STANDARDIZATION OF KSHAMATVA CHURNA:

A NUTRACEUTICAL FORMULATION

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BY

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DECLARATION BY THE CANDIDATE

I declare that this thesis "**Standardization of Kshamatva Churna : A Nutraceutical formulation** " submitted for the award of Master of Science to THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY, Bengaluru, is my original work, conducted under the supervision of Dr. Subrahmanya Kumar K. I confirm that no part of the work reported herein has been submitted for a degree or examination at any other university. References, funding and material obtained from other sources have been duly acknowledged, and no part of this dissertation has been plagiarised.

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CERTIFICATE FROM THESIS SUPERVISOR/S

This is to certify that the work incorporated in this thesis **"Standardization of Kshamatva Churna : A Nutraceutical formulation**." submitted by DIPIN V M was carried out under my/our supervision. No part of this thesis has been submitted for a degree or examination at any other university. References, help and material obtained from other sources have been duly acknowledged. I confirm the originality of the work and that there is no plagiarism in any part of the thesis.

Dr. Subrahmanya Kumar K Guide Signature

Associate Professor

TDU

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SUMMARY

The thesis titled "**Standardization of Kshamatva churna :** A **Nutraceutical formulation**" is an attempt to standardize the Ayurvedic formulation Kshamatva Churna, and converting it into easy to use nutraceutical forms. This research was motivated by the need to enhance the palatability, transportation, and administration of Kshamatva Churna, particularly due to its ability to enhance resilience immunomodulatory effects which was already evaluated.

Kshamatva Churna, composed of nine herbal ingredients including Guduchi, Aswagandha and Yastimadhu, has shown potential to support resilience and mitigating respiratory symptoms. The study highlights the challenges encountered with consumption the traditional dosage form of Kshamatva Churna, which inspired to convert into user-friendly nutraceutical forms such as sugar candy, palm jaggery candy, crystals, dip tea bags, and easily soluble powders.

The thesis emphasizes the importance of standardization in Ayurveda to ensure the quality and efficacy of herbal formulations. Standardization involves establishing consistent parameters for raw material collection, processing, and storage. This process is crucial to maintain the therapeutic efficacy and safety of the formulations, thereby building consumer trust and enhancing the credibility of Ayurvedic medicine.

Network pharmacology was employed to explore the molecular interactions and potential targets of Kshamatva Churna, revealing its broad-spectrum immunomodulatory effects. This approach provided insights into how the formulation interacts with key genes involved in immune regulation, cell survival, pathogen response and its effect in Upper respiratory diseases.

PERSONAL REFLECTION

I learned a great deal in my project, both in terms of technical and social skills. From a technical standpoint, I gained valuable experience in several areas. Engaging in extensive literature reviews and scientific writing enhanced my understanding of Ayurveda and sanskrit literature, improving my ability to present scientific data clearly and concisely. Maintaining data and using different software, including “R”, significantly enhanced my data handling and analytical skills. Developing and standardizing protocols, coupled with working on In-Silico studies such as Network Pharmacology, provided me with a solid foundation in computational drug design.

Phytochemistry labs gave me practical experience with high-tech equipment like extraction techniques, chromatography including HPTLC, and HPLC. This exposure was invaluable for gaining proficiency in advanced laboratory techniques and studies. Documenting the preparation of nutraceutical forms through photography and critical observation helped me develop skills in photo editing and detailed documentation. Also working in scientific kitchen, pilot plant, and dealing with spray drying machines gives me more exposures. The greatest privilege which I had earned as a part of my study was getting to know more about the nutraceutical forms and the beauty of developing nutraceutical Ayurveda formulations, its importance in current scenario.

In terms of social skills, I gained valuable insights into effective teamwork. Collaborating under the supervision of multiple seniors allowed me to improve my communication and negotiation skills, fostering a productive and cooperative working environment. Overall, my project was an excellent opportunity to enhance my technical and social competencies. The skills and lessons I have acquired, from working in both lab and field settings, have prepared me well for future endeavours. I am confident that these experiences will be incredibly useful in my career.

ABBREVIATIONS

KC	Kshamatva churna
NP	Network Pharmacology
BA	Bio Actives
GT	Gene Targets
BP	Biological Pathway
DA	Disease Association
TLC	Thin Layer Chromatography
HPLC	High Pressure Liquid Chromatography
FRLHT	Foundation for Revitalization of Local Health Traditions
I-AIM	Institute of Ayurveda and Integrative Medicine
TDU	The University of Trans-Disciplinary Health Sciences and Technology
HX	Hexane
CH	Chloroform
EA	Ethyl Acetate
MH	Methanol
WA	Water
BK	Baishajya Kalpana

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1.INTRODUCTION

Ayurveda is one of the oldest natural healing systems which originated in India about 5000 years ago and is often called the “Mother of All Healings”. The literal meaning of Ayurveda is science of life and the term "Ayurveda" comes from the combination of two Sanskrit words:” *Ayush* ” means life or longevity and” *Veda*" means knowledge or science (Charaka & Sharma, 1981).

“हिताहित सुखं दुःखमायुस्तस्य हिताहितम् ।
मानं च तच्च यत्रोक्तमायुर्वेदः स उच्यते ।” (Ca.Su.1/41)

The Science deals with everything which is favourable or unfavourable, happy or unhappy for a lifespan as well as analysing what is conductive and non-conductive for that life. It addresses both the good and bad aspects of life, and its measurement also addresses the happy and unpleasant states of existence (Sushruta & Murthy, 2005).

समदोषः समाग्निश्च समधातु मलक्रियाः।
प्रसन्नात्मेन्द्रियमनाः स्वस्थः इत्यभिधीयते ॥ (Su. Su.15/41)

When all of the body's tissues (*Sapthadhathus*), all excretory functions (*mala kriya*), and the mind (*Mana*), senses (*Indriyas*), and spirit (*Atma*) are in perfect balance and the three *doshas* (*Vata, Pitta*, and *Kapha*) are balanced (*sama*), an organism is said to be perfectly healthy.

"स्वास्थस्य स्वास्थ्य रक्षणं आतुरस्य विकार प्रशमनं चा" || (Ca.Su.30/26)

The purpose of Ayurveda is to maintain health of healthy individuals and to relieve disease of sick people. Ayurveda is a form of intellectual coherence that addresses the harmony or balance of the mind and body as a necessary condition for living a purposeful and healthy life as well as for achieving the three main human objectives of *dharma, artha*, and *kama* (Charaka & Sharma, 1981).

1.1. Immunity, Immune System, Immunomodulation In Ayurveda

Plant-based remedies and medications are emphasized in Ayurveda. An abundance of pharmacological characteristics can be found in the many plants recognized in the Indian Ayurvedic medical system. One of the first medical systems in the world, Ayurveda encompasses a variety of ethnopharmacological practices, including immunostimulant, neurostimulation, tonic, anti-aging, antibacterial, antiviral, antirheumatic, anticancer, and adaptogenic.(Panossian & Wikman, 2010)

1.1.1. Immunity and immune System

Immunity can be defined as the body's defence mechanism against infectious potentially harmful microorganisms, enabling the body to prevent or resist diseases and inhibit organ and tissue damage. Innate, adaptive, and passive immunity are the three categories. Skin and mucous membranes are examples of barriers found in innate immunity that prevent pathogens from entering the body. It is the immune system's initial reaction to an alien material. In reaction to contracting a microbe or receiving a vaccination against one, adaptive immunity develops. The immunological response that the body produces can stop the microbe from infecting others in the future. A person can have adaptive immunity throughout their whole life. When a person acquires antibodies against a disease instead of producing them on their own through the immune system, this is known as passive immunity. While passive immunity offers protection right away, its duration is limited to a few weeks or month. (National Institute of Health (NIH))

Multi-layered defences on multiple levels comprise the immune system's basic architecture. The skin, the first line of defence against infection, is most visible and important. There are also physiological circumstances, wherein the body's pH and temperature give unwanted living conditions for external organisms. The immune system, either innate, acquired, or adaptive, deals with pathogens once they have effectively penetrated the body. Numerous cells and chemicals make up both systems, which work together intricately to identify and get rid of infections. The process of chemical bonding is necessary for both detection and eradication. The immune system cells' surfaces are coated in several types of receptors, some of which attach chemically to pathogens and others of which attach to other immune system cells or molecules to facilitate complex signalling.

1.1.2. Immunomodulators

These are chemical or biological agents that have the ability to alter, suppress, or activate any component of the immune system, including its innate and adaptive components.(Goyal et al., 2011)

1.2 Plant as Immunomodulators

Around the world, scientists have become interested in a number of medicinal herbs that are employed in the *Rasayana* traditional Indian method, which aims to strengthen the body's resilience. According to the discussion that follows, a number of medicinal plants have a variety of therapeutic properties, including antifungal, hypo-cholesterolemic, hepatoprotective, anti-asthmatic, anti-inflammatory, antioxidant, and diuretic.(H. Sharma et al., 2007)

A full chapter in the Ayurvedic Materia Medica (Dash, 2005) is devoted to "*Rasayana*" medications, which are said to increase body resistance. Included in the literature on traditional Indian medicine, *Rasayana* is a class of plants said to strengthen the body's defences, improve physical and mental health, and lengthen life.(H. Sharma et al., 2007) These characteristics bear resemblance to the contemporary notion of adaptogenic agents, which are recognized for their ability to provide defence of the human physiological system against various stresses.(Goyal et al., 2011) Many medicinal plants known as *rasayanas*, such as *Withania somnifera, tinospora cordifolia*, and *mangifera indica*, have been reported to exhibit immunomodulatory properties. They can particularly react to an alien material, or they can weaken or increase the host's general resistance to infection and malignancies.(Mishra et al., 2001)

Nowadays, immunomodulators are regarded as one of contemporary medicine's most effective instruments for managing both health and disease. The contemporary concept is becoming closer to the Ayurvedic principles of *Vyadhi-ksamatva, Ojas, Bala* and *Rasayana*, to the more recent understanding of the neuro-endocrine-immune axis, the impact of exercise, circadian rhythms, seasonal variations, and different psychological states on immune system.

a) *Vyadhikshamatva* refers to the body's ability to resist a disease in one of two ways: (Charaka & Sharma, 1981)

I. *Vyadhi-Bala-Virodhitvam*, which is the body's ability to resist and withstand the strength, severity, or progression of a disease

II. *Vyadhi-Utpada-Pratibandhakatvam*, which is the body's ability to resist and prevent the manifestation of a disease.

व्याधिक्षमत्वं व्याधिबलविरोधित्वं व्याध्युत्पाद प्रतिबन्धकत्वमिति यावत्। (Ch .Su.28/7)

b) *Ojas* is the finest product of all the seven *dhathus*. It is obtained through dhathwagnipaka and is transported through all the *dhathuvaha srothas* (*siras*, *dhamanis* including *hridaya* (Vagbhata & Murthy, 2007).

ओजस्तु तेजो धातूनां शुक्रान्तानां परं स्मृतम्।
हृदयस्थमपि व्यापि देहस्थितिनिबोधताम्।
स्निग्धं सोमात्मकं शुद्धं ईषत् लोहित पीतकम्। (AH. SU 11/27)

c) The broad word "*Rasayana*" refers to Ayurvedic immunomodulatory therapies. In Ayurveda, the word "*Rashyana*" refers to a broad category of herbs, formulations, and treatments that are used to maximise body resistance through the attainment of an ideal level of *Rasadi Dhatu* (Charaka & Sharma, 1981). This is accomplished by using certain medicinal plants, concoctions, and behavioural rules. *Rasayana* supports the preservation of maximum physical and sensory strength as well as strength, vitality, lifespan, memory, intelligence, and youthfulness (Sushruta & Sharma, 1999).

लाभोपायो हि शस्तानाम रसादीनां रसायनम् । (Ch. Chi)

Using the *Rasayana* and *Vajikarana* therapy, along with the *Achara Rasayana* measures and *Ojovardhaka* medicines, is how Ayurvedic practice aims to increase immunity (Charaka & Sharma, 1981).

“बलं ह्यलं दोषहरं निग्राहाय दोषानाम्।**”** (Ch.Chi. 3/16)

1.3 Novel Discovery of Immunomodulatory Properties of Kshamatva Churna.

The COVID-19 pandemic has presented us a chance to demonstrate the efficacy of Ayurveda in two areas: prophylaxis and prevention using immunomodulators *(Rasayana*). The outbreak was quick and within a short span of time the disease spread rapidly among population. The pandemic condition which was similar to that created by COVID-19 was explained by Acharya Charaka in his treatises "*Janpadhodhwasa***".** The word '*Janapadodhwamsa*'is made up of two sanskrit words *Janapada* (large population) & *Udhvamsa* (destruction) which means the diseases affecting & causing damage of a large number of people; similar to Covid19 pandemic. It was believed that COVID-19 is a *Sannipatika vyadhi,* arising from the vitiation of *Vata* with *Kapha* in the early stages and with pitta in the latter stages. Main *Dushya* include *Rasa*, *Rakta*, and *Mamsa*; however, *Ojakshaya* can also be seen in some conditions.

During the rapid spread of pandemic, IAIM Hospital and TDU proposed the concept of giving Kshamatva Churna as *kashaya* to help people get immune. They did this by choosing two villages' populations for the study. As agreed upon, the people were given 3 grams of Kshamatva Churna in 120 ml of water as *kashaya* for 30 days. After a month, the study's analysis revealed a dramatic drop in the number of cases from that specific hamlet. It also revealed that individuals who had the disease had minor issues with the least upper and lower respiratory symptoms.

1.4 Ingredients of Kshamatva Churna

Table 1: Ingredients of Kshamatva Churna

Ingredients	***Scientific name***	**Part used**	**Qunatity(5gm)**
Guduchi	*Tinospora cordifolia (*Thunb.) Miers	Dry stem	1000mg
Aswagandha	*Withania somnifera* (L.) Dunal	Dry root	1000 mg
Yastimadhu	*Glycyrrhiza glabra* L.	Dry stem& Dry root	1000 mg
Haridra	*Curcuma longa* L.	Dry rhizome	500 mg
Shunti	*Zingiber officinale* Roscoe	Dry rhizome	500 mg
Pippali	*Piper longum* L.	Dry spike (fruit)	250 mg
Maricha	*Piper nigrum* L.	Dry fruit	250 mg
Dhanyaka	*Coriandrum sativum* L.	Dry seed	250 mg
Jeeraka	*Cuminum cyminum* L.	Dry seed	250 mg

Fig 1:Kshamatva Churna and its ingredients

1.4.1 Guduchi *(Tinospora cordifolia* (Thunb.) Miers)

Guduchi, also known as Giloy, is a climber plant that holds a significant place in traditional Ayurvedic medicine. Its historical use in treating various ailments, including infectious diseases, has sparked interest in its immunomodulatory potential.

Guduchi contains several bioactive compounds, such as alkaloids, glycosides, steroids, and polysaccharides, which contribute to its therapeutic properties. These compounds play a crucial role in modulating immune responses, influencing both the innate and adaptive immune systems (Saqib & Janbaz, 2016).

Studies have shown that Guduchi exerts its immunomodulatory effects through various mechanisms. It enhances the activity of macrophages, natural killer cells, and T lymphocytes, leading to increased production of cytokines, such as interleukins and

interferons. These effects contribute to a strengthened immune response against pathogens (S. S. Singh et al., 2003).

Guduchi's antioxidant properties also play a vital role in its immunomodulatory effects. By scavenging free radicals and reducing oxidative stress, Guduchi helps maintain the balance of the immune system and prevents excessive inflammation.(Goyal et al., 2011)

PROPERTIES AND ACTION(API, 2001(1))

- Rasa (Taste) : Bitter (*Tikta*), Astringent (*Kashaya*)
- Guna (Property) : Light (*Laghu*)
- Virya (Potency) : Hot (*Ushna*)
- Vipaka (Post-digestive Taste) : Sweet (Madhura)
- Karma (Action) : Balances all three d*oshas* (*Tridosasamaka*), Strengthening (*Balya*) Rejuvenating (*Rasayana*), Absorbent (*Samgrahi*), Digestive (*Dipana*), Blood purifier (*Raktasodhana*), Fever reducer (*Jvaraghna*)
- Therapeutic uses : Skin diseases (*Kusta*), Gout (*Vatarakta*), Fever (*Jvara*), Jaundice (*Kamala*), Anemia (*Pandu*), Diabetes (*Prameha*)

1.4.2 Aswagandha (*Withania somnifera* (L.) Dunal)

Ashwagandha, also known as Indian ginseng, is a widely used herb in traditional Ayurvedic medicine. Its adaptogenic properties and historical use in enhancing vitality and longevity have prompted investigations into its immunomodulatory potential.(Panossian & Wikman, 2010)

Ashwagandha contains bioactive compounds such as withanolides, alkaloids, and steroidal lactones, which contribute to its pharmacological effects. These compounds are believed to influence the immune system and contribute to Ashwagandha's adaptogenic properties.

Studies have indicated that Ashwagandha exerts immunomodulatory effects by regulating the activity of immune cells, including macrophages, T cells, and natural killer cells. The herb appears to enhance the production of cytokines, such as interleukins and interferons, promoting a balanced and responsive immune system.

Ashwagandha's anti-inflammatory properties also play a role in its immunomodulatory effects. By modulating inflammatory pathways and reducing oxidative stress, the herb helps maintain immune homeostasis and may be beneficial in conditions associated with inflammation(N. Singh et al., 2011).

The paper reviews relevant clinical studies investigating Ashwagandha's impact on immune function in various health conditions. While research is ongoing, preliminary findings suggest potential benefits in enhancing immune responses and supporting overall immune health (Lima et al., 2020).

PROPERTIES AND ACTION(API, 2001a)

- Rasa (Taste) **:** Bitter (*Tikta*), Astringent (*Kashaya*)
- Guna (Property) **:** Light (*Laghu*)
- Virya (Potency) **:** Hot (*Ushna*)
- Vipaka (Post-digestive Taste)**:** Sweet (*Madhura*)
- Karma (Action) **:** Alleviates *Vata* and *Kapha (Vatakaphapaha*), Strengthening (*Balya*), Rejuvenating (*Rasayana*), Aphrodisiac (*Vajikaranakara*)
- Therapeutic uses **:** Emaciation (*Karshya*), Weakness (*Daurbalya*), *Vata* disorders (*Vataroga*), Swelling (*Sotha*), Impotence (*Klaibya*)

1.4.3 Yastimadhu (*Glycyrrhiza glabra* L.)

Yastimadhu, also referred to as licorice, has a rich history in traditional medicine, including Ayurveda and traditional Chinese medicine. Known for its sweet taste, Yastimadhu has been recognized for its diverse therapeutic properties, including potential immunomodulation.

Yastimadhu contains bioactive compounds such as glycyrrhizin, glycyrrhetinic acid, flavonoids, and polysaccharides, which contribute to its pharmacological effects. These compounds are believed to play a role in Yastimadhu's immunomodulatory action(Khan et al., 2015) .

Studies indicate that Yastimadhu exerts immunomodulatory effects by influencing the activity of immune cells, including macrophages, T cells, and dendritic cells. The herb is thought to modulate cytokine production, enhancing the balance of immune responses and contributing to immune system regulation. Yastimadhu's anti-

inflammatory properties are also integral to its immunomodulatory effects. By inhibiting inflammatory pathways and reducing oxidative stress, the herb helps maintain immune homeostasis and may be beneficial in conditions associated with inflammation (Shan et al., 2005).

The paper reviews relevant clinical studies investigating Yastimadhu's impact on immune function in various health conditions. Preliminary findings suggest potential benefits in enhancing immune responses and supporting overall immune health.

PROPERTIES AND ACTION(API, 2001a)

- Rasa (Taste) **:** Sweet (*Madhura*)
- Guna (Property) **:** Heavy (*Guru*), Unctuous (*Snigdha*)
- Virya (Potency) **:** Cold (*Sita*)
- Vipaka (Post-digestive Taste)**:** Sweet (Madhura)
- Karma (Action) **:** Alleviates *Vata* and *Pitta* (*Vatapithajith*), Strengthening (*Balya*), Blood purifier (*Rakthaprasadanam*), Promotes fertility and vitality (*Varshya*)
- Therapeutic uses **:** Cough (*Kasa*), Hoarseness of voice (*Svarabheda*), Emaciation (*Karshya*), Wounds (*Vruna*), Gout (*Vatarakta*)

1.4.4 Pippali *(Piper longum* L.*)*

Pippali, also known as long pepper or Piper longum, holds a prominent place in Ayurvedic medicine for its diverse therapeutic properties, particularly as an immunomodulator. This essay explores its bioactive components and their impact on the immune system (V. , T. Sharma & Chauhan, 2013)

Central to Pippali's medicinal efficacy are bioactive compounds such as alkaloids, flavonoids, and essential oils, with piperine notably enhancing bioavailability (Srinivasan, 2014) .These compounds collectively contribute to its immunomodulatory effects. Pippali influences various immune components, including macrophages, T cells, and natural killer cells, while enhancing the production of key cytokines crucial for immune response coordination (V. , T. Sharma & Chauhan, 2013).

Its anti-inflammatory properties are integral to maintaining immune balance by inhibiting inflammatory pathways and reducing pro-inflammatory molecules, thereby

supporting immune homeostasis and potentially benefiting inflammatory conditions (Rehman, 2011).Traditionally used for respiratory ailments, Pippali supports the respiratory tract and may provide immune-boosting benefits for lung and airway health. While rooted in Ayurvedic tradition, recent scientific studies are validating Pippali's immunomodulatory properties. Initial findings suggest that Pippali supplementation enhances immune responses, making it a promising natural compound for immune system support (V. , T. Sharma & Chauhan, 2013). In conclusion, Pippali's rich history in Ayurveda coupled with modern research underscores its potential as an effective immunomodulator, offering new insights into natural approaches to enhance immune function and overall health.

PROPERTIES AND ACTION(API, 2004)

- Rasa (Taste) : Sweet (*Madhura*), Bitter (*Tiktha*), Pungent (*Katu*)
- Guna (Property) : Light *(Laghu*), Unctuous (*Snigdha*)
- Virya (Potency) : Neither hot nor cold (*Anushna*)
- Vipaka (Post-digestive Taste): Sweet (*Madhura*)
- Karma (Action) : Alleviates *Vata* and *Kapha (Vatakaphahara)*, Digestive (*Dipana)*, Appetizer (*Ruchya*), Rejuvenating (*Rasayana*), Heart tonic (*Hridya*), Aphrodisiac (*Vrishya*)
- Therapeutic uses: Cough (*Kasa*), Asthma (*Swasa*), Abdominal tumors (*Gulma*), Fever (*Jvara*), Hemorrhoids (*Arsha*), Emaciation (*Karshya*), Abdominal diseases (*Udararoga*), Hiccups (*Hikka*), Worm infestations (*Krimi*), Skin diseases (*Kushta*), Colic pain (*Shula*), Rheumatoid arthritis (*Amavata*)

1.4.5 Haridra *(Curcuma longa* L. *)*

Haridra, more commonly known as turmeric, is celebrated not only for its vibrant colour and culinary uses but also for its potent medicinal properties, particularly as an immunomodulator. This essay explores its bioactive components and their impact on the immune system (S. C. , et al. Gupta, 2011).

At the heart of turmeric's health benefits is curcumin, a polyphenol renowned for its antioxidant and anti-inflammatory properties (Jagetia & Aggarwal, 2007). Alongside

curcumin, turmeric contains various bioactive compounds that synergistically enhance its therapeutic potential.

Haridra's immunomodulatory capabilities are rooted in its ability to influence immune cells and signalling pathways. Curcumin modulates the activity of macrophages, lymphocytes, and dendritic cells, while also regulating cytokine production crucial for immune function (Jagetia & Aggarwal, 2007).

The anti-inflammatory effects of Haridra, mediated by curcumin, are pivotal in maintaining immune balance and supporting a robust immune response (Hewlings & Kalman, 2017). By mitigating inflammatory pathways, turmeric helps the body manage chronic inflammation associated with compromised immune function. Turmeric's antioxidant properties are vital for immune health, as they protect immune cells from oxidative stress and ensure their optimal function (Hewlings & Kalman, 2017).

Extensive research underscores Haridra's role in enhancing immune responses across various health conditions, highlighting its potential as a natural immunomodulator (S. C. , et al. Gupta, 2011). Its widespread culinary use, from curries to teas, offers a convenient and flavourful means to incorporate these immune-supporting compounds into daily diet.

Overall, Haridra's profound immunomodulatory effects, coupled with its safety and culinary versatility, position turmeric as a promising ally in promoting immune system resilience and overall health.

PROPERTIES AND ACTION(API, 2001a)

- Rasa (Taste) : Bitter (*Tiktha*), Pungent (*Katu*)
- Guna (Property) : Dry (*Ruksa*)
- Virya (Potency) : Hot (*Usna*)
- Vipaka (Post-digestive Taste): Pungent (*Katu*)
- Karma (Action) : Alleviates Kapha and Pitta (*Kaphapittanut*), Antitoxic (*Visaghna*), Enhances complexion (*Varnya*), Treats skin diseases (*Kushtaghna*), Anthelmintic (*Krimighna*)
- Therapeutic uses : Toxic conditions (*Vishavikara*), Skin diseases (*Kushta*), Anemia (*Pandu*), Skin disorders (*Tvagroga*), Diabetes (*Prameha*),

Urticaria (*Sitapitta*)

1.4.6 Maricha *(Piper nigrum* L. *)*

Maricha, commonly known as black pepper, transcends its role as a culinary spice with emerging recognition for its medicinal properties, particularly its potential as an immunomodulator. This essay explores its bioactive components and their impact on the immune system (Srinivasan, 2014) .

Central to Maricha's health benefits is piperine, its primary bioactive compound renowned for antioxidant, anti-inflammatory, and immunomodulatory properties (J. Singh & Dubey, 2013). Piperine influences immune cell activity, including macrophages and T lymphocytes, and regulates cytokine production, pivotal in orchestrating effective immune responses.

Maricha's anti-inflammatory prowess is crucial for immune modulation, helping maintain immune balance amidst chronic inflammation (Prasad & Tyagi, 2016). Its antioxidant properties further shield immune cells from oxidative stress, ensuring their optimal function and integrity (J. Singh & Dubey, 2013).

Widely available in kitchens worldwide, Maricha's integration into various culinary preparations offers a practical and enjoyable avenue for incorporating potential health benefits into daily life. While research on Maricha's immunomodulatory effects continues, initial studies underscore its promise as a natural agent for supporting immune function and managing inflammatory responses (Srinivasan, 2014). Continued exploration will deepen our understanding of black pepper's role in enhancing immune health.

PROPERTIES AND ACTION(API, 2001b)

- Rasa (Taste) : Bitter (*Tiktha*), Pungent (*Katu*)
- Guna (Property) : Dry (*Ruksa*), Light (*Laghu*), Sharp (*Tikshna*)
- Virya (Potency) : Hot (*Usna*)
- Vipaka (Post-digestive Taste): Pungent (*Katu*)
- Karma (Action) : Alleviates *Kapha* and *Vata* (*Kaphavatajith*), Increases Pitta

(*Pittakara*), Cutting or expectorant (*Chedana*), Digestive (*Dipana*), Appetizer (*Ruchya*), Reduces fat (*Medhohara*), Treats heart diseases *(Hrdroga*), Antimicrobial (*Janthunashana*)

• Therapeutic uses: Asthma (*Svasa*), Worm infestations (*Krumiroga*), Skin disorders (*Tvagroga*)

1.4.7 Shunti *(Zingiber officinale* Roscoe *)*

Shunti, commonly known as ginger, holds a revered place in both culinary traditions and traditional medicine, with emerging recognition for its immunomodulatory potential. This essay explores its bioactive compounds and mechanisms supporting immune system health (Shahwar & Nawaz, 2021).

Ginger owes its distinct flavour and therapeutic properties to bioactive compounds like gingerol, shogaol, and paradol, which possess antioxidant and anti-inflammatory effects crucial for immune support (Shahwar & Nawaz, 2021). These compounds bolster immune function by enhancing the activity of immune cells such as macrophages and lymphocytes, thus fortifying the body's defences against infections (J. A. Smith, 2023).

Moreover, ginger's ability to modulate inflammatory pathways helps maintain a balanced immune response, crucial for optimal immune function (J. A. Smith, 2023). Its antioxidant properties further protect immune cells from oxidative stress, preserving immune integrity (Shahwar & Nawaz, 2021).

Traditionally valued for respiratory benefits, ginger's role in alleviating symptoms of respiratory infections underscores its potential for supporting immune health, especially in respiratory conditions (J. A. Smith, 2023). An appealing aspect of ginger is its versatility in culinary applications, making it easy to incorporate into daily meals such as teas and stir-fries, offering not only flavour but also potential health benefits.

While much of the evidence supporting ginger’s, immunomodulatory effects derive from preclinical studies, preliminary research suggests promising avenues for exploring ginger as a natural immune-supporting agent (Shahwar & Nawaz, 2021).

Continued investigation will further elucidate its role in enhancing immune responses and maintaining immune balance.

PROPERTIES AND ACTION(API, 2001a)

- Rasa (Taste) : Pungent (*Katu*)
- Guna (Property) : Light (*Laghu*), Unctuous (*Snigdha*)
- Virya (Potency) : Hot (*Usna*)
- Vipaka (Post-digestive Taste): Sweet (*Madhura*)
- Karma (Action) : Digestive (*Dipana*), Digestive stimulant (*Pachana*), Carminative (*Anuloma*), Removes toxins (*Amadosahara*), Heart tonic (*Hridya*), Alleviates *Vata* and *Kapha* (*Vatakaphapaha*)
- Therapeutic uses: Digestive weakness (*Agnimandya*), Bloating (*Adhmana*), Anemia (*Pandu*), Asthma (*Svasa*), Abdominal diseases (*Udararoga*), Rheumatoid arthritis (*Amavata*)

1.4.8 Dhanyaka *(Coriandrum sativum* L.*)*

Dhanyaka, commonly known as coriander, has been cherished for centuries in culinary and traditional medicinal practices. This article explores its potential as an immunomodulator, emphasizing its bioactive constituents and mechanisms (Williams & Jones, 2021).

Rich in compounds like linalool, geraniol, and flavonoids, coriander offers not only flavour but also therapeutic properties, including antioxidant and anti-inflammatory activities (J. Smith & Brown, 2023). These characteristics suggest Dhanyaka's ability to modulate immune responses by supporting immune system components.

Coriander's antioxidants help combat oxidative stress, crucial for maintaining immune cell function and overall immune balance (Green & Clark, 2022). Its anti-inflammatory properties further contribute to a responsive immune system. Traditionally valued for digestive health benefits, coriander's positive influence on the gut may indirectly support immune modulation (Lee & Kim, 2020).

Versatile in culinary applications, coriander can easily be incorporated into daily meals, enhancing both flavour and potential health benefits. While ongoing research

continues to explore Dhanyaka's immunomodulatory effects, early studies show promise in understanding its role in supporting immune function and managing inflammation (Zhao & Wang, 2019).

PROPERTIES AND ACTION(API, 2001a)

- Rasa (Taste) : Pungent (*Katu*), Sweet (*Madhura*), Bitter (*Tikta*), Astringent (*Kashaya*)
- Guna (Property) **:** Light (*Laghu*), Unctuous (*Snigdha*)
- Virya (Potency) **:** Hot (*Usna*)
- Vipaka (Post-digestive Taste)**:** Sweet (*Madhura*)
- Karma (Action) **:** Digestive (*Dipana*), Digestive stimulant (*Pachana*), Heart tonic (*Hridya*), Absorbent (*Grahi*), Balances all three *doshas* (*Tridosanut*), Diuretic (*Mutrala*)
- Therapeutic uses: Fever (*Jvara*), Thirst (*Trsna*), Burning sensation (*Dahahera*), Indigestion (*Ajirna*), Diarrhea (*Atisara*)

1.4.9 Jeeraka *(Cuminum cyminum* L. *)*

Jeeraka, or cumin, renowned for its culinary role, holds promise beyond flavour enhancement—it serves as a potential immunomodulator. This essay delves into its bioactive compounds, such as cuminaldehyde and essential oils, highlighting their therapeutic potential (Patel & Patel, 2020).

Research indicates that Jeeraka influences immune responses through compounds like thymoquinone, enhancing cytokine production and regulating immune cell activity (P. Sharma & Kumar, 2022). Its anti-inflammatory properties help balance immune function by modulating inflammatory pathways, crucial for combating chronic inflammation.

Additionally, Jeeraka's antioxidant capabilities protect immune cells from oxidative stress, supporting their optimal function (P. Sharma & Kumar, 2022). Its traditional use in aiding digestion indirectly supports immune health, given the gut's role in immune function. Jeeraka's versatility in cuisine makes it accessible for daily consumption,

potentially amplifying its health benefits through flavourful integration into various dishes.

Ongoing scientific exploration underscores Jeeraka's potential as an immune-supporting agent, reinforcing its place beyond the spice rack (S. Gupta & Singh, 2021).

PROPERTIES AND ACTION (API, 2001a)

- Rasa (Taste) **:** Pungent (*Katu*)
- Guna (Property) **:** Light (*Laghu*), Unctuous (*Snigdha*), Dry (*Ruksa*)
- Virya (Potency) **:** Hot (*Usna*)
- Vipaka (Post-digestive Taste)**:** Sweet (*Madhura*)
- Karma (Action) **:** Digestive (*Dipana*), Digestive stimulant (*Pachana*), Appetizer (*Ruchya*), Anthelmintic (*Krimighna*), Alleviates *Kapha* and *Vata* (*Kaphavatahara*)
- Therapeutic uses **:** Digestive weakness (*Agnimandya*), Diarrhea (*Atisara*), Worm infestations (*Krimiroga*)

1.5 Action on Respiratory System

The respiratory system is essential for gas exchange because it allows the body to exhaust carbon dioxide and absorb oxygen. This function can be hampered by a number of respiratory conditions, including bronchitis, asthma, and chronic obstructive pulmonary disease (COPD)(Dalimi et al., 2015). Plant-derived medications have a long history of usage in both conventional and alternative medicine to treat respiratory conditions because of their wide range of pharmacological characteristics, which include bronchodilator, expectorant, and anti-inflammatory actions (Ahmad et al., 2019). Through these diverse mechanisms, herbal medicines exert their therapeutic effect on the respiratory tract and are therefore effective in the treatment of respiratory diseases. Ongoing research and growing interest in herbal medicine continue to support the use of these herbal remedies in respiratory health and highlight their importance as a natural alternative for the treatment of respiratory diseases (Brunelli, 2016).

1.6 Necessity of Converting Kshamatva Churna to Different Nutraceutical Forms.

Beyond the amazing outcomes, many challenges were encountered when researching the immunomodulatory effects of Kshamatva churna during the COVID-19 pandemic. Palatability (many found Kshamatva Churna in the form of kashaya to be extremely difficult to consume) and transportation of the medication, as well as the distribution of churna in packets and instructions for storage and administration, were some of the significant challenges to which we had to pay attention.

The need to resolve all of these issues emerged as the main challenge after analysing and contrasting them with Kshamatva Churna's immunomodulatory qualities. Converting Kshamatva churna into different nutraceutical forms was the best way to solve this issue without sacrificing the product's effectiveness or dosage, but rather by compromising the traditional preparation. The significance of standardizing formulations and nutraceutical forms then arises. It is crucial to implement quality control and standardization measures to guarantee the quality of herbal formulations.

1.7 Standardization of Ayurveda Formulations

Standardization is crucial in ensuring the quality of herbal formulations by establishing a set of replicable parameters that must be met during the formulations' stability period. Ayurveda, the most widely recognized traditional medicinal system, is increasingly being adopted by individuals worldwide who are seeking alternatives to modern medicine. However, the growing demand for herbal remedies, coupled with differences in raw material availability and a profit-driven mindset among pharmaceutical companies, has led to the production of inadequate and sometimes dangerous goods. The primary issues contributing to the production of inferior and unsafe products include drug adulteration and substitution, improper methods of gathering, storing, and preserving raw materials, differing interpretations of formulations in traditional texts, addition of preservatives, and improper purification of toxic herbal and mineral drugs. To address these issues, organizations such as the World Health Organization (WHO)(WHO (World Health Organization), 2011) and the Indian government have acted to standardize individual medications and formulations (Indian Pharmacopoeia Commission, 2018). The Indian Ayurvedic Pharmacopoeia includes certain standardized individual formulations, and the Ministry of AYUSH has established

standard guidelines for various types of preparations. Given that most Ayurvedic medicines are used in polyherbal formulations, it is vital to standardize each formulation to ensure the quality of the medicines and to promote the global popularity of Ayurveda. Standardization not only helps in maintaining the therapeutic efficacy and safety of the formulations but also builds consumer trust and enhances the credibility of traditional medicinal systems (Baragi et al., 2011).

1.8 Network Pharmacology

Network pharmacology (NP) is a burgeoning field that utilizes computational methods to elucidate the intricate interactions between drugs and multiple targets within biological systems (LI & ZHANG, 2013). By harnessing the power of computer algorithms, NP enables a systematic exploration of the molecular interactions of drug molecules within living cells, providing insights into their mechanisms of action (Hopkins, 2008).

In the context of Ayurvedic formulations, NP proves to be a valuable tool for understanding the holistic effects of botanical compounds on the entire body. Through network analysis, NP facilitates an unbiased exploration of potential target areas, paving the way for the discovery of new drug leads and the repurposing of existing drug molecules for diverse therapeutic aplications (LI & ZHANG, 2013).

However, the success of NP initiatives hinges on the ability to identify appropriate targets and novel therapeutic molecule scaffolds. Here, traditional wisdom plays a crucial role in guiding the discovery and repurposing processes of Ayurvedic formulations and approved medications (Zhang et al., 2016).

Looking ahead, the integration of NP with systems biology holds promise for the rational design of next-generation medications. By leveraging advancements in both fields, researchers can develop promiscuous drugs with enhanced safety and efficacy profiles, thereby expanding the repertoire of treatment options for various diseases (LI & ZHANG, 2013)

1.9 Nutraceuticals

Food and nutrition have always been crucial to understanding the pathophysiology of disease and preventing it. The goal of public health is shifting from a “cure” to a “prevention.” The majority of people feel far more comfortable using drugs than eating, and Hippocrates' “food as medicine” theory has received little attention. But over the last decade, this perspective has evolved and the importance of “nutraceuticals” has steadily increased. *“Let food be thy medicine and medicine be thy food'* was quoted by Hippocrates in 400 BC, which has great relevance in today's world (Yates, 2010).

Nutraceuticals is a combination of the word’s "nutrition" and "pharmaceuticals.". The phrase "nutraceutical" was first used in 1989 by Dr.Stephen DE Felice, who founded the Foundation of Innovation Medicine in Crawford, New Jersey. The potential benefits of nutritional supplements for therapy, safety, and nutrition have drawn a lot of attention in recent years. These nutraceuticals have an impact on several biological processes, such as antioxidant defences, cell proliferation, gene expression, and preserving mitochondrial integrity. Therefore, they can be employed to preserve the body's integrity and functionality, as well as to fend off chronic illnesses, postpone aging, and increase life expectancy (Jalleh et al., 2020).

Improving the dietary status of Indian citizens has been the government's main priority. The global health crisis caused by the coronavirus disease 2019 (COVID-19) has brought attention to the relationship between the nation's economic and nutritional standing (Ratanachamnong et al., 2020). As a result of the pandemic, public health initiatives, healthcare plans, and disease prevention strategies are probably going to give nutrition more consideration and emphasis than in the past. Another change brought about by this pandemic is a greater emphasis on health and wellbeing in consumer behaviour. It is now much more important to strengthen one's immunity. Along with our nation's rich history of using Ayurvedic and herbal medicine, it portends well.

1.9.1 Kshamatva Churna - Different Nutraceutical forms

1. Kshamatva Churna Sugar candy
2. Kshamatva Churna Palm Jaggery candy
3. Kshamatva Churna Crystals
4. Kshamatva Churna Dip tea bags
5. Kshamatva Churna Easily soluble powder
6. Kshamatva Churna Granules
7. Kshamatva Churna Kashayam Spray dried Powder
8. Kshamatva Churna Syrup

In this study we are going to discuss various steps that we had done to analyse the immunomodulatory action of Kshamatva churna such as

- Standardization of the formulations
- Fingerprinting of Kshamatva churna
- In-Silico study of the formulations
- Mode of preparation of various nutraceutical forms

2.METERIALS AND METHODS

2.1 Selection of Plant materials

The Plant samples are selected and identified by the Bhaishajya Kalpana (BK) department of I-AIM and other experts. After collection accurate scientific identification was done by using the reported papers and literature survey.

2.2 Collection and Authentication of Plant materials

The samples used in this study were collected from the Department of Bhaishajya Kalpana, I-AIM hospital Yelahanka. Authenticated by experts of I-AIM and TDU.

2.3 Drying of Drugs and Powdering

The all 9 ingredients were weighed separately, made into small pieces, washed well and dried under sunlight, to make sure it is devoid of fungus and other impurities. The dried samples were made into crushed powder using a pulveriser. Then mix the 9 ingredients one by one by considering the mentioned ratio. From the half part was taken and made to fine powder using a pulveriser, packed and stored.

Fig 2: Collection, Drying, Powdering and Packing of Kshamatva churna

Zingiber officinale

Withania somnifera

Piper longum

Curcuma longa

Glycyrrhiza glabra

Tinospora cordifolia

Piper nigrum

Coriandrum sativum

Cuminum cyminum

Fig 3: 9 collected ingredients of Kshamatva Churna

2.4 Phytochemistry (Qualitative analysis)

2.4.1 Extraction of the Plant material

- Successive Soxhlet extraction
- A thimble was prepared with thick filter paper with 10gm of plant sample in it and kept in the Soxhlet extractor, and 200ml solvent was poured from non-polar to polar respectively.
- And the set of apparatus was kept in the water bath at a specific temperature according to the solvents boiling point.
- After a specific time (7 to 8 hr), the apparatus was taken out, and the solvent was stored in a conical flask.
- The same thimble was dried and used for the subsequent solvent extraction (Successive Extraction) (Srivastava et al., 2015).

Table 2 : Successive Extraction

SOLVENT	WATER BATH TEMP °C	DURATION	CYCLE
Hexane Chloroform Ethyl acetate Methanol Water	70 60 80 65 100-110 frequency in a heating mantle	7 to 8 hrs.	For all samples, an average of 8 cycles were performed.

2.4.2 Preparation of extracts

After successive extraction, the extracted samples are kept for evaporation. The china dishes were weighed before adding the solvent. The solvent extracts from the Soxhlet were poured into a china dish and left in a water bath for evaporation. The temperature of the water bath is set according to the solvent's boiling point. After evaporation, the china dish was weighed again to get the extract yield. The samples were scraped with the help of blades and stored in clean test tubes. These samples were used for further test

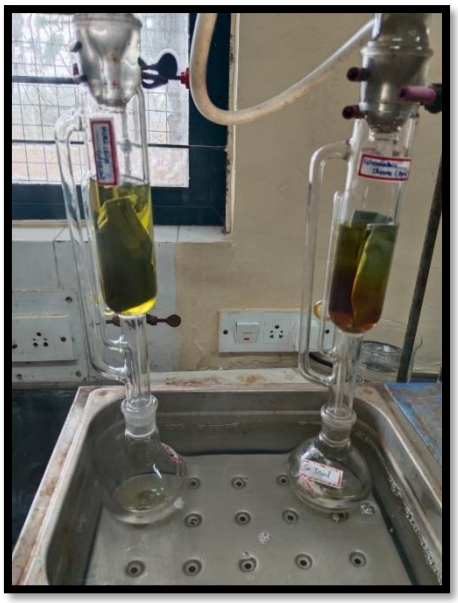

Fig 4 : Soxhlet Extraction of Kshamatva churna

2.5 Preliminary Phytochemical Detection

- The test was carried out as described below from Table-3 to Table- 10.

Alkaloids test

- Solvent-free extract of 50 mg is stored with a few mL of H_2SO_4 acid, and a filter. Filtrate is used in various alkaloids tests.

Table 3 : Alkaloids test

SR.No	TEST	PROCEDURE	OBSERVATION
i	Mayer 's test	A drop of Mayer's reagent was added along the sides of the test tube	A white or creamy precipitate indicated the presence of alkaloids. (Evans,1997)
ii	Wanger test	A drop of Wagner's reagent was added along the sides of the test tube	A reddish-brown precipitate indicated the presence of Alkaloids. (Wanger,1993)
iii	Hager's test	A drop of Hager's reagent was added along the sides of the test tube	A yellow precipitate indicated the presence of alkaloids.
iv	Dragendorff's test	A drop of Dragendorff's reagent was added along the sides of the test tube	A prominent yellow precipitate indicated the presence of Alkaloids. (Waldi, 1965)

Carbohydrates and glycosides test

- Extract (100mg) dissolved in 5 mL of water and filtered (Ramakrishna et al. 1994)

Table 4 : Carbohydrates and glycosides test

SR.No	TEST	PROCEDURE	OBSERVATION
i	Molisch's test	Filtrates were treated with two drops of alcoholic α-naphthol solution in a test tube. To this mixture, 2 mL of concentrated sulphuric acid was added along the sides of the test tube.	A violet ring at the junction of two solutions indicates the presence of carbohydrates.
ii	Benedict's test	1 ml of filtrate was treated with 1mll of Benedict's reagent and heated in a boiling water bath.	Orange-red precipitate indicates the presence of carbohydrates.
iii	Barfoed's test	To 1 mL of filtrate, 1 mL of Barfoed's reagent was added and heated on a boiling water bath.	A red precipitate indicates the presence of reducing sugars.
iv	Fehling's test	1 mL of filtrate was boiled in the water bath with 1 mL of each of Fehling's solutions A and B.	The formation of a red precipitate indicates the presence of reducing sugars.

Glycosides test

- 50mg of the extract is hydrolysed in concentrated H_2SO_4 for 2 hours in a water bath, then filtered, and the hydrolysate is subjected to the following tests.

Table 5: Glycosides test

SR.No	TEST	PROCEDURE	OBSERVATION
I	Borntrager's test	To 2 mL of filtrate, 3 mL of chloroform+shaken. The chloroform layer was separated, and an equal amount of 10% ammonia was added to it.	A pink colour indicates the presence of anthraquinone glycosides.

Ii	Legal's test	To 2 mL of filtrate, one drop of pyridine, and 1 mL of sodium nitroprusside solution were added. This mixture was made alkaline using 10% sodium hydroxide.	The pink to blood-red colour indicates the presence of glycosides.
Iii	Liebermann Burchard's test	To 2 mL filtrate, 3 mL of chloroform and a few drops of acetic anhydride were added. This mixture was boiled and cooled in an ice bath. To this, 2 mL concentrated acetic anhydride was added along the sides of the test tube.	brown ring at the junction of the two layers, along with the formation of. violet/green/ blue the colour indicates the presence of steroidal glycosides.

Detection of saponins test

Table 6 : Detection of saponins test

SR.No	TEST	PROCEDURE	OBSERVATION
I	Foam test	50 mg extract is diluted in Distilled water and make up to 20 ml	A 2 cm layer of form

Proteins and amino acids test

- The extract(100m) is dissolved in 10 mL of distilled water. And filtered through Whatman No.1 filter paper.
- The filtrate is subjected to a test for protein and amino acids

Table 7: Proteins and amino acids test

SR. No	TEST	PROCEDURE	OBSERVATION
I	Millon's test	To 2 mL of extracts, a few drops of Millon's reagent	white precipitate indicates the presence of proteins. (Rasch and Swit,1960)

Ii	Biuret test	2 mL of extracts were treated with one drop of 2% copper sulphate solution and 1 mL of 95% ethanol, and an excess of potassium hydroxide (KOH) pellets.	The pink colour in the ethanolic layer indicates the presence of proteins.
Iii	Ninhydrin test	To 2 mL of filtrate, two drops of ninhydrin solution were added.	Purple the colour indicates the presence of amino acids. (Yasuma and Ichikawa,1953)

Phenol and Tannins test

Table 8 : Phenol and Tannins test

SR.No	TEST	PROCEDURE	OBSERVATION
I	Ferric chloride test	To 2 mL of filtrate, a few drops of neutral 5% ferric chloride	The dark green colour indicates the presence of tannins. (Mac,1963)
Ii	Gelatin test	To 2 mL of filtrate, 2 mL of a 1% gelatine solution containing 10% sodium chloride	A white precipitate indicates the presence of tannins. (Evans,1997)
Iii	Lead acetate test	To 2 mL of filtrate, 3 mL of 10% lead acetate solution	white precipitate indicates the presence of phenolic compounds.

Flavonoids test

Table 9 : Flavonoids test

SR.No	TEST	PROCEDURE	OBSERVATION
I	Alkaline reagent test	2 mL of filtrate was treated with 2 mL of 10% ammonia.	Yellow fluorescence indicates the presence of flavonoids.
Ii	Magnesium and HCl reduction (Shinoda test)	To 2 mL of filtrate, 1 mL of Methanol a few fragments of magnesium ribbon and concentrated hydrochloric acid	Pink to crimson colour indicates the presence of flavanols. (Harborne,199)

Iii	Lead acetate Test	2 mL of extract was treated with a few drops of lead acetate solution.	Yellow precipitate indicates the presence of flavonoids.

Gums and mucilage test

Table 10: Gums and mucilage test

SR.No	TEST	PROCEDURE	OBSERVATION
I	Alcoholic precipitation test	25mL of absolute alcohol was slowly added to the extracts with constant stirring.	The formation of a precipitate indicates the presence of gums & mucilages

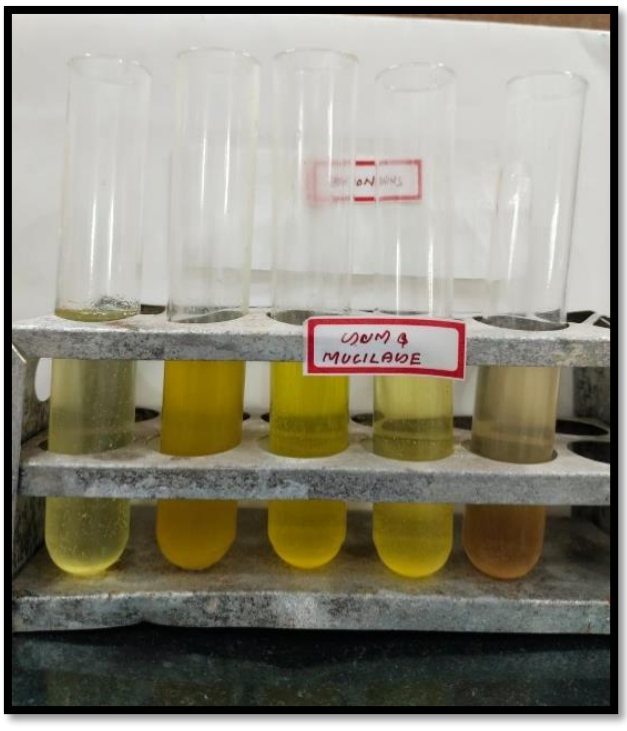

Fig 5*:* Preliminary Analysis for Phytochemicals of Kshamatva churna

2.6 Thin Layer Chromatography (TLC)

In many experiments, it is important to be able to separate a mixture into its chemical components in order to isolate one compound or to assess the purity of the mixture. Thin layer chromatography (TLC) is one of the easiest and most versatile methods of doing this because of its low cost, simplicity, quick development time, high sensitivity, and good reproducibility.(Sherma, 2003) TLC is used by many industries and fields of research, including pharmaceutical production, clinical analysis, industrial chemistry, environmental toxicology, food chemistry, water, inorganic, and pesticide analysis, dye purity, cosmetics, plant materials, and herbal analysis. In its simplest form, glass plates are coated with a uniform layer of silica gel (SiO2). The dissolved sample is placed on the plate, and the plate is inserted into a glass chamber (TLC chamber) containing the developing solvent and a piece of TLC plate. When the solvent has risen to near the top of the plate, the plate is removed, dried, and visualized using UV light. (Touchstone, 1992)

TLC Condition

- Samples : Successive extracts of Kshamatva churna
- Stationary phase: Merck ,TLC plate, Silica gel 60F 254
- Plate format : 10cm * 10cm
- UV detector :LINOMAT 5 (TDU)
- Wavelength :254mm & 366 mm
- Solvent system :

Table 11 :TLC Solvent system

PHYTOCONSTITUENTS	SOLVENT SYSTEM	RATIO
Alkaloids	Toluene: Ethyl acetate: Methanol: 25% Ammonia	(30: 30: 15: 1)
Glycosides	Ethyl Acetate: Methanol: water	(20: 2.8: 2)
Flavonoids	Ethyl Acetate: Formic Acid: Glacial Acetic Acid: Water	(10: 0.5: 0.5:1.3)
Terpenes	n-hexane: Ethyl Acetate	(1: 1)
Polyphenols	Tetra Hydro Furan: Toluene: Ethyl Acetate: Water	(16: 8: 2: 1)

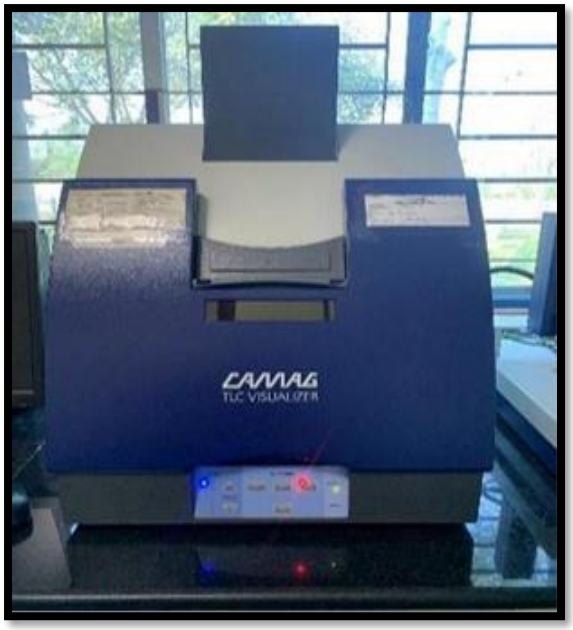

Fig 6: CAMAG TLC visualizer (TDU)

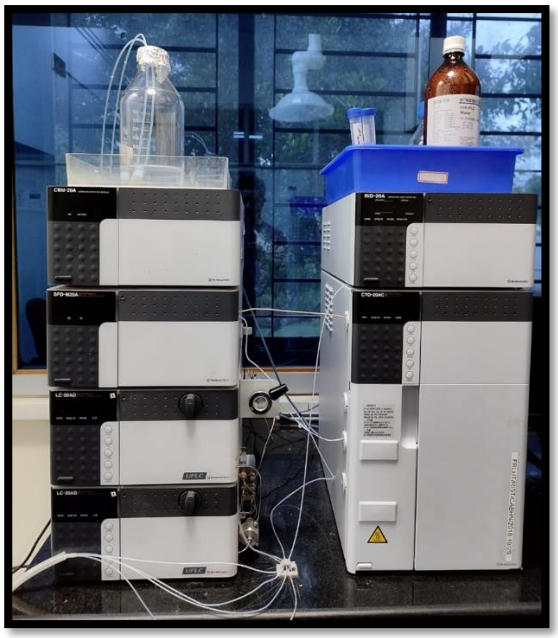

Fig 7: HPLC system (TDU)

2.7 Physico-Chemical Parameters

2.7.1 Total Ash value

Ash values are useful for assessing the quality and purity of crude drugs, particularly in powder form. The purpose of ashing herbal drugs is to eliminate all organic matter, which might otherwise interfere with analytical determinations. Upon incineration, crude drugs typically leave ash that consists of carbonates, phosphates, and silicates of sodium, potassium, calcium, and magnesium. The total ash content indicates the amount of minerals naturally present in the medicinal plants and the extent of foreign materials introduced during processing or handling (Indian Pharmacopoeia Commission, 2018).

Powdered ingredients of Kshamatva churna weighed 5 gms and White crucibles made of quartz and marble were used for charring. After proper charring keep the crucibles for ashing in the Muffle furnace for 6 hours at 600 c. weigh the crucibles and find the amount of Ash.

Fig 8: Charring of KC

Fig 9: Muffle furnace

Fig 10: Ashing of KC

2.7.2 Water-soluble extractive value

The water-soluble extractive value is crucial in evaluating crude drugs. A lower extractive value suggests the addition of exhausted material, adulteration, or errors in processing during drying, storage, or formulation (WHO (World Health Organization), 2011)

Demineralized water was added to the crucibles mix with ash and heated in low temperature in a heating pan. Filtered the water containing Ash through No.1 filter paper and weighed it after drying to calculate the water-soluble ash content.

2.7.3 Acid Insoluble Ash

Acid-insoluble ash content indicates the presence of fine soil and sand particles. Acid-insoluble ash (AIA) serves as a marker in digestibility studies. Three variations of the original gravimetric method are commonly used to determine AIA content: these involve ashing the sample to burn off organic matter, boiling the residue in hydrochloric acid, and then re-ashing the sample (WHO (World Health Organization), 2011).

To the charred Ash Hydrochloric acid (HCL) were added drop by drop when it was fully digested filtered through No 1 Filter paper. Weigh the filter paper after drying to detect the acid insoluble contents.

2.7.4 pH

The pH of the drug delivery system affects the drug's penetration route, making it essential to understand the primary pathways of drug absorption. An increase in pH decreases the solubility and percentage of the unionized form of a weakly basic drug, while increasing its distribution coefficient (WHO (World Health Organization), 2011).

3gm of Kshamatva churna was boiled with 120ml of water take 50ml in a glass biker and checked the pH value using pH meter (TDU)

Fig 11: pH meter (TDU)

2.8 High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) is an analytical technique used to separate, identify, and quantify components in a mixture, relying on a liquid mobile phase passing through a column filled with a solid stationary phase.(Snyder et al., 2011) It is widely used in pharmaceuticals, environmental monitoring, food and beverage industry, and clinical research due to its precision, accuracy, and versatility. Components are separated based on their interactions with the stationary and mobile phases, with retention time aiding in identification and quantification. HPLC consists of the mobile phase, pump, injector, column, and detector, with common detectors including UV-Vis, fluorescence, and mass spectrometers. There are various types of HPLC: normal-phase, reverse-phase, ion-exchange, and size-exclusion, each suitable for different applications.(Kazakevich & LoBrutto, 2007) Method development involves selecting the appropriate column and mobile phase, and optimizing flow rate and temperature. HPLC is crucial in the pharmaceutical industry for drug analysis, environmental analysis for detecting pollutants, the food and beverage industry for analysing additives, and clinical research for measuring biomarkers. Despite its advantages of high resolution and sensitivity, HPLC requires expensive equipment and maintenance, high purity solvents, and can be time-consuming. Continuous

advancements in technology are expanding its capabilities and applications.(Skoog et al., 2018)

2.8.1 Standard Solution Preparation:

- Accurately weighed 1 mg of piperine, Gallic acid, and Curcumin.
- Dissolved the weighed powders in 1 ml of methanol to make a 1 mg/ml solution.
- Mixed thoroughly until the solution was clear.
- Filtered the solution through a 0.2 µm syringe filter to remove any particulate matter, ensuring it was suitable for HPLC analysis.
- Made a mixture of Piperine, Gallic acid, and Curcumin standards.

2.8.2 Test Solution Preparation:

- Accurately weighed 10 mg of Kshamatva Churna and performed methanolic extraction using the Soxhlet extraction method.
- Kept the extract for concentrating purposes and reconstituted it with HPLC grade methanol to make a 1 mg/ml solution.
- Mixed thoroughly until the solution was clear.
- Filtered the solution through a 0.2 µm syringe filter to remove any particulate matter.

2.8.3 HPLC Analysis Conditions:

- Column: SHISEIDO SOLAR, C-18, 4.6 mm x 250 mm
- Mobile Phase: Used a simple isocratic mobile phase of 50% HPLC grade water with 0.1% OPA (A) and 50% Acetonitrile (B).
- Flow Rate: 1 mL/min
- Detection: UV detection at 254 nm
- Injection Volume: 10 µL
- Column Temperature: Ambient or controlled at 30°C
- Running Time: 10 min
- HPLC System: UFLC -L2010510221AE
- Software: Lab Solutions

2.8.4 Procedure :

System Equilibration:

- Equilibrated the HPLC system with the isocratic mobile phase (50% water, 50% acetonitrile) for at least 30 minutes prior to the first injection to ensure a stable baseline.

Sample Injection:

- Injected 10 µL of the filtered piperine, Gallic acid, and Curcumin standard mix solution and the filtered Kshamatva Churna extract solution into the HPLC system separately.
- Recorded the chromatograms to analyse the peaks corresponding to piperine in both solutions. Noted the retention time, peak shape, and any other notable features.

Documentation and Reporting:

- Recorded all conditions, including mobile phase preparation, injection volumes, and any adjustments made during the analysis.
- Ensured that all data, including chromatograms and graphs, tables were saved and appropriately documented for review or regulatory compliance.

2.9 Network Pharmacology- Kshamatva Churna

A comprehensive dataset of phytochemicals found in nine ingredients of Kshamatva Churna was meticulously developed through an extensive review of literature and mining of public databases such as PubMed-NCBI and Google Scholar. Relevant research articles were carefully selected and scrutinized manually. Potential active phytochemicals from these ingredients were identified using databases like the IMPACT database (Indian Medical Plants, Phytochemistry And Therapeutics), Dr. Duke's Phytochemical and Ethnobotanical Databases, and the KNApSNaK Family Database. Chemical information, including ChEMBL ID and SMILE equations (Simplified Molecular Input Line Entry System), for these phytochemicals was gathered from PubChem and ChEMBL databases. Duplicate entries were then filtered out from the compiled dataset.

Gene targets for all phytochemicals present in the nine ingredients (Guduchi, Aswagandha, Yastimadhu, Haridra, Shunti, Pippali, Maricha, Dhanyaka, Jeeraka) of Kshamatva churna were data mining carried out using resources such as ChEMBL and Binding DB along with their respective UniProt IDs, were extracted from UniProt, ChEMBL, NCBI-GENE, and STRING.

The structural representation of the data, including interactions between plants and biological activity, as well as biological activity and gene targets, was visualized using software Cytoscape v3.10.2.

Furthermore, the DAVID (Database for Annotation, Visualization and Integrated Discovery) Database was utilized to ascertain disease associations and pathway associations by analyzing the gene associations of Kshamatva Churna with disease names sourced from the DisGeNet Database and pathways sourced from the KEGG database.

Strategy:

Literature Survey & Data Mining

Use PubMed, Google Scholar to select relevant research articles

Screening Potential Phytochemical

Use IMPACT, Dr. Duke's, KNApSNaK databases

Compile Chemical Information

Gather data from PubChem, ChEMBL including CheMBL ID and SMILE equations

Identify Gene Targets of Phytochemicals

Utilize CheMBL, Binding DB to identify gene targets.

Retrieve Gene Target Information

Collect short names and UniProt IDs from UniProt, ChEMBL, NCBI-GENE, STRING

Visualization of Interactions

Use Cytoscape_V3_10.2 to visualize Plants-Biological Activity and Biological Activity-Gene Targets interactions.

Find Disease and Pathway Associations

Use DAVID to find disease associations (DisGeNet) and pathway associations (KEGG).

2.9.1 Network Construction and Analysis

To investigate the pharmacological action of the Phytochemicals, various network showing the interaction among Bio actives (BA), Gene targets (GT), Biological pathway (BP), and Disease Association (DA) were constructed and analyzed using Cytoscape_V3_10.2.

2.10 Different nutraceutical forms of Kshamatva Churna

2.10.1 Sample 1- KC- Kashayam

To prepare Kshamatva Churna Kashayam, 200 ml of water was taken and 5 grams of Kshamatva Churna was added to it. This mixture was placed in a suitable vessel and brought to a boil. The boiling process was maintained consistently until the water reduced to approximately 150 ml. This reduction ensured that the active constituents of the churna were thoroughly extracted into the water, creating a potent kashayam. Once the boiling process was complete, the mixture was filtered to remove any solid residues, resulting in a clear liquid. This filtered liquid, now a concentrated kashayam, was to be consumed while warm. The standard dosage was 150 ml, taken as directed, to achieve the desired therapeutic effects. This method ensured that the preparation was both effective and palatable for consumption.

Mode of Administration: Oral ,3gm boiled with warm water, can add sugar or jaggery
Dose : 3gm powder per day

Fig 12: Kshamatva churna Kashaya making

2.10.2 Sample 2 – KC-Sugar Candy

Two parts of sugar candy powder were added to one part of Kshamatva Churna, in the ratio of 1:2. One part of Kshamatva Churna was boiled with 5 parts of water until the contents were properly extracted. Then, 2 parts of the sugar candy powder were added to this concentrated water and boiled at a temperature of 140-160 degrees celsius, while being stirred continuously until the desired consistency was achieved. Lemon juice was added for flavour, and then the mixture was kept on a mild fire. The product was immediately transferred to the mould while it was hot. After waiting for 30 minutes, the candy was checked to ensure it had the right consistency, then coated with fine sugar candy powder to avoid stickiness and stored in an airtight container.

Mode of Administration: Oral (Chewable form)
Dose : 4 candy per day (Average weight- 2.1 gm

Fig 13: Kshamatva Churna Sugar candy

2.10.3 Sample 3- KC- Palm Jaggery Candy

To prepare Palm Jaggery Candy, two parts of palm jaggery were added to one part of Kshamatva Churna in a 2:1 ratio. One part of Kshamatva Churna was boiled with five parts of water until the contents were properly extracted. Then, two parts of palm jaggery were added to this concentrated extract and boiled at a temperature of 140-160 degrees celsius, stirring continuously until the desired consistency was achieved. A small quantity of ginger powder was added for flavour, and the mixture was kept on a mild fire. The hot mixture was immediately transferred to moulds and allowed to set for 30 minutes. Once the candy achieved the right consistency, it was coated with fine sugar candy powder to avoid stickiness and then stored in an airtight container.

Mode of Administration: Oral (Chewable form)
Dose : 4 candy per day (Average weight- 2.1 gm)

Fig 14 : Kshamatva Churna Palm Jaggary candy

2.10.4 Sample 4- KC-Crystals

To prepare KC crystals, 1 part Kshamatva Churna was combined with 2 parts sugar and lightly sprinkled with water. The mixture was gently heated until the sugar melted, then sunti (dried ginger) powder was added. The mixture was spread thinly and left to dry for 5 hours. The result was a sweet, healthful crystal with the beneficial properties of Kshamatva Churna and the warm flavour of ginger.

Mode of Administration: Orally, can be taken directly or with warm water

Dose : 6gm per day

Fig 15 : Kshamatva Churna Crystals

2.10.5 Sample 5 - KC Dip- Tea Bag

In a high-quality tea bag, there was a meticulous blend of 1.5 grams each of tea powder and Kshamatva Churna. Kshamatva Churna, a traditional Ayurvedic herbal powder known for its health benefits and immunomodulation, was carefully combined with tea powder to create a balanced and flavorful tea mixture. Once the tea powder and Kshamatva Churna were blended together, they were used to fill each tea bag. This ensured that every tea bag contained a consistent mixture, providing a uniform taste and quality in every cup. To further enhance the flavour, cardamom powder was added. This combination of tea powder, Kshamatva Churna, and cardamom aimed to deliver a rich and healthful tea experience, balancing the strong, earthy flavours of the tea and herbal mix with the aromatic sweetness of cardamom.

Mode of Administration: Oral, tea bag dipped in hot water for 5 mins, after straining it can be used as beverage

Dose : 2 Dip tea bag per day

Fig 16 : Kshamatva Churna Dip- Tea bags and Cubes

2.10.6 Sample 6- KC Easily Soluble Powder

To prepare an easily soluble powder, the following ingredients were gathered: one part Kashaya extract powder, one part sugar powder, and one part glucose. These ingredients formed the base of the soluble powder.

First, one part of Kashaya extract powder was combined with one part of sugar powder in a large mixing bowl. Next, one part of glucose was added to the mixture. The dry ingredients were thoroughly blended together using a blunt instrument such as a pestle or a mechanical blender to achieve a uniform mixture. This blending process was essential to ensure that the Kashaya extract powder, sugar powder, and glucose were evenly distributed throughout the mixture.

Once the base mixture was well-blended, a natural mint extract was added for flavouring. The quantity of mint extract was adjusted according to taste preference, added gradually to avoid overpowering the flavour. The mint extract not only enhanced the taste but also provided a refreshing aroma to the powder.

The mint extract was mixed thoroughly with the dry ingredients to ensure an even distribution of flavour. After blending, the powder was fine and homogeneous, with no lumps or uneven patches. The final product, an easily soluble powder, was stored in an airtight container to maintain its freshness and prevent moisture from affecting its solubility. This preparation method ensured that the powder dissolved easily in water or other liquids, providing a convenient and pleasant-tasting supplement that retained the therapeutic benefits of the Kashaya extract while being enhanced by the sweetness of sugar and glucose, and the refreshing flavour of natural mint.

Mode of Administration: oral and sublingually or after mix with honey
Dose : 5 gm per day

Fig 17 : Kshamatva Churna Easily soluble powder

2.10.6 Sample 7- KC Granules

To prepare granules using Kshamatva Churna and jaggery, begin by taking two parts of jaggery and 1.5 parts of Kshamatva Churna. Place the jaggery in a suitable vessel and add five parts of water to it. Heat the mixture to a temperature between 140 to 160 degrees Celsius, ensuring the jaggery fully dissolves in the water.

Once the jaggery solution reached the desired temperature, 1.5 parts of Kshamatva Churna were gradually added to the boiling solution. The churna was added little by little while maintaining a medium flame, helping to evenly distribute the churna and prevent clumping. The mixture was continuously stirred to ensure a uniform consistency, which was crucial for converting the mixture into a granular form.

After sufficient stirring, the mixture began transitioning into granules. Once this granular form was achieved, the flame was turned off. While the mixture was still hot, the required quantity of sunti powder (ginger powder) was added, ensuring it was evenly mixed throughout the granules.

The mixture was allowed to cool down completely. The resultant granules were then stored in an airtight container to maintain their freshness and potency. This method ensured the preparation of Kshamatva Churna granules that were free from

preservatives and additives, retaining the traditional therapeutic benefits of the ingredients used.

Mode of Administration: Can be taken directly or with warm milk
Dose: 7gm per day

Fig 18: Kshamatva Churna Granules

2.10.7 Sample 8 - KC Kashayam Extract Chunam

In this study, the spray drying process was employed at the TDU's pilot plant in Bangalore to transform the traditional Kwatha formulation into a more convenient and stable dosage form known as Kshamatva churna kashaya. Initially, the Kashaya was prepared by combining 1 part of Kshamatva churna with 16 parts of water, then reducing it to 8 parts. This prepared Kashaya was transferred into a vessel, where a peristaltic pump tube submerged in the mixture facilitated its feeding into the spray dryer.

Simultaneously, air at a pressure of 5 kg/m³ was directed to the nozzle of the dryer. The spray drying chamber had been preheated to 150°C prior to introducing the Kashaya slurry. Upon entering the chamber, the Kashaya underwent rapid drying,

resulting in the formation of dried powder. This powder was subsequently collected at the bottom of the chamber either through a cyclone pump that directed it into a collector box or by dismantling the attached pipe for direct collection from the chamber bottom.

The use of spray drying was chosen for its capability to produce a stable, easy-to-use, and portable product without the need for preservatives or other potentially harmful additives, addressing the modern challenges associated with traditional Kwatha formulations.

Mode of Administration: Oral,1.5 gm powder mix with 100 ml warm water
Dose : 1.5 gm per day

Fig 19: Spray drying machine and Kashaya extract making of Kshamatva churna

2.10.8 Sample 9- KC Syrup

To prepare KC syrup, 10 grams of churna was taken and boiled in 200 ml of water. The mixture was boiled until the water volume reduced to 100 ml, ensuring that the essence of the churna was well-extracted and concentrated. Next, 80 grams of sugar candy was added to the reduced mixture, and the temperature was maintained between 120-140°C to ensure the sugar candy dissolved completely and integrated well with the churna extract. For flavouring, 2 ml of lemon juice was incorporated, which added a refreshing citrus note to the syrup. The mixture was then stirred and cooked until it reached the desired syrupy consistency. Once the consistency was obtained, the syrup was filtered to remove any solid residues, ensuring a smooth final product. Finally, the filtered KC syrup was stored in a clean, airtight container to maintain its quality and potency.

Mode of Administration: 10 ml of syrup added with warm water or normal water
Dose : 30 ml per day

Fig 20 : Kshamatva Churna Syrup

2.11 Hedonicity Evaluation

To conduct a sensory evaluation of nine product samples by a diverse panel of 10 individuals from different disciplines, the following methodology was employed. First, a panel comprising participants from various fields such as ayurvedic people and non-ayurvedic was selected to ensure a wide range of perspectives. The sensory evaluation was carried out using a standardized sensory evaluation sheet, which included a 5-point scale to assess the attributes of colour, aroma/flavour, texture, taste, and overall acceptability. Each attribute had specific criteria ranging from 'dislike very much' to 'like very much' or equivalent descriptions for each attribute.

Table 12: Sensory evaluation table

Scale/ Attribute	Colour	Aroma/ Flavour	Texture	Taste	Acceptability
1	Dark	Dislike	Very poor	Very poor	Dislike
2	Slightly Dark	Neither Like	Poor	Poor	Neither Like
3	Moderate	Like slightly	fair	fair	Like slightly
4	Pale	Like moderately	Good	Good	Like moderately
5	Slightly Pale	Like Very much	Very good	Very good	Like Very much

The preparation of the nine samples was done under standardized conditions to maintain uniformity, and each sample was assigned a unique code to prevent bias. Participants were given detailed instructions on using the sensory evaluation sheet. After completing the evaluations, the sensory evaluation sheets were collected, and the scores were entered into a spreadsheet for statistical analysis. Total score was

calculated to assess the consistency and reliability of the evaluations. The results were interpreted to understand the sensory profile of each sample, identifying strengths and weaknesses based on the panel's perceptions. This methodology ensured a systematic, transparent, and reproducible process, providing valuable insights for product development and improvement.

3.RESULT AND DISCUSSION

3.1 Extraction of the Plant material

The extractive values for each solvent used in the successive extraction of Kshamatva Churna are presented in the table below:

Table 13: Extractive values of KC in different solvents

SR. NO	Solvent	Extractive value
1	Hexane	0.370
2	Chloroform	0.210
3	Ethyl acetate	0.240
4	Ethanol	0.850
5	Water	0.630

The extractive values show a significant variation across different solvents, indicating the differential solubility of active constituents in these solvents.

- Hexane (0.370%): Being a non-polar solvent, hexane extracted a relatively small amount of non-polar compounds.
- Chloroform (0.210%): Chloroform, which is moderately polar, also extracted a small number of compounds, likely due to its limited solubility range for the constituents present in Kshamatva Churna.
- Ethyl acetate (0.240%): Another moderately polar solvent, ethyl acetate showed slightly higher extractive values compared to chloroform, possibly due to better solubility for certain compounds.
- Ethanol (0.850%): Ethanol, a polar solvent, extracted the highest number of constituents. This indicates the presence of a significant number of polar compounds in Kshamatva Churna that are soluble in ethanol.
- Water (0.630%): Water, the most polar solvent used, extracted a considerable number of constituents, second only to ethanol. This suggests a high content of hydrophilic compounds in the formulation.

The successive extraction of Kshamatva Churna indicates that ethanol and water are the most effective solvents for extracting its active constituents, with extractive values of 0.850% and 0.630%, respectively. This highlights the predominance of polar compounds in the formulation. These findings can guide the selection of solvents for the preparation and standardization of Kshamatva Churna to ensure the maximum extraction of its beneficial components.

3.2 Preliminary Analysis

The phytochemical screening of n-hexane, chloroform, ethyl acetate ethanol, and water extracts of Kshamatva churna were done (Gul et al., 2017). The test gave positive and negative results in all the phytoconstituents

Table 14: Preliminary Phytochemical Analysis

SR. NO	PHYTOCHEMICAL CONSTITUENTS	SUCCESSIVE EXTRACTION KSHAMATVA CHURNA				
		HEXANE	CHLOROFORM	ETHYL ACETATE	METHANOL	WATER
1	**Phenol and Tannins**					
	a) Ferric chloride test	-	-	+	+	+
	b) Gelatin test	-	-	+	+	+
	c)Lead acetate test	-	-	+	+	+
2	**Flavonoids**					
	a) Alkaline reagent test	+	+	+	+	+
	b) Magnesium and HCl reduction (Shinoda test)	+	+	+	+	+
	c)Lead acetate test	+	+	+	+	+

3	**Glycosides**					
	a) Borntrager's test	+	+	+	+	-
	b) Legal test	-	+	+	+	-
4	**Alkaloids**					
	a) Mayer's test	+	+	+	+	+
	b) Wanger test	+	+	+	+	-
5	**Carbohydrates**					
	a) Molisch's test	+	+	+	+	+
	b) Benedict's test	+	+	+	+	+
	c) Barfoed's test	+	+	+	+	+
	d) Fehling's test	-	-	+	+	+
6	**Saponins**					
	a) Foam test	-	-	+	+	+

3.3 Thin Layer Chromatography (TLC)

- Alkaloids' most common bands were seen in Kshamatva churna chloroform ethyl acetate extract and Methanol.
- Flavonoids' most common band were seen in Kshamatva churna ethyl acetate and Methanol extracts.
- Glycosides' most common bands were seen in Kshamatva churna chloroform, ethyl acetate and Methanol extract.
- Terpenes' most common band were seen in Kshamatva churna Hexane, chloroform and ethyl acetate extract.
- Polyphenols' most common band were seen in Kshamatva churna chloroform, ethyl acetate and Methanol extract.

- **Polyphenols**

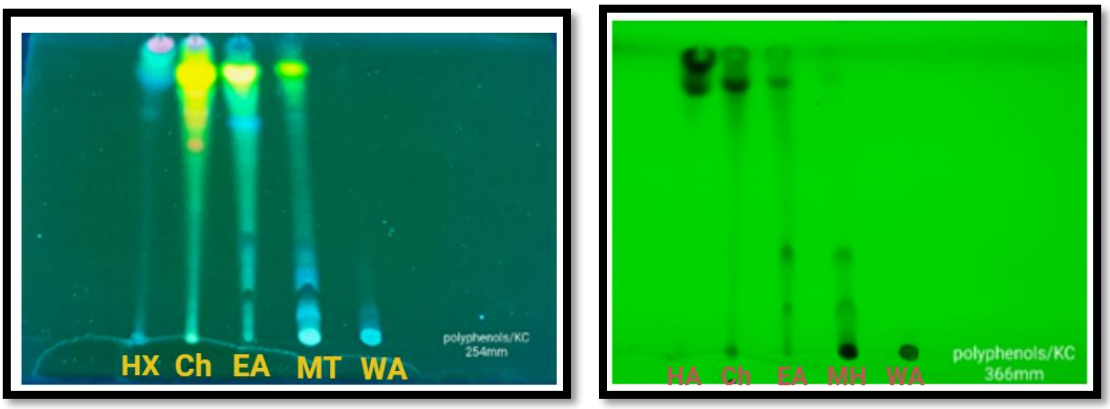

Fig 21: The image is captured at two different wavelengths 254mm and 366 mm
HX- Hexane, Ch- Chloroform, EA- Ethyl acetate, MH- Methanol, WA- Water

- **Terpenes**

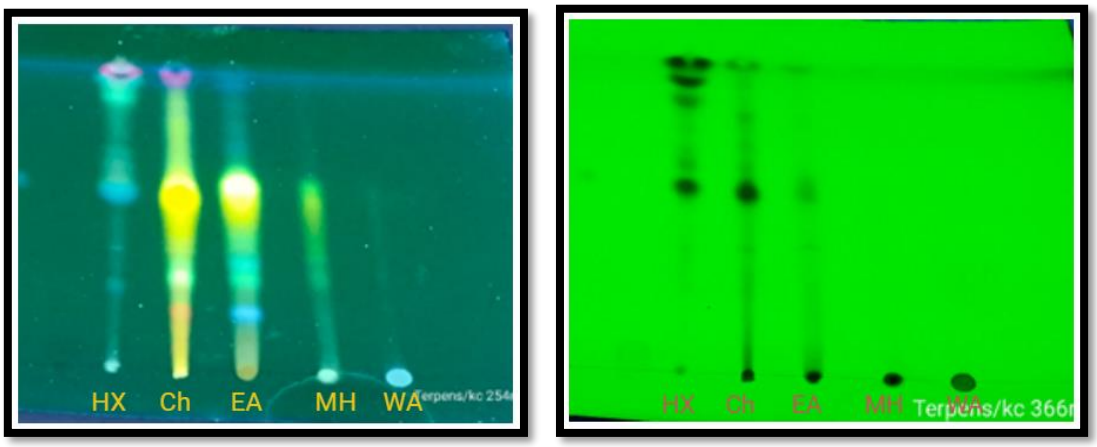

Fig 22: The image is captured at two different wavelengths 254mm and 366 mm
HX- Hexane, Ch- Chloroform, EA- Ethyl acetate, MH- Methanol, WA- Water

- **Glycolysis**

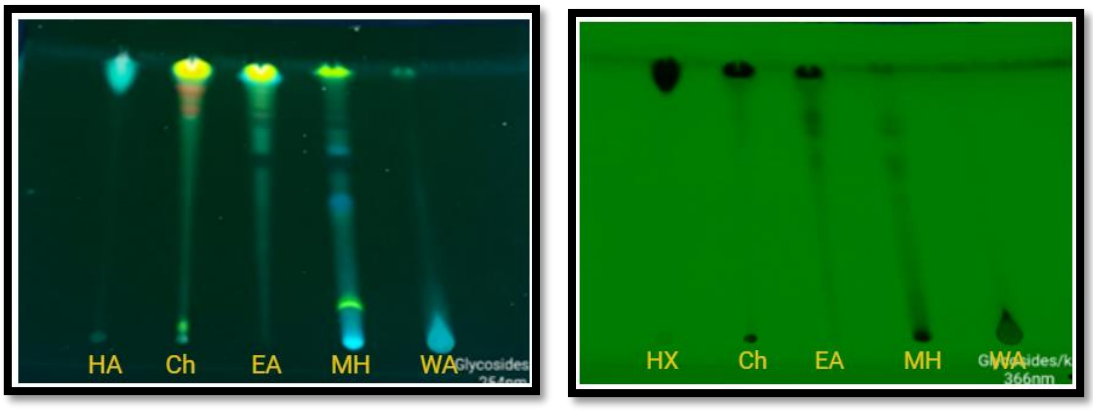

Fig 23: The image is captured at two different wavelengths 254mm and 366 mm
HX- Hexane, Ch- Chloroform, EA- Ethyl acetate, MH- Methanol, WA- Water

- **Alkaloids**

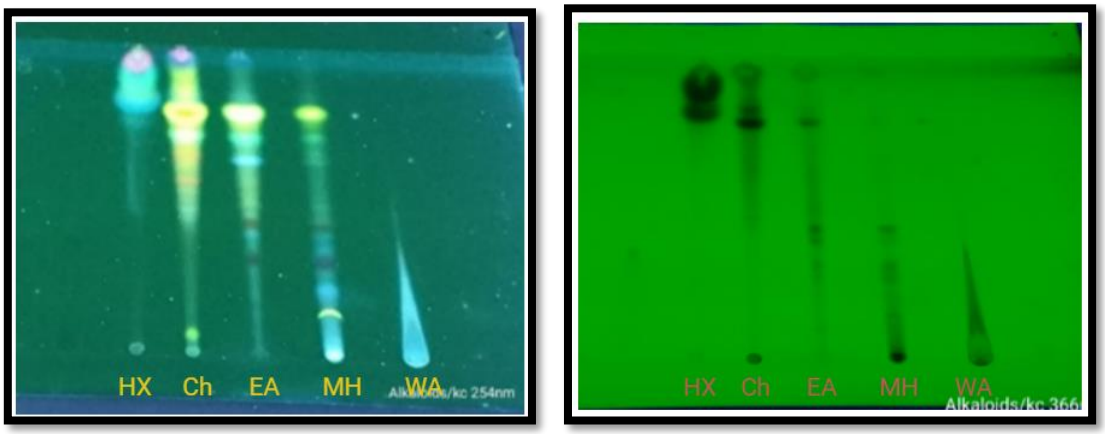

Fig 24: The image is captured at two different wavelengths 254mm and 366 mm
HX- Hexane, Ch- Chloroform, EA- Ethyl acetate, MH- Methanol, WA- Water

- **Flavonoids**

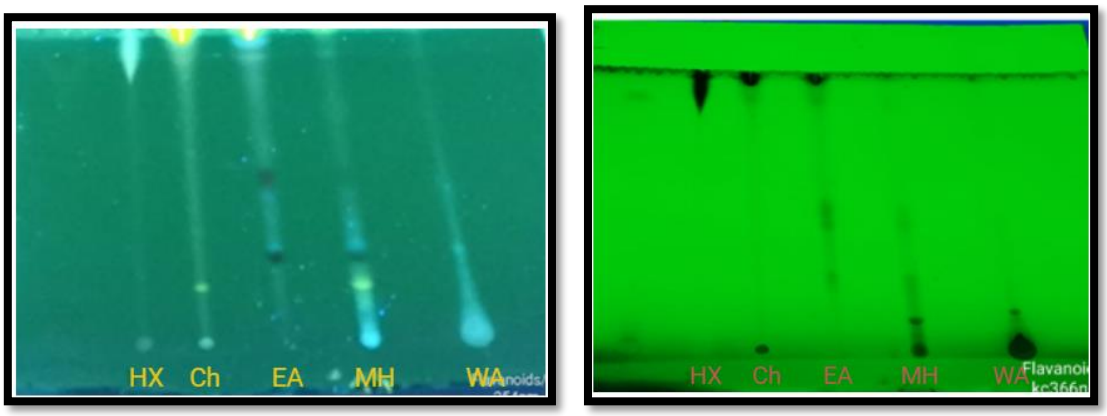

Fig 25: The image is captured at two different wavelengths 254mm and 366 mm
HX- Hexane, Ch- Chloroform, EA- Ethyl acetate, MH- Methanol, WA- Water

3.4 Physico-Chemical Analysis

The analysis of Kshamatva churna reveals significant insights into its composition and quality. The total ash value of 5.1% mg indicates a substantial presence of inorganic compounds, which reflects the overall mineral content of the Churna. A high water-soluble ash value of 94.2 % suggests that most of these minerals are soluble in water, pointing to the presence of bioavailable inorganic substances that can be readily absorbed by the body. The acid in-soluble ash value, at 4.2%, indicates the presence

of acid-insoluble materials, such as silicates, which are generally considered impurities with normal limit. This lower value compared to the water-soluble ash is desirable, as it signifies fewer contaminants. The pH of 5.78 shows that the Churna is slightly acidic, which is typical for many herbal formulations and can influence the solubility and stability of its active components. Overall, these results suggest that Kshamatva Churna has a high mineral content with a majority being bioavailable, making it potentially effective for its intended therapeutic uses, while maintaining a balance in its acid-insoluble impurities.

Table 15: Physio-chemical parameters

SR.NO	Parameters	Value
1	TOTAL ASH VALUE	5.1 ± 0.4%
2	WATER SOUBLE ASH	94.2 ± 1.52 %
3	ACID INSOLUBLE ASH	4.2 ± 7.3 %
4	pH	5.78 ± 0

3.5 High Pressure Liquid Chromatography (HPLC)

The HPLC analysis was performed on two samples: "MIX" (used as the standard, containing equal amount of Piperin, Curcumin and Gallic acid) and "KC" (the test sample, Methanolic extract of Kshamatva Churna) on May 7, 2024, with the data processed on May 22, 2024. For the "MIX" standard, key peaks were observed at retention times (RT) of 2.808 min, 3.563 min, and 9.204 min, with corresponding peak areas of 14934, 124299, and 251937, and peak heights of 2949, 20532, and 17201 respectively. These peaks indicate the presence of specific compounds within the "mix" sample, serving as a reference for comparison.

In the "KC" sample, peaks were detected at RTs of 2.675 min, 2.962 min, 3.586 min, and 9.227 min, with areas of 36868, 8899, 19432, and 33859, and peak heights of 3318, 1013, 2764, and 2415 respectively. The primary peaks at RT 3.563 min and 9.204 min in the "mix" standard match closely with peaks at RT 3.586 min and 9.227 min in the "KC" sample. This qualitative match indicates that the "KC" sample contains key components of the "MIX" standard. The early peak in the "KC" sample (RT: 2.675

min) aligns closely with the early peak in the "MIX" standard (RT: 2.808 min), further supporting the qualitative similarity

The slight differences in retention times can be attributed to minor variations in the experimental setup or sample matrix, but these do not detract from the overall qualitative match. The "KC" sample shows an additional peak at RT 2.962 min, which is not present in the "MIX" standard, suggesting the presence of an additional compound or impurity. Despite this, the primary peaks in both samples correspond well, supporting a positive qualitative identification.

The differences in peak areas and heights between the samples indicate that the concentration of the compounds varies, with the "MIX" standard showing higher areas, implying higher concentrations of these analytes. However, the presence of peaks at similar retention times confirms that the major components in the "KC" sample are the same as those in the "MIX" standard.

In conclusion, the HPLC analysis demonstrates that the "KC" sample contains the primary compounds found in the "MIX" standard. The consistent retention times and peak characteristics validate a positive qualitative match, confirming the similarity between the samples despite the presence of an additional compound in the "KC" sample. This analysis successfully identifies the primary analytes in the "KC" sample, affirming its qualitative similarity to the "MIX" standard.

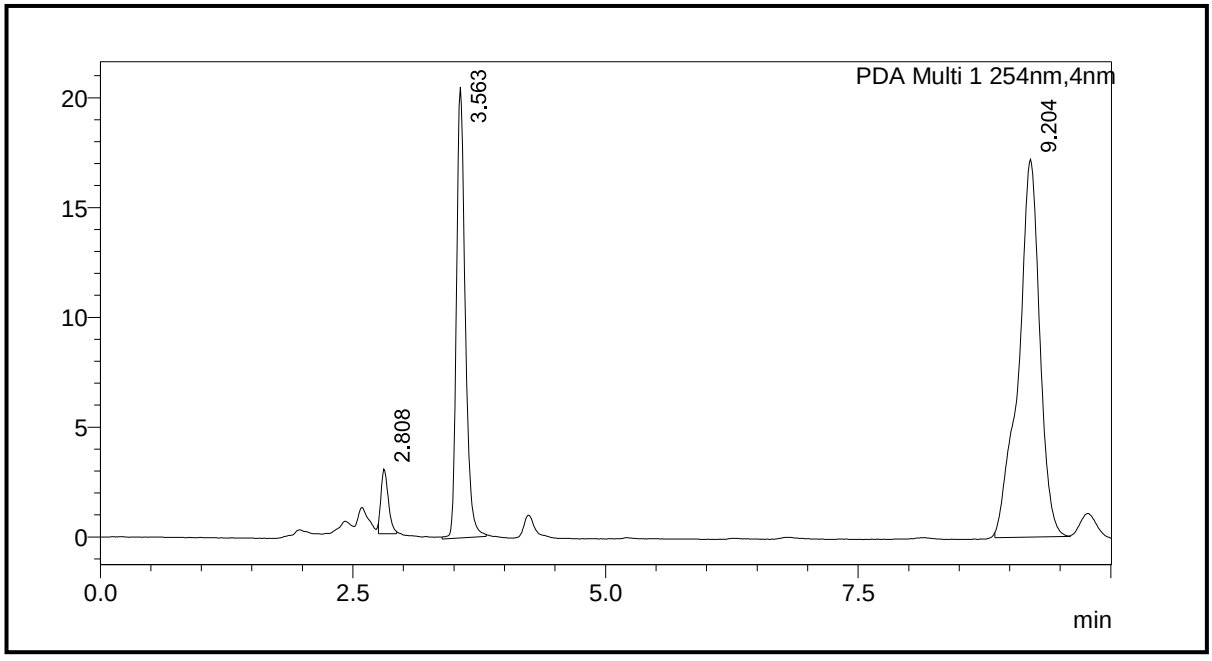

Fig 26: Chromatogram of Standard mix (MIX)

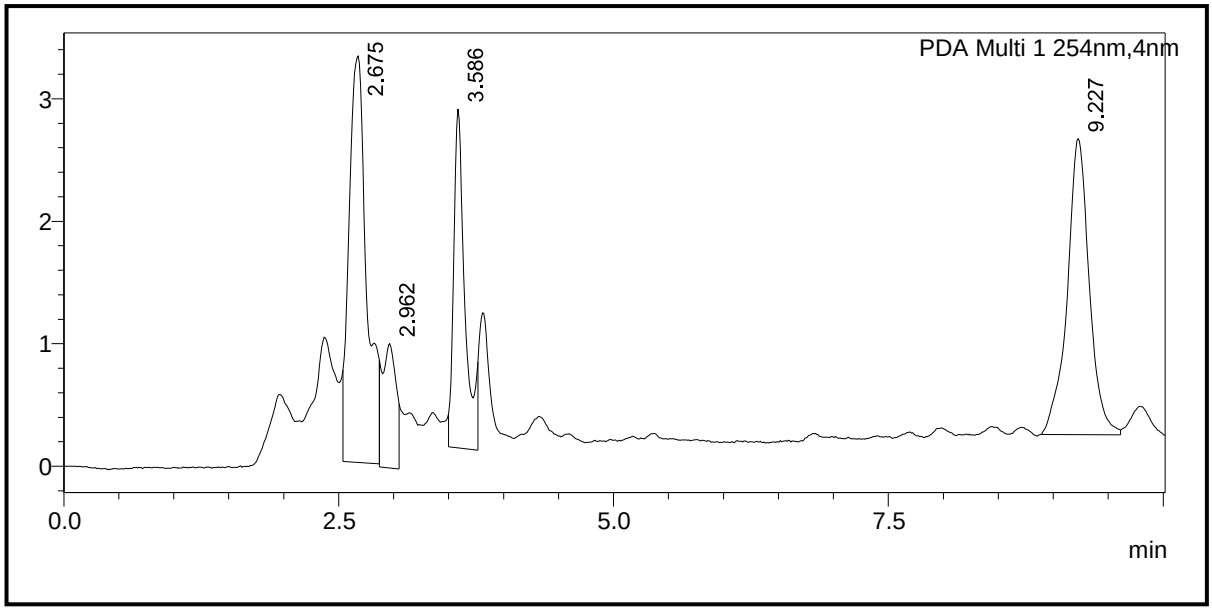

Fig 27: Chromatography of Kshamatva Churna (KC)

Table 16: Peak Table of Standard mix

Peak	Rt. Time	Area	Height	Name
1	2.808	14934	2949	Piperin
2	3.563	124299	20532	Curcumin
3	9.204	251937	17201	Gallic Acid
Total		391171	40682	

Table 17: Peak table of Kshamatva churna (KC)

Peak	Rt. Time	Area	Height	Name
1	2.675	36868	3318	Piperin
2	2.962	8899	1013	Unknown
3	3.586	19432	2764	Curcumin
4	9.227	33859	2415	Gallic Acid
Total		99058	9511	

3.6 Network Pharmacology Analysis

3.6.1 Identification of Phytochemicals

A total of 1,151 phytochemicals have been identified from nine different plants that are used to prepare Kshamatva Churna. This identification was carried out through an extensive literature review and by consulting various scientific databases. Each phytochemical has been assigned a unique ID for reference and further study.

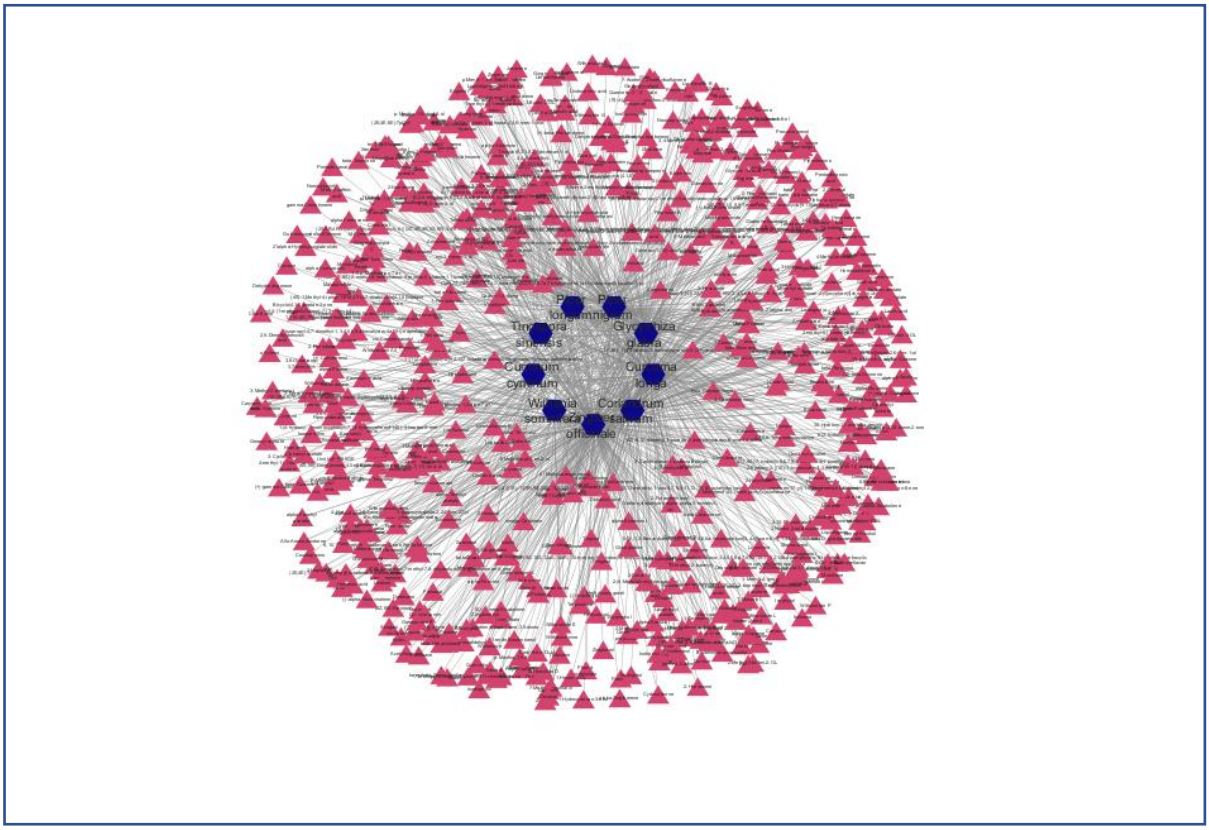

Fig 28:Plant- Bioactive (BA) network , Blue indicate the 9 Plants of KC and 1151 Bio actives

3.6.2 Phytochemical – Gene Target Net work

Through extensive study, it has been discovered that 1,151 bioactive compounds present in Kshamatva Churna interact with a total of 5,855 gene targets. This indicates a complex network where each phytochemical can potentially interact with multiple gene targets. These interactions suggest that each phytochemical may exhibit either activation or inhibitory effects on different genes, suggesting that these

phytochemicals can modulate gene expression in various ways. Consequently, this indicates that the bioactive compounds in Kshamatva Churna have multiple biological actions, contributing to its therapeutic potential through diverse mechanisms of action.

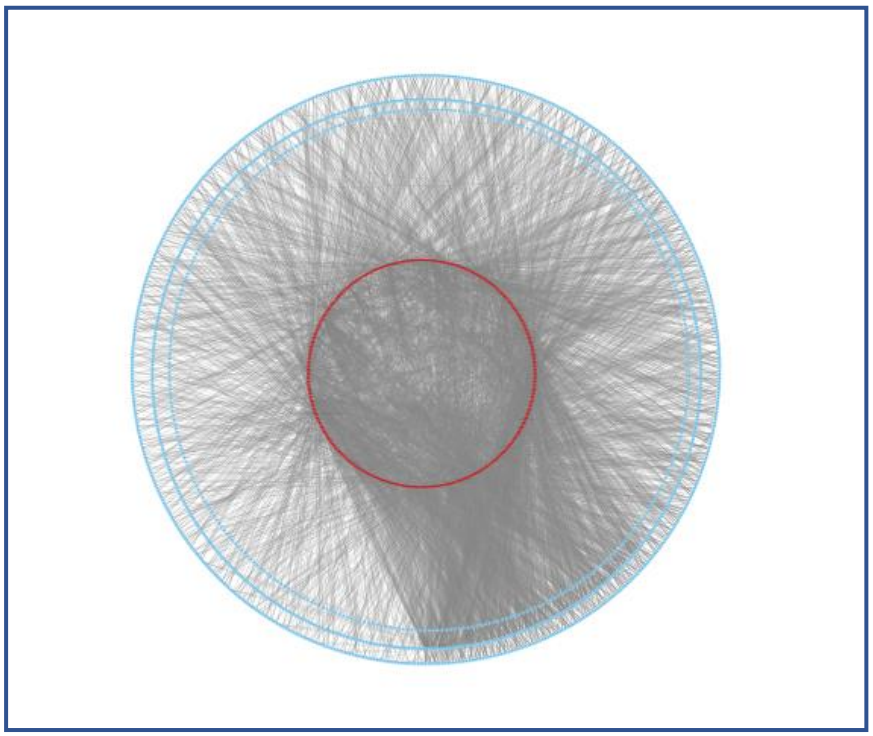

Fig 29 *:* Bio actives – Gene target network ; Red represents 1151 bio actives and Blue represents 5855 Gene targets of KC

3.6.3 Biological Pathways (BP) of Kshamatva churna

Further analysis has shown that these interactions affect 224 distinct biological pathways. This extensive involvement in multiple pathways underscores the broad-spectrum efficacy of Kshamatva Churna, as these pathways are often critical for various physiological and pathological processes. The ability of the phytochemicals to influence such a wide range of pathways explains their potential in treating or preventing different diseases, supporting the use of Kshamatva Churna in traditional medicine for promoting overall health and resilience.

3.6.4 Disease Association (DA) of Kshamatva Churna

Study has identified 306 bioactive from 1,151 bioactive compounds in Kshamatva Churna (KC) that influence 1026 out of 5,855 gene targets. These findings demonstrate that each phytochemical can interact with multiple genes, revealing complex interactions where the compounds can either activate or inhibit gene expression. This multifaceted modulation suggests that the bioactive compounds in Kshamatva Churna contribute to its therapeutic effects through various biological actions.

Further investigation has shown that these gene interactions impact 224 different biological pathways. The extensive involvement of these pathways highlights the broad-spectrum efficacy of Kshamatva Churna, given their critical roles in numerous physiological and pathological processes. Through these 224 pathways, 998 distinct diseases are found to be influenced by the bioactive compounds in Kshamatva Churna.

The wide-ranging impact of Kshamatva Churna's bioactive compounds on 998 diseases underscores its potential in addressing various health conditions. These diseases span a broad spectrum, including Metabolic disorders, Malignancy, Immunological disorders, Respiratory disorders, Neurological disorders, Infectious disorders, Auto immune disorders etc. The ability of Kshamatva Churna's bioactive compounds to influence such a wide range of pathways and diseases supports its traditional use in promoting overall health and resilience. This broad therapeutic potential makes Kshamatva Churna a valuable tool in both preventive and therapeutic healthcare settings.

3.6.5 Disease Classification

The Genetic Association Database (GAD) classifies diseases based on genetic associations and pathways, providing a robust framework to understand the genetic underpinnings of various conditions. In the context of Kshamatva Churna (KC), the identification of 5,855 gene targets influenced by 1,151 bioactive compounds allows us to map these interactions onto the GAD classification system. This mapping of 18 classes of Disease reveals significant insights into the potential therapeutic applications of KC.

By leveraging the GAD classification, we can categorize the 998 diseases influenced by KC's bioactive compounds into distinct groups. This classification helps in understanding the broader impact of these compounds on disease mechanisms and their potential roles in therapy. For instance, metabolic disorders, cardiovascular diseases, and neurological disorders emerge as primary categories where KC shows significant influence. The GAD system enables a detailed analysis of how these bioactive compounds interact with gene targets and pathways specific to these disease categories.

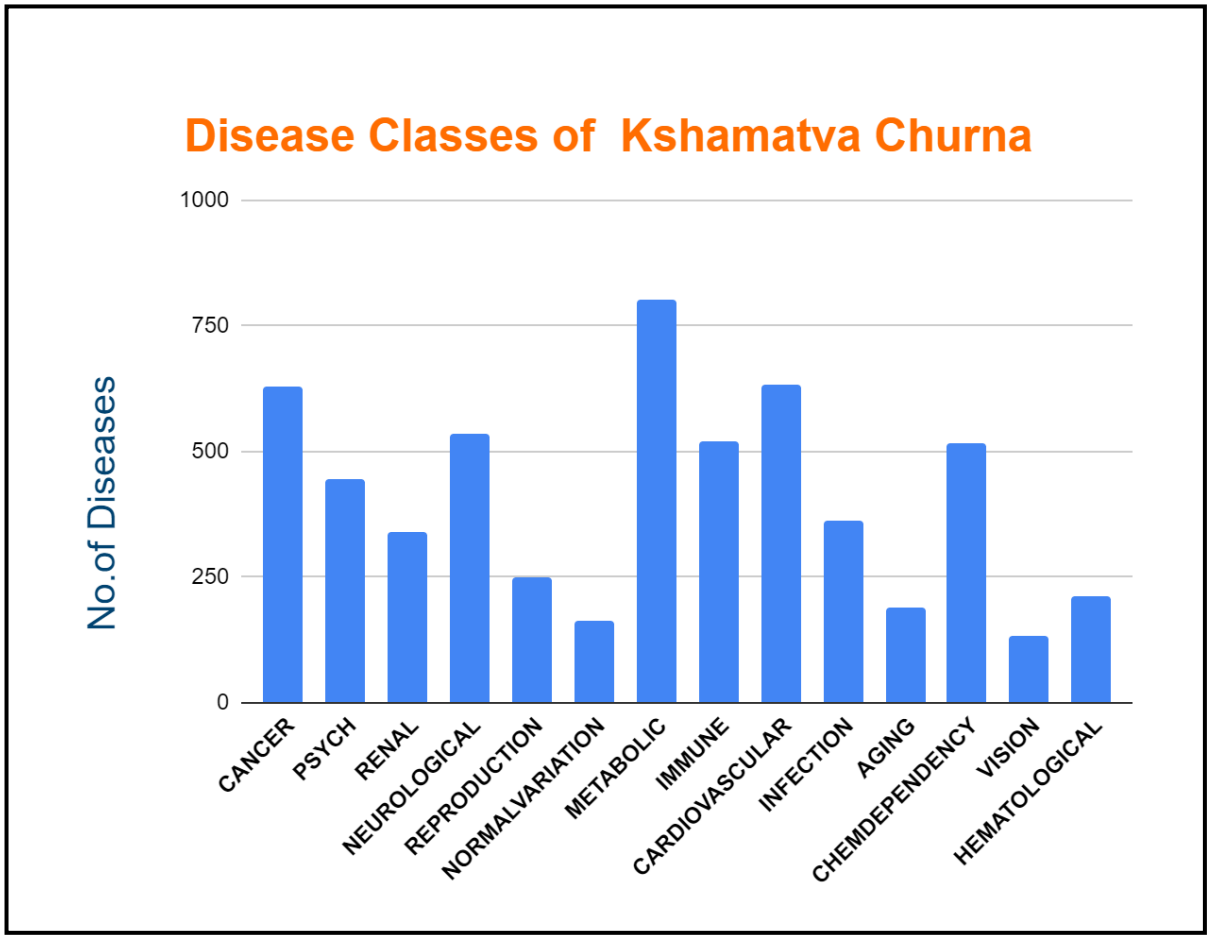

Fig 30: Disease Classes where in Kshamatva churna

The GAD classification system provides a comprehensive framework to understand the diverse therapeutic potential of Kshamatva Churna. By categorizing the affected diseases and elucidating the underlying genetic and pathway interactions, this analysis supports the broad-spectrum efficacy of KC in traditional medicine and modern therapeutic contexts.

3.6.6 Action of Kshamatva Churna in Upper respiratory Diseases

Kshamatva Churna (KC) is a traditional formulation containing various bioactive compounds that interact with a multitude of gene targets and pathways. By analyzing the data through the Disease Association and biological pathways, we can understand the broad therapeutic potential of KC, particularly in the context of respiratory disorders and other diseases.

The analysis reveals the following key metrics:

The compounds in KC are associated with 27 different upper respiratory disorders. These disorders are influenced through various pathways, indicating the extensive potential of KC in managing respiratory health. A total of 715 distinct biological pathways are impacted by the bioactive compounds in KC. This extensive pathway involvement highlights the broad-spectrum efficacy of KC, as these pathways are crucial for numerous physiological and pathological processes. The bioactive compounds in KC interact with 698 unique gene targets. This interaction suggests a complex network of gene modulation, which underpins the diverse therapeutic actions of KC. There are 267 unique phytochemicals in KC that act through these pathways. Each phytochemical's ability to influence multiple pathways and targets underscores the formulation's multifaceted therapeutic potential.

The 27 upper respiratory disorders affected by KC include conditions such as asthma, chronic obstructive pulmonary disease (COPD), bronchitis, and other respiratory tract diseases. The bioactive compounds in KC modulate gene targets and pathways involved in inflammation, immune response, and respiratory function. This modulation can lead to improved management and treatment of these disorders, supporting the traditional use of KC in respiratory health.

The 715 pathways impacted by KC's bioactive compounds include critical pathways involved in immune regulation, inflammation, metabolic processes, and cellular signaling. For example, pathways related to cytokine production and regulation are essential in managing inflammatory responses in respiratory diseases. The broad involvement of pathways indicates that KC can influence a wide range of biological functions, contributing to its therapeutic versatility.

The interaction with 698 gene targets suggests that KC compounds can modulate various genetic expressions, leading to diverse biological effects. These gene targets are involved in key physiological processes such as cell proliferation, apoptosis, immune response, and metabolic regulation. The ability to modulate these genes can help in treating a variety of diseases, from inflammatory conditions to metabolic disorders and cancer.

The 267 unique phytochemicals in KC demonstrate the formulation's rich chemical diversity. Each phytochemical's interaction with multiple pathways and gene targets highlights the synergistic effects of KC. This synergy is likely responsible for the enhanced therapeutic outcomes observed in traditional medicine practices.

The analysis using the GAD classification system provides a comprehensive understanding of Kshamatva Churna's therapeutic potential. By categorizing the affected diseases and elucidating the underlying genetic and pathway interactions, the results support the broad-spectrum efficacy of KC. The findings underscore its potential in treating and managing a wide range of diseases, particularly upper respiratory disorders, through its multifaceted biological actions. This comprehensive interaction network affirms the traditional use of Kshamatva Churna in promoting overall health.

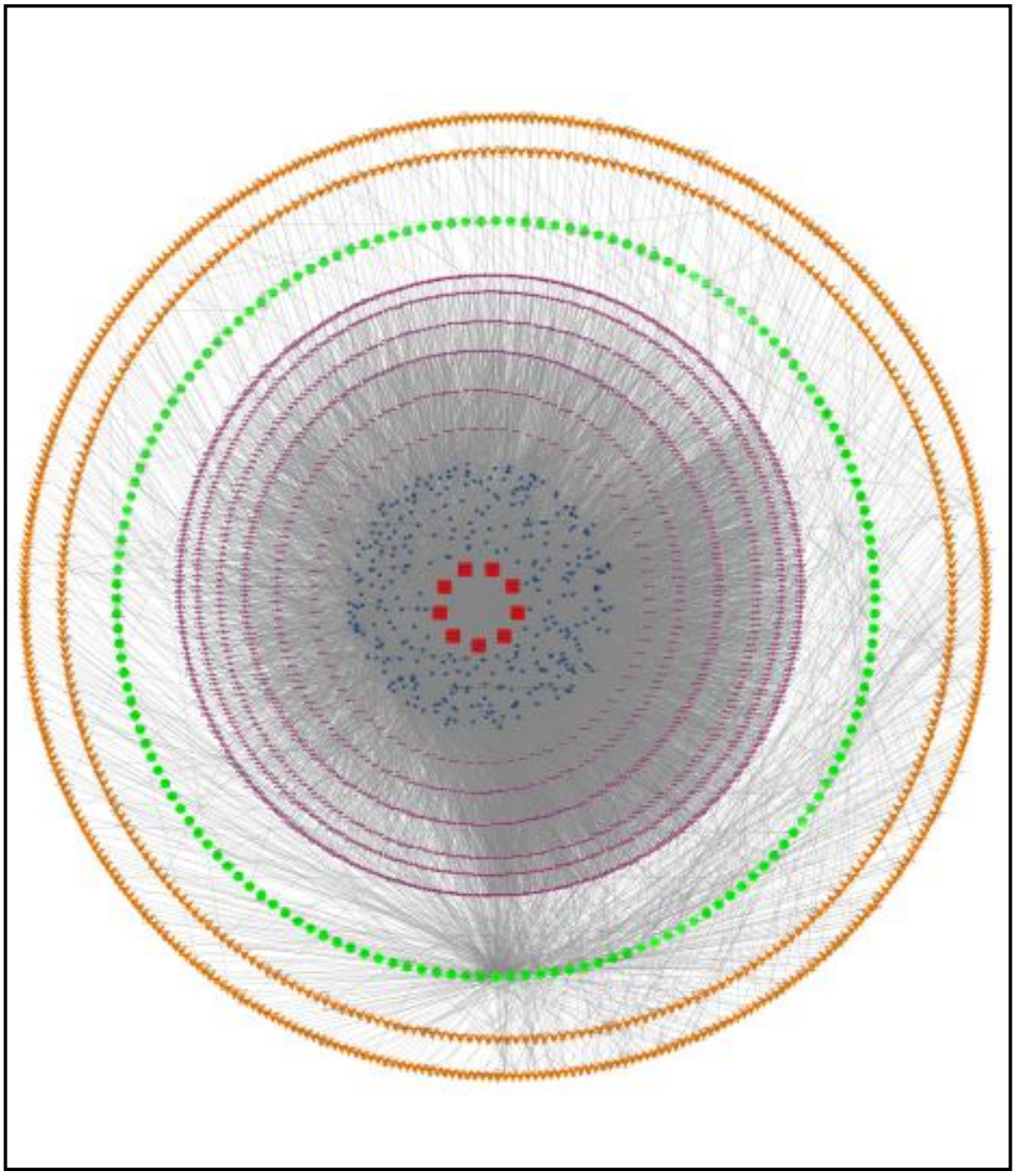

Fig 31 : Upper respiratory disease association ; Red represents 9 plants, Blue represents 267 Bio actives, Violet represents 698 Gene targets, Green represent 27 upper respiratory diseases, and Orange represents respective Path ways

3.6.8 Immunomodulatory Sub Network of Kshamatva Churna

The subnet work of the immune system influenced by Kshamatva Churna is illustrated in Figure 1. The bio actives of Kshamatva Churna interact with multiple targets and pathways that play crucial roles in modulating the immune system. Table 19 provides a detailed enumeration of the immune pathways, the number of gene targets, and the specific gene targets involved

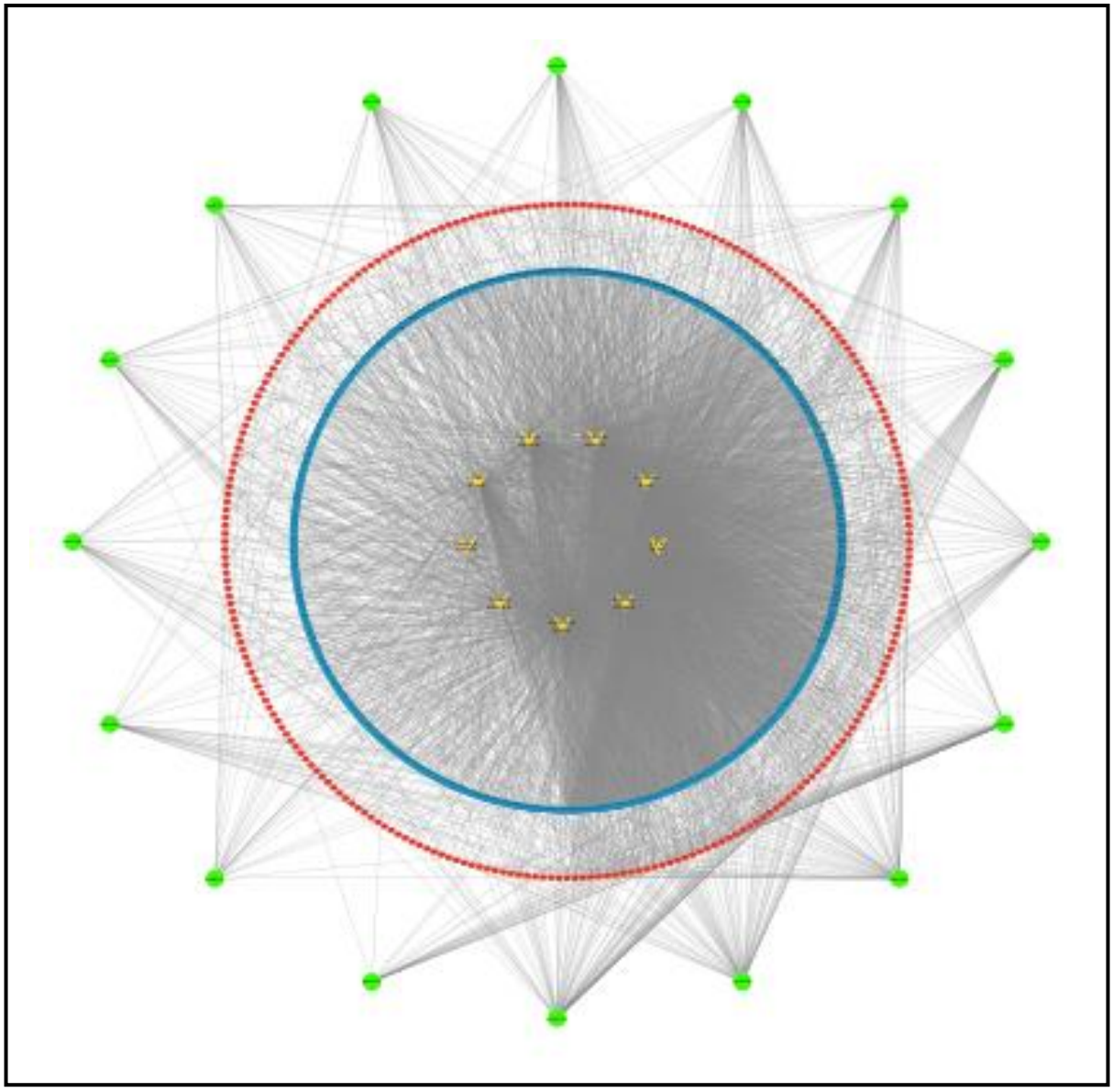

Fig 32*:* Sub- Network of Immune system: Yellow colour represents 9 Plants of KC, Blue represents 130 Bio actives, Red represents 266 Gene Targets and Green represents 15 immune pathways from KEGG pathway

Immune pathways - Gene targets

Table18: Immune pathways with respective Gene targets

Immune Pathway	ID	Target number	Gene Target
T cell receptor signaling pathway	hsa04660	52	GSK3B, ITK, PPP2R2A, PIK3CB, CD3E, TNF, PPP3CA, AKT2, AKT3, AKT1, MAP3K8, IKBKG, HRAS, MAP3K7, NCK1, MAP2K1, MAP2K2, CHUK, PDPK1, PPP2R5A, RHOA, ZAP70, PIK3CA, LCK, RAF1, PIK3R2, PIK3R1, PPP2CA, PPP2CB, MAPK9, PPP3R1, PAK1, MAPK8, MAPK1, FYN, PLCG1, MAP2K7, PAK2, PAK4, MAPK3, PTPN11, MAPK14, MAPK12, MAPK13, MAPK11, PTPRC, TEC, CDK4, KRAS, PTPN6, NFKBIE, LAT
Human T-cell leukemia virus 1 infection	hsa05166	73	RB1, CD40, ITGB2, PTEN, SLC2A1, PIK3CB, TNFRSF13C, CD3E, ITGAL, ELK1, TNF, POLB, CDC20, PPP3CA, CCND3, CCND1, PRKACG, CHEK2, AKT2, AKT3, CHEK1, AKT1, EP300, IKBKG, PRKACA, JAK3, PRKACB, HRAS, JAK1, MAP2K1, MMP7, MAP2K2, CHUK, IL1R1, HLA-A, TGFBR2, CCNA2, KAT2B, KAT2A, CREB1, ADCY9, PIK3CA, CCNE1, LCK, CANX, LTA, TP53, XIAP, ADCY3, PIK3R2, ADCY2, PIK3R1, ADCY1, ADCY8, ADCY5, MAPK9, PPP3R1, MAPK8, TERT, MAPK1, MAPK3, FDPS, CREBBP, CDKN2A, CDK4, IL2RA, CDK2, BAX, KRAS, ATM, HLA-DRB3, RAN, BCL2L1
Human immunodeficiency virus 1 infection	hsa05170	78	PIK3CB, CD3E, TNF, CASP9, PPP3CA, TBK1, CASP8, CASP3, AKT2, AKT3, CHEK1, AKT1, RAC3, IKBKG, CCR5, HRAS, MAP3K7, MAP2K3, MAP2K1, MAP2K2, CHUK, PRKCB, AP1B1, HLA-A, IRAK4, TNFRSF1B, CDC25C, PIK3CA, RAF1, TLR4, CRK, TLR2, GNAI3, CXCR4, PIK3R2, ITPR3, PIK3R1, GNAI1, MAPK9, PPP3R1, PAK1, MAPK8, CCNB1, IRAK1, GNA11, PLCG2, PTK2B, MAPK1, PLCG1, MAP2K7, BID, PAK2, PAK4, MAPK3, MAP2K6, BAD, MAPK14, MAPK12, MTOR, PTK2, MAPK13, GNAO1, MAPK11, WEE1, WEE2, RPS6KB1, GNAQ, RPS6KB2, BCL2, CDK1, BAX, KRAS, ATM, TAB1, CALM1, CUL4B, BCL2L1, APOBEC3B

Chemokine signaling pathway	hsa04062	65	GSK3B, ITK, PIK3CB, CX3CL1, PRKACG, AKT2, AKT3, AKT1, RAC3, IKBKG, CCR6, PRKACA, CCR5, JAK3, PRKACB, HRAS, CCR4, CCR2, MAP2K1, CHUK, PRKCB, RHOA, FGR, TIAM1, ADCY9, PIK3CA, RAF1, PLCB1, CRK, CX3CR1, SHC2, ROCK1, ROCK2, SRC, SHC1, GNAI3, CXCR4, ADCY3, PIK3R2, ADCY2, PIK3R1, ADCY1, FOXO3, ADCY8, GNAI1, ADCY5, GRK1, PAK1, GRK2, GRK5, CXCR1, GRK4, CXCR2, PLCG2, PTK2B, MAPK1, PLCG1, MAPK3, CCR1, BAD, STAT1, BRAF, PTK2, GNAQ, KRAS
Fc epsilon RI signaling pathway	hsa04664	37	PIK3R2, PIK3CB, PIK3R1, TNF, MAPK9, MAPK8, AKT2, ALOX5, AKT3, PLCG2, AKT1, MAPK1, FCER1A, RAC3, FYN, PLCG1, MAP2K7, HRAS, MAPK3, MAP2K6, MAP2K3, MAP2K1, MAP2K2, SYK, PDPK1, PLA2G4B, PLA2G4C, MAPK14, MAPK12, MAPK13, MAPK11, PIK3CA, ALOX5AP, BTK, KRAS, RAF1, LAT
Toll-like receptor signaling pathway	hsa04620	40	CD40, PIK3R2, PIK3CB, PIK3R1, TNF, MAPK9, MAPK8, CASP8, TBK1, IRAK1, AKT2, CTSK, AKT3, AKT1, MAPK1, MAP3K8, IKBKG, MAP2K7, MAP3K7, IKBKE, JAK1, MAPK3, MAP2K6, MAP2K3, MAP2K1, MAP2K2, CHUK, STAT1, IRAK4, MAPK14, MAPK12, MAPK13, MAPK11, PIK3CA, IL1B, TLR9, TAB1, TLR4, TLR2, IFNAR1
Natural killer cell mediated cytotoxicity	hsa04650	39	SHC2, SHC1, ITGB2, PRF1, PIK3R2, PIK3CB, PIK3R1, ITGAL, TNF, PPP3CA, PAK1, PPP3R1, FCGR3B, CASP3, PLCG2, PTK2B, MAPK1, RAC3, FYN, PLCG1, BID, HRAS, MAPK3, MAP2K1, MAP2K2, SYK, PRKCB, TNFRSF10B, GZMB, PTPN11, BRAF, ZAP70, PIK3CA, LCK, KRAS, PTPN6, RAF1, LAT, IFNAR1
IL-17 signaling pathway	hsa04657	31	GSK3B, HSP90AB1, PTGS2, TNF, MAPK9, MAPK8, CASP8, MAPK7, TBK1, CASP3, MAPK1, IKBKG, MAPK6, MAP3K7, IKBKE, MAPK4, MAPK3, CHUK, MMP1, MMP3, MAPK14, MMP9, MAPK12, MUC5AC, MAPK13, MAPK11, MMP13, IL1B, FOSB, TAB3, IL17F
Th17 cell differentiation	hsa04659	36	HSP90AB1, RORA, CD3E, HIF1A, RXRB, PPP3CA, MAPK9, PPP3R1, MAPK8, RXRA, MAPK1, IKBKG, PLCG1, JAK3, RXRG, JAK1, MAPK3, CHUK, IL1R1, STAT1, MAPK14, MAPK12, MTOR, TGFBR2, MAPK13, MAPK11, ZAP70, LCK, IL1B, IL2RA, RARA, IL17F, IL6ST, HLA-DRB3, NFKBIE, LAT
Th1 and Th2 cell differentiation	hsa04658	24	CHUK, STAT1, CD3E, MAPK14, MAPK12, MAPK13, DLL3, MAPK11, MAPK9, ZAP70, PPP3CA, PPP3R1, MAPK8, LCK, IL2RA, MAPK1, IKBKG, PLCG1, HLA-DRB3, NFKBIE, JAK3, LAT, JAK1, MAPK3

NOD-like receptor signaling pathway	hsa04621	45	HSP90AB1, XIAP, ITPR3, TNF, GABARAP, GPRC6A, MAPK9, MAPK8, CASP8, MAP1LC3A, TBK1, CASP4, NAMPT, CASP1, MFN2, MAPK1, IKBKG, MAP3K7, IKBKE, JAK1, MAPK3, CTSB, GABARAPL2, CASR, CHUK, STAT1, TRPV2, IRAK4, MAPK14, IRGM, RHOA, MAPK12, MAPK13, P2RX7, MAPK11, AIM2, IL1B, BCL2, TAB3, TAB1, PLCB1, TLR4, BIRC2, BCL2L1, IFNAR1
Complement and coagulation cascades	hsa04610	27	CFH, C1R, SERPINC1, C5AR1, ITGB2, F13A1, PLAT, PLG, C3, PLAU, C9, C3AR1, BDKRB1, CD55, FGB, F10, F2R, F11, PLAUR, F2, F3, F7, F9, PROC, KLKB1, F2RL2, F2RL3
Leukocyte trans endothelial migration	hsa04670	30	ITGB1, ITK, ROCK1, ROCK2, TXK, ITGB2, GNAI3, CXCR4, PIK3R2, PIK3CB, PIK3R1, ITGAL, GNAI1, PLCG2, PTK2B, PLCG1, VCAM1, ITGA4, PRKCB, MMP2, PTPN11, MAPK14, MMP9, RHOA, MAPK12, PTK2, MAPK13, MAPK11, PIK3CA, EZR
C-type lectin receptor signaling pathway	hsa04625	44	SRC, PIK3R2, ITPR3, PIK3CB, PIK3R1, PTGS2, TNF, PPP3CA, MAPK9, PAK1, PPP3R1, MAPK8, CASP8, AKT2, AKT3, CASP1, PLCG2, AKT1, MAPK1, IKBKG, HRAS, IKBKE, MAPK3, PLK3, ARHGEF12, SYK, CHUK, STAT1, RRAS2, PTPN11, MAPK14, RHOA, MAPK12, MAPK13, CYLD, MAPK11, PIK3CA, IL1B, BCL3, MAPKAPK2, MDM2, KRAS, RAF1, CALM1
Fc gamma R-mediated phagocytosis	hsa04666	31	PIK3R2, ASAP1, PIK3CB, PIK3R1, PLD1, PLD2, PAK1, FCGR3B, AKT2, AKT3, PLCG2, AKT1, MAPK1, PLCG1, MAPK3, MAP2K1, SYK, PRKCB, PRKCE, SPHK1, PLA2G4B, PLA2G4C, PTPRC, FCGR2A, PIK3CA, RPS6KB1, RPS6KB2, RAF1, CRK, LAT, ARF6
B cell receptor signaling pathway	hsa04662	28	BLK, GSK3B, CD81, PIK3R2, PIK3CB, PIK3R1, PPP3CA, PPP3R1, AKT2, AKT3, PLCG2, AKT1, MAPK1, RAC3, IKBKG, HRAS, MAPK3, MAP2K1, MAP2K2, SYK, CHUK, PRKCB, PIK3CA, BTK, KRAS, PTPN6, RAF1, NFKBIE

The analysis of the immune pathways impacted by Kshamatva Churna reveals significant interactions with key signaling pathways that regulate immune responses. Notably, the pathways with the highest number of gene targets include the Human immunodeficiency virus 1 infection, Human T-cell leukemia virus 1 infection, and Chemokine signaling pathway, which are critical in understanding the immune modulation effects of Kshamatva Churna.

Highly Interacted Pathways and Targets:

- **Human Immunodeficiency Virus 1 Infection (hsa05170)**: The highest interaction with 78 gene targets. Key genes include TLR 4, PIK3CB, CASP9, TBK1, CASP8, AKT2, and others.
- **Human T-cell Leukemia Virus 1 Infection (hsa05166)**: 73 gene targets with significant interactions including RB1, CD40, ITGB2, PTEN, SLC2A1, and others.
- **Chemokine Signaling Pathway (hsa04062)**: 65 gene targets, crucial for chemotaxis and immune cell signaling. CXCR4, CCR5, JAK2 are the Key targets.

These interactions suggest that Kshamatva Churna has broad-spectrum immunomodulatory effects, potentially beneficial in enhancing immune responses and modulating immune-related diseases. Future research should focus on validating these interactions through experimental studies and clinical trials to confirm the efficacy and therapeutic potential of Kshamatva Churna in immunomodulation.

These key gene targets play significant roles in various aspects of immune system regulation:

- **Immune Activation and Regulation:** Genes like CD40, TLR4,NFkB and MYD88 are central to activating immune cells and regulating their responses to pathogens.
- **Cell Survival and Apoptosis:** Genes such as CASP9, CASP8, and AKT2 influence cell survival, apoptosis, and immune cell turnover, critical for maintaining immune homeostasis.
- **Pathogen Recognition and Response:** Genes like TBK1, IFNAR1, and TRAF6 are essential for recognizing and responding to viral and bacterial infections, highlighting their importance in innate immunity.
- **Chemotaxis and Cell Migration:** Genes such as CXCR4, CCR5, and ITGB2 are involved in directing immune cells to infection sites, which is crucial for effective immune surveillance and response.

The interaction of Kshamatva Churna with these key gene targets suggests its potential to modulate various immune pathways, enhancing immune responses,

controlling inflammation, and providing broad-spectrum immunomodulatory effects. Future studies should further explore these interactions to validate the therapeutic potential of Kshamatva Churna in immune-related diseases.

3.7 Sensory Analysis

The sensory evaluation of various samples, including Palm Jaggery Candy, was conducted based on several attributes: colour, aroma/flavour, texture, taste, and overall acceptability. The evaluation used a scale from 1 to 5, where higher scores indicate better sensory quality. Below are the results for each sample:

Table 19: Sensory analysis grading of KC (n=10- No of Participants, Maximum score 50, Green box indicate most Acceptable and Red indicate Least Acceptable)

Sample	Colour	Aroma/ Flavour	Texture	Taste	Overall Acceptability
KC-KASHAYAM	32 ± 0.79	30 ± 1.25	37 ± 0.68	30 ± 0.82	25 ± 0.85
KC-SUGAR CANDY	20 ± 1.05	33 ± 1.34	26 ± 1.13	38 ± 0.63	38 ± 0.67
KC-PALM JAGGERY CANDY	30 ± 1.33	35 ± 0.10	39 ± 0.74	39 ± 0.82	37 ± 1.25
KC - CRYSTALS	28 ± 0.63	30 ± 0.82	32 ± 1.03	34 ± 0.84	32 ± 1.03
KC-DIP-TEA BAG	19 ± 0.57	37 ± 0.67	33 ± 0.67	31 ± 0.74	27 ± 0.97
KC_EASILY SOLUBLE POWDER	41 ± 0.57	41± 0.74	33 ± 1.35	36 ± 1.07	33 ± 1.25
KC-GRANULES	22 ± 0.79	36 ± 0.70	34 ± 1.07	36 ± 0.85	36 ± 0.84
KC-KASHAYAM EXTRACT POWDER	36 ± 0.70	24 ± 0.70	27 ± 1.06	25 ± 0.85	21 ± 1.20
KC-SYRUP	28 ± 0.79	37 ± 0.95	40 ± 1.25	42 ± 1.03	42 ± 1.32

The sensory evaluation results provide insights into the quality and consumer acceptability of the different samples. Here's a detailed of the findings:

Colour:

- Palm Jaggery Candy scored 30(60%), indicating a desirable colour, as it falls in the higher range. This suggests that the candy had an appealing visual appearance, which is crucial for consumer attraction.
- The highest score for colour was achieved by the Easily Soluble Powder (42-82%), indicating it had the most appealing colour among the samples.

Aroma/Flavour:

- Palm Jaggery Candy received a score of 35 (70%), which is quite high, indicating that its aroma and flavour were well-liked by the evaluators.
- The highest score in this category was given to the Easily Soluble Powder (41-82%), suggesting a very pleasant aroma and flavour profile.

Texture:

- Palm Jaggery Candy scored 39 (78%), the second-highest score in this category, indicating that it had an excellent texture, which is important for the overall mouthfeel and consumer satisfaction.
- The highest texture score was achieved by the KC Syrup (40-80%), suggesting it had the best texture among all samples.

Taste:

- Palm Jaggery Candy received a score of 39 (78%), indicating a highly favourable taste, which is critical for consumer acceptance.
- The highest score in taste was achieved by KC Syrup (42-84%), indicating the most preferred taste among the samples.

Acceptability:

- Palm Jaggery Candy scored 37 (74%) in overall acceptability, indicating that it was well-received by the evaluators.

- The highest overall acceptability score was given to the KC Syrup (42-84%), showing that it was the most liked product overall.

Palm Jaggery Candy performed exceptionally well across all sensory attributes, particularly in texture and taste, where it scored 39 (78%) in both categories. Its overall acceptability score of 37 (74%) suggests it is a highly favourable product among the samples tested. The high scores in aroma/flavour and texture indicate that the product is not only pleasing to the taste buds but also has a desirable mouthfeel and aroma.

Comparatively, KC Syrup received the highest scores in texture, taste, and overall acceptability, making it the most preferred product among the evaluated samples. The Easily Soluble Powder also performed well, particularly in colour and aroma/flavour.

In conclusion, KC Syrup is the most preferred product due to its outstanding sensory attributes and overall acceptability. Palm Jaggery Candy follows closely behind, being a highly acceptable product with excellent sensory attributes, particularly in texture and taste. While it is already well-received, slight improvements in colour and aroma could potentially enhance its overall acceptability even further. These findings suggest that both KC Syrup and Palm Jaggery Candy are favourable products for consumers, with potential for even greater acceptance with minor enhancements.

4.CONCLUSION

1. **Standardization**: The research underscores the critical need for standardizing Ayurvedic formulations. The rigorous standardization protocols developed ensure the consistent quality, safety, and efficacy of Kshamatva Churna across various nutraceutical forms, making it reliable for consumer use.
2. **Transformation to Nutraceutical Forms**: The successful conversion of Kshamatva Churna into palatable and convenient forms such as candies, crystals, dip tea bags, easily soluble powders, syrup etc enhances its acceptability and usability without compromising its therapeutic activity.
3. **Network Pharmacology**: Network pharmacology analysis revealed that Kshamatva Churna components interact with several key genes involved in immune regulation, such as NF-kB and TLR4, supporting its broad-spectrum immunomodulatory effects.
4. **Insight into Mode of Action**: The analysis of Kshamatva Churna (KC) reveals its complex mode of action through the interaction of 1,151 bioactive compounds with 5855 unique gene targets and 224 biological pathways. These interactions suggest that KC modulates various gene expressions, contributing to its broad-spectrum therapeutic potential.
5. **Significant Involvement in Respiratory Health**: The compounds in KC are associated with 27 different upper respiratory disorders, including asthma, COPD, and bronchitis. This indicates a significant involvement in respiratory health, supporting traditional claims of KC's efficacy in managing respiratory conditions.
6. **Enhancement of Overall Immunity**: KC impacts pathways related to immune regulation and inflammation, which are critical for overall immunity. The modulation of these pathways by KC compounds highlights its potential in enhancing immune responses and preventing a wide range of diseases.
7. **Comprehensive Overview of Therapeutic Actions**: The interaction of KC's bioactive compounds with multiple pathways and gene targets provides a comprehensive overview of its therapeutic actions. This rich chemical diversity and synergistic effects likely contribute to the enhanced therapeutic outcomes observed in traditional medicine practices.

8. **Public Health Impact**: The findings underscore the potential of Kshamatva Churna as a nutraceutical that promotes health and wellness, emphasizing the preventive aspect of healthcare, which aligns with the principle of using food as medicine.
9. **Global Acceptance of Ayurveda**: The standardization and successful transformation of Kshamatva Churna into various nutraceutical forms contribute significantly to the global acceptance and popularity of Ayurveda, offering natural and effective alternatives to medicine.
10. **Future Research scope**: The detailed mapping of bioactive compounds, gene targets, and biological pathways provides a valuable foundation for future experimental studies and clinical trials to further validate and expand on the therapeutic potential of Kshamatva Churna.

This comprehensive approach not only validates the traditional use of Kshamatva Churna but also establishes it as a standardized, effective, and commercially viable nutraceutical product for integration into modern healthcare practices.

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1.6 Appendix

SOP-HPLC – KASHAMATWA CHURNA

Equipment and Reagents

- HPLC system equipped with a UV detector
- Column: SHISEIDO SOLAR, C-18, 4.6 mm x 250 mm
- Mobile Phase:
- Solvent A: HPLC grade water
- Solvent B: Acetonitrile(100 %)
- Piperine Standard: 1 mg/mL solution prepared in methanol
- Test Solution: Extract powder of Kshamatwa Churna (1 mg/mL solution prepared in methanol)
- Injection syringe
- Syringe filters: 0.2 µm
- Methanol: HPLC grade
- HPLC grade water and Acetonitrile

Preparation of Solutions

Standard Solution Preparation:

- Accurately weigh 1 mg of piperine powder.
- Dissolve the weighed powder in 1 mL of methanol to make a 1 mg/mL solution.
- Mix thoroughly until the solution is clear.
- Filter the solution through a 0.2 µm syringe filter to remove any particulate matter, ensuring it is suitable for HPLC analysis.

Test Solution Preparation:

- Accurately weigh 10 mg of Kshamatwa Churna and do methanolic extraction by soxlet extraction method.
- Keep the Extract for concentrating purpose and reconstitute it with HPLC grade methanol to make a 1 mg/mL solution.
- Mix thoroughly until the solution is clear.
- Filter the solution through a 0.2 µm syringe filter to remove any particulate matter.

HPLC Analysis Conditions

- Column: SHISEIDO SOLAR, C-18 , 4.6 mm x 250 mm

Mobile Phase:

- Use a simple isocratic mobile phase of 50% HPLC grade water (A) and 50% Acetonitrile (B).
- Flow Rate: 1 mL/min
- Detection: UV detection at 254 nm
- Injection Volume: 20 µL
- Column Temperature: Ambient or controlled at 30°C, if temperature control is available on the HPLC system.

Procedure

System Equilibration:

- Equilibrate the HPLC system with the isocratic mobile phase (50% water, 50% acetonitrile) for at least 30 minutes prior to the first injection to ensure a stable baseline.

Sample Injection:

- Inject 20 µL of the filtered piperine standard solution and the filtered Kshamatwa Churna extract solution into the HPLC system separately.
- Record the chromatograms to analyze the peaks corresponding to piperine in both solutions. Note the retention time, peak shape, and any other notable features.

Data Analysis:

- Compare the chromatograms of the standard and test solutions to determine the presence and quantity of piperine in the test solution.
- Note any differences in retention time or peak shape between the standard and test solutions.

Documentation and Reporting

- Record all conditions, including mobile phase preparation, injection volumes, and any adjustments made during the analysis.
- Ensure that all data, including chromatograms and Graphs, Tables are saved and appropriately documented for review or regulatory compliance.

Reference

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Progress Report 1

Project Title

Standardization of Nutraceutical forms as Kshamatva churna.

Objectives

- Manufacturing of Kshamatva churna (Formulation developed by I-AIM as Immunomodulator) in different Nutraceutical forms like Candy, Dip Tea bag, Chewable tablet, Crystalized Form etc
- Make the extract of Kshamatva churna by Spray drying method.
- Standardization, Sensory analysis and Quality analysis of every Single Ingredients of Kshamatva churna and Final products.
- Clinical validation and effectiveness of Kshamatva Churna Nutraceutical formulations (as Dip Tea , Candy and Chewable Tablets)

Progress made

- Study about the Standardization Data (HPLC, GC-MS) of the original Kshamatva churna which is already available . Read the research papers on chromatography , sensory analysis , quality control protocols etc.
- Do Soxhlet extraction of Kshamatva churna in different polarities for Phytochemical studies
- Done preliminary chemical test for phytochemical analysis of kshamatva churna in both sox let extraction and water extraction
- TLC of kshamatva churna is done in different solvent system
- Start making and standardization of Kshamatva churna candy, Kshamatva churna Dip tea in different concentrations.
- Read some protocols for clinical study and roughly plan the method of study with Dr.Subrahmanya kumar.

Skill Learned

- Selection of authentic drug
- Standardization of single drug / Formulation and Standardization of does
- Idea to formulate different Nutraceutical forms

- Improved communication skill
- Learn more about Pharmaceutical side like purchase, manufacturing, standardization , funding , packing, clinical trial procedures etc.
- Learn about pharmacological studies
- Learn about Phytochemistry technique TLC, HPTLC, HTLC

Challenges

- Making more and more sample products to get the final product and for standardization of products.
- Taking little more time to understand chemistry part at initial later become easier.
- To find chemicals, Handling chemicals, Machineries make some confusions at starting.
- Sometimes extractions may take more time than expected. It makes me some difficulties for time management

Thesis Progress Report-2

Project Title

Standardization of Nutraceutical forms as Kshamatva churna.

Objectives

- Manufacturing of Kshamatva churna (Formulation developed by I-AIM as Immunomodulator) in different Nutraceutical forms like Candy, Dip Tea bag, Chewable tablet, Crystalized Form etc
- Chemical test for Phytochemical analysis of Kshamatva churna (TLC, HPTLC, HPLC, GC-MS)
- Standardization, Sensory analysis and Quality analysis of every Single Ingredients of Kshamatva churna and Final products.
- Clinical validation and effectiveness of Kshamatva Churna Nutraceutical formulations (as Dip Tea , Candy and Chewable Tablets)

Progress made

- Study about the Standardization Data (HPLC, GC-MS) of the original Kshamatva churna which is already available . Read the research papers on chromatography , sensory analysis , quality control protocols etc.

- Do Soxhlet extraction of Kshamatva churna in different polarities for Phytochemical studies
- Done preliminary chemical test for phytochemical analysis of kshamatva churna in both sox let extraction and water extraction
- TLC of kshamatva churna is done in different solvent system
- Start making and standardization of Kshamatva churna candy, Kshamatva churna Dip tea in different concentrations.
- Read some protocols for clinical study and roughly plan the method of study with Dr.Subrahmanya kumar.

Skill Learned

- Selection of authentic drug
- Standardization of single drug / Formulation and Standardization of does
- Idea to formulate different Nutraceutical forms
- Improved communication skill
- Learn more about Pharmaceutical side like purchase, manufacturing, standardization , funding , packing, clinical trial procedures etc.
- Learn about pharmacological studies
- Learn about Phytochemistry technique TLC, HPTLC, HTLC

Challenges

- Making more and more sample products to get the final product and for standardization of products.
- Taking little more time to understand chemistry part at initial later become easier.
- To find chemicals, Handling chemicals, Machineries make some confusions at starting.
- Sometimes extractions may take more time than expected. It makes me some difficulties for time management

Thesis Progress Report -3

State the objectives of your project

- Standardization of Kshamatwa Churna (An immunomodulatory formulation developed by I-AIM) through literature review , Phytochemistry, Network pharmacology and Cell biology
- Standardization of different nutraceutical forms of Kshamatwa churna

Elaborate on the progress made in the objectives.

- For Literature review – Collect all information about the ingredients (both Ayurveda and modern) from Samhitas and research papers .Chemical compounds are listed out using different data base and Pubmed. Later forms the Clams on Kshamatva churna as hypothesis
- For Phytochemistry- Successive extraction done , Preliminary analysis, TLC, HPTLC, Physio chemical analysis are completed . HPLC , GC-MS on progression stage.
- For Network Pharmacology – Found the phytochemicals and protein interactions from data bases. We are looking for Disease association , Action on immunological pathway and upper respiratory involvement. Also Planning to do ADMET profiling study.
- For Cell biology- Not yet started, reading part is done and decided to brought Upper respiratory cells and Do RTPCR to detect how Kshamatva churna works on Upper respiratory conditions.
- For nutraceutical forms – Manufacturing of different forms are done, try to make that into finalized form and prepare for doing organoleptic study

Skills learned

- Handling of HPTLC , HPLC, GC-MS
- Idea to do computerized Drug discovery
- Analyse network pharmacology Data from different Data base
- Making of different types of Nutraceuticals using Kahamatwa churna
- Managing different work simultaneously

Challenges that you have faced while doing your work.

- Take more time than expected
- Different data and duplicate data from different source
- Delay in getting samples and standards for procedures