetric flask was kept at normal condition for 90 minutes. After 60 min. time interval, 1 ml of solution was pipette out from this flask, neutralised and diluted with methanol in order to make the volume up to 10 ml and the dilution was carried out to achieve the appropriate concentration (30µg/ml). This solution was taken in cuvette. For the blank, 0.5 ml solution of 3N HCl and 0.5 ml solution of 3N NaOH were diluted with methanol in 10 ml of volumetric flask. After 90 minutes, again 1ml of the solution was pipetted out from the flask and the above procedure was repeated.

7.3(D) Stress degradation by hydrolysis under alkaline condition

To 3 ml of stock solution of gallic acid, tannic acid 1 ml of 0.1 N NaOH was added in 10 ml of volumetric flask and made up the volume to the mark with methanol. Volumetric flask was kept

at normal condition for 90 min. After 60 min time interval, 1 ml of solution was pipette out from this flask, neutralized and diluted with methanol in order to make the volume up to 10 ml and the dilutions were carried out to achieve the appropriate concentration (20µg/ml). The solution was then taken in cuvette. For the blank, 0.5 ml solution of 0.1N HCl and 0.5 ml solution of 0.1N NaOH diluted with methanol in 10 ml of volumetric flask. After, 90 minutes 1ml of solution was again pipette out from the flask and the above procedure was repeated.

7.3(E) Dry heat induced degradation

Gallic acid and tannic acid were taken in a petriplate and exposed to a temperature of 70°c for 48 hours in an oven. After 48 hours, 10 mg of the sample was diluted with methanol in order to make the volume up to 10 ml. From this solution, dilutions were carried out to achieve the appropriate concentration (20µg/ml) and the solution was taken in cuvette for the UV-VIS Analysis.

7.3(F) Oxidative degradation

To 1.5 ml of the stock solution of gallic acid and tannic acid (1000µg/ml), 1 ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with methanol. The volumetric flask was then kept at room temperature for 15 min. For the blank, 1ml of the 30 % w/v of hydrogen peroxide was kept at normal condition for overnight in 10 ml of volumetric flask. Both solutions were heated on boiling water bath to remove the excess of hydrogen peroxide. Finally, after 15 minutes dilutions were made from the stock solution to achieve the required concentration (30µg/ml). The solution was then taken in a cuvette and analysed in UV.

CHAPTER 8

SUMMARY & CONCLUSION

CHAPTER 8 SUMMARY & CONCLUSION

The formulation HEPASAVE syrup is a polyherbal formulation. It contains Andrographolide, Kutkin, Gallic acid, Tannic acid and Vasicine. The various Quality control parameters were studied for the formulation like organoleptic, physicochemical, microbiological, phytochemical screening , estimation of phytoconstituents, heavy metal screening , in which the result came within the limit.

The study also was performed for the biomarkers present in it which includes fractionation of the formulation, HPLC studies, Identification through U.V and forced degradation studies. Appropriate result of it was found.

The present work deals with Development of Quality Control Parameter of HEPASAVE Syrup. Hepasave syrup is a polyherbal formulation. It contains 5 active constituents that are Gallic acid, Tannic acid, Vasicine, Andrographolide, and Kutkin.

The Evaluation of the formulation was in 2 ways

1. Evaluation of the formulation
2. Evaluation of the biomarkers present in the formulation.

1. Evaluation of the formulation

   It includes Organoleptic characters, Physicochemical parameters, Phytochemical screening, Detection of heavy metals, Estimation of phytoconstituents, Extractive values, Bitterness value, & microbiological studies.

2. Evaluation of the biomarkers present in the formulation

   It includes Identification of biomarkers, Preliminary Thin Layer Chromatography, HPLC studies & Forced Degradation Study.

The results of all these parameters were

- Evalution of physicochemical parameters like pH, density, viscosity, which were found about 4.16,1.33gm/ml and 1560poise respectively.
- Detection of secondary metabolites like alkaloids, glycosides, etc by performing phytochemical screening.
- Estimation of total phenolic content and total flavonoid content
- Microbiological studies shows total viable count was found to be in limit in fungi and near to the limit in bacteria as per WHO guidelines.
- The zone of inhibition was performed on E.Coli, B.Subtilus, and St.aureus and found to be more effective against st.aureus.
- Extractive value has performed taking 4 solvents and maximum extractive value was found in toluene.
- Bitterness value was performed in reference to quinine sulphate and the value was found to be in limit.
- The maximum wavelength of the gallic acid, tannic acid and vasicine were found to be 271, 280,260.
- Preliminary investigation of the biomarkers were performed using TLC and the Rf value were found 0.38 in gallic acid, 0.2875 in vasicine and 0.27 in andrographolide.
- RP-HPLC method was performed to detect presence of biomarkers like gallic acid, tannic acid using mobile phase acetonitrile :water (10:90) maintaining pH 3 and Rt value was found to be near 4.5 and 6 minute.
- The Photolytic studies were performed on gallic acid and tannic acid which were found to be 31.31µg/ml & 60.48µg/ml respectively.
- In the pH degraradation study it was noted that as pH increases (alkaline condition) k value & % degradation decreases & concentration increases.
- The % degradation in acidic condition (3N HCl for 60& 90 minute) was 30 & 50% at 60& 90 minutes respectively for gallic acid & tannic acid was 22.22, 44.44, 26.46, 29.47 % respectively.
- In alkaline condition (0.1N NaOH for 60& 90 minute) was 33& 53.3 % at 60& 90 minutes. For gallic acid & tannic acid it was 10, 20 & 68.17%. The dry heat degradation was 65.66% for the extract & for the gallic acid & tannic acid was 4% & no change in tannic acid.

- The oxidative degeneration was 33% for the extract & for gallic acid & tannic acid was 26 &22 % respectively.

CHAPTER 9

FUTURE SCOPE

CHAPTER 9 FUTURE SCOPE

The Polyherbal formulation, HEPASAVE SYRUP, is an Ayurvedic Proprietary Medicine, used particularly as a Powerful Hepato-protective, an Antioxidant, and a Bitter tonic Detailed quality control parameters has been developed which includes morphological evaluation, phytochemical screening, estimation of phytoconstituent, determination of heavy metal and total viable count and bacterial count etc. The Future scope to the study includes the HPLC method employed in the current study can be validated as per Official guidelines, i.e ICH guidelines or U.S. Pharmacopoeia, for the estimation of Andrographolide, Gallic acid and Kutkin in Hepasave Tonic, or a new method can also be validated for the same. Various HPTLC and HPLC simultaneous estimation methods can be developed and Validated for the estimation of Andrographolide, Gallic acid and Kutkin in Hepasave Tonic. The formulation can be administered with a dosage regimen of 10ml three times a day for the Adults and 5 ml three times a day for the Children as prescribed by the concerned physician. The polyherbal formulation remains in the best form when stored below 30˚C and when protect from exposure to Direct Sunlight. In addition Due to presence of various active phytoconstituents this syrup can also be checked for different activities especially hepatoprotective effect and clinical trials can also be performed.

CHAPTER 10

BIBLIOGRAPHY

Chapter 10 Bibliography

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