

Formulation and Evaluation of Polyherbal for Reducing the Viral Load of Hepatoprotective Activity

Tarun Thakur^{1*}, Dr. Abhishek Soni¹, Dr. Chinu Gautam¹, Mrs. Akanksha Sharma¹, Mr. Vineet Kapoor¹, Aman Katoch²

Student, Corresponding Author

M. Pharm, PhD in Pharmaceutics, Dean of Pharmacy

M. Pharm, PhD in Pharmacology, HOD of Pharm

M. Pharm, Pharmacognosy, Assistant Professor

M. Pharm Pharmaceutics, Assistant Professor

Student, 2nd Author

School of Pharmacy, Abhilashi University, Mandi, HP, India

tarunthakur6721@gmail.com, abhisoni.phd@gmail.com, chinu990@gmail.com

akshusharma026@gmail.com, vineetkapoor47@gmail.com, amankatoch1709@gmail.com

Abstract: *The aim of this current study is to formulate and evaluate polyherbal syrup comprising Black Cummin, Garlic, Kutki, and Turmeric for its potential hepatoprotective activity. The medicinal plants selected for preparation exhibit hepatoprotective, antioxidant, and anti-inflammatory activity. The powdered plant material was extracted with methanol by the decoction method. The syrup is finalized by adding fructose, Citric acid, Sodium Benzoate, and Honey. The phytochemical study of the polyherbal syrup reveals the presence of alkaloids, flavonoids, glycosides, terpenoids, and tannins. The post-formulation studies of formulated syrup include pH, density, viscosity, specific gravity, color, odor, and appearance. The result of the formulation provides a satisfactory result with density which is 1.27g/ml, viscosity 2.62cP, and pH, which was found to be 6.7 or organoleptic property was also acceptable. The prepared herbal formulation also supports liver health. Further, pharmacological and clinical investigations are necessary to determine its hepatoprotective activity.*

Keywords: Polyherbal Syrup, Hepatoprotective Activity, Nigella sativa, Curcuma longa, Phytochemical Screening, Herbal Formulation, Liver Health

I. INTRODUCTION

Human beings require housing, clothes, food, scents, and, last but not least, medications to survive. Plants are a common source of medication. Ayurvedic, Unani, and Chinese traditional medicine architectures are among the many that have been influenced by plants. Plants were considered to have a divine and supernatural healing ability and served as the cornerstone of care systems in Indian, Egyptian, Chinese, Roman, and Greek civilizations. [1,2]

The Edwin Smith Papyrus, an ancient Egyptian medical document about wound healing, dates back to 1700 BC [3] Herbs have been utilized as natural treatments for a variety of physiological conditions since ancient times. They were valued in traditional medical literature as nature's gift to humanity for healing of diseases. [4] Traditional herbal medicine refers to the use of plants or plant material, either in its raw or processed form, to treat illness or injury. The therapeutic potential of the medicinal plants with ethnomedicinal properties is presently being investigated. [5] The social and natural sciences are both included in the Ethnopharmacological approach to the comprehension and evaluation of traditional and herbal medicines. Ethnopharmacological investigations are based on anthropological field



observations detailing the regional use of medicines obtained from nature. [6] Research on polyherbal formulation revealed that certain individual plants have high levels of phenolic, flavonoids, and their polyherbal combination. Out of all the separate extracts, green tea was shown to produce the highest antioxidant activity. [7] Because of their synergism and less adverse effects, polyherbal herb combinations are preferable to single herbs in the majority of traditional approaches for managing diabetes. [8] Similar to the conventional silver sulphadiazine cream, diabetic foot ulcers were reported to be effectively and safely healed by diabetic wound cream made with a polyherbal formulation. [9] Carrageenan-induced carrageenan was used to test the anti-inflammatory properties of the polyherbal formulation Entox, which contains *Allium cepa*,

Allium sativum, *Aloe vera*, *Cajanus cajan*, *Coccinia indica*, *Caesalpinia bonducella*, *Ficus bengalensis*, *Gymnema sylvestris*, *Mordica charantia*, *Ocimum sanctum*, *Pterocarpus marsupium*, *Swertia chirayita*, *Syzigium cumini*, *Tinospora cordifolia*, and *Trigonella foenumgraecum*. Using carrageenan-induced rat paw edema and cotton pellet granuloma techniques, respectively, *Momordica charantia*, *Ocimum sanctum*,

Pterocarpus marsupium, *Swertia chirayita*, *Syzigium cumini*, *Tinospora cordifolia*, and *Trigonella foenum graecum* were studied in rats for acute and sub-acute models of inflammation at oral doses of 300 mg/kg and 600 mg/kg. In all experimental models, the formulation demonstrated a significant anti-inflammatory effect, and its efficacy was on par with that of the common medication, indomethacin. [10] By inhibiting cyclooxygenase-2 and lipoxygenase-5, BHUx, a proprietary polyherbal formulation made of the aqueous fraction of five medicinal herbs from the ayurveda system, has notable anti-inflammatory qualities. [11]

Boswellia serrate, *Commiphora*, *mukul*, the leaves of *Cissus quadrangularis*, *Vitex negundo*, *Centella Asiatica* *Tinospora cordifolia*, as well as *Curcuma longa* and *Euphorbia hirta*.

Black Cumin:

The annual flowering plant *Nigella sativa*, also known as black caraway, black cumin, nigella, or kalonji, is indigenous to eastern Europe (Bulgaria and Romania) and the western part of Asia, Saudi Arabia, the Levant, Cyprus, Iran, Turkey, and Iraq). It has spread throughout the northern part of Africa, portions of Europe, and even as far east as Myanmar. In many different food preparations, particularly in Arab and Halal cuisine, it is utilized as a spice. [12, 13]



Figure 1: *Black Cumin*.

Chemical composition:

Millions of people utilize the oil from *N. sativa* seeds as a natural treatment for healing and protection in the waters of Indian subcontinent. The chemical makeup of the seeds is extremely varied and rich. Proteins, carbohydrates, fixed plus volatile oils, and amino acids are also present. Quinone components in the seed have been implicated in a number of the pharmacological actions listed above. Chopra et al. discovered thymoquinone [14]

Uses:

It is used as anti-cancer, antimicrobial, anti-inflammatory, anti-oxidant and hepatoprotective effect analgesic properties. [15]

Turmeric:

Curcuma longa is a leafy, rising perennial herb that grows up to 1 m tall with a short stem. It has yellow flowers in the shape of a funnel and oblong, pointed leaves. It is a member of the Zingiberaceae family. It grows widely in Asiatic nations, mainly China and India, and is found in tropical and subtropical areas of the world. In India, "haldi" is a traditional plant has rectangular, ovate, pyriform, and frequently short-branched rhizomes [16]





Figure 2: *Turmeric.*

Chemical Composition:

Turmeric has the following list of components: protein (6.3%), fat (5.1%), minerals (3.5%), carbs (69.4%), and moisture (13.1%). The essential oil (5.8%) is made from steam-distilled rhizomes and has C-phellandrene (1%), sabinene (0.6%), cineol (1%), boneol (0.5%), zingiberene (25%) and sesquiterpenes (53%). Curcumin (diferuloy methane) gives the yellow hue. It is a mix of curcumin I (94%), curcumin II (6%), and curcumin III (0.3%) °. Also, the deme-thoxy and bisdemethoxy derivatives of curcumin have been isolated. Curcumin® was first identified in 1815, and its chemical structure was identified by Roughley and Whiting® in 1973. At 176–177°C, it fuses, reacts with alkali producing a reddish-brown salt, and mixes with acetic acid, ethanol, alkali, ketone, and chloroform. (17).

Uses:

It is used in liver disorder, cancer, bacterial infection, wound healing and eye disorders.(18)

Garlic:

Garlic is a perennial flowering plant that is native to Central Asia, South Asia and north eastern Iran. It grows from a bulb, with a tall, erect flowering stem that reaches up to 1 m (3 ft). The leaf blade is flat, linear, solid, and approximately 1.25–2.5 cm (0.5–1.0 in) wide, with an acute apex. The plant may produce pink to purple flowers from July to September in the Northern Hemisphere. It produces hermaphroditic flowers. It is pollinated by butterflies, moths, and other insects. (19)



Figure 3: *Garlic.*

Chemical Composition:

Bulbs of *A. sativum* are known to contain hundreds of phytochemicals, including sulfurcontaining compounds (such as ajoenes (E-ajoene, Z-ajoene), thiosulfates (allicin), vinyldithiins (2-vinyl-(4H)-1,3-dithiin, 3-vinyl-(4H)-1,2-dithiin), sulfides (diallyl disulfide (DADS), diallyl trisulfide (DATS)), and others that cosmetics 82% of the total sulfur content in garlic . After garlic is cut and the parenchyma is destroyed, the allinase enzyme turns alliin, the main cysteine sulfoxide, into allicin S-propyl-cysteine-sulfoxide (PCSO), allicin, and Smethyl cysteine-sulfoxide (MCSO) are the main odor-causing molecules found in freshly milled garlic homogenates [20]

Uses:

It is majorly used for anticancer, immunity booster, antihypertensive, anti-oxidant and cardiac health. (21)



Kutki:

Picrorhiza kurroa Royle ex Benth is one of the many notable medicinal plants found around the world that play a major role in traditional medicine systems (figure 1). It belongs to the Scrophulariaceae family and is referred to as Kutki in several languages, including Hindi. The plant is indigenous to the western Himalayas. The Greek word "picross," which means bitter, and the word "rhiza," which means roots, make up the phrase Picrorhiza. The plant's Punjabi name, "Karu," conveys the same meaning as "rhiza."(22)



Figure 4: *Kutki.*

Chemical constituents:

Picrorhiza kurroa has been reported to contain over 50 secondary metabolites, including iridoid glycosides, cucurbitacins, and phenolic compounds. The dried rhizome of Picrorhiza kurroa comprises at least 60% of a blend of Picoside-I and Kutkoside, with the remaining 40% consisting of iridoid and cucurbitacin glycosides [23].

Uses:

It is used as hepatoprotective, anti-cancer, anti-oxidant, anti-inflammatory and anti-asthematic. (24)

Primary Studies on Hepatitis:

A lot viruses affect liver function. However, only a few of those are genuinely infectious to the liver, resulting in clinically is Ignificant hepatitis. Diseases caused by this subgroup are described as viral hepatitis. Five human viruses have been understood, which include hepatitis A (HAV), B (HBV), C (HCV), D (HDV), and E (HEV). All types of viral hepatitis exhibit similar pathology, leading to an acute inflammation of the whole liver.

Hepatitis A: It is the main able to acute hepatitis internationally, accountable for 20-25% of clinical hepatitis cases in developed countries. HAV expands through the fecal-oral route. Its incubation period lasts 2-7 weeks.

Hepatitis B: In the USA and Britain, hepatitis B (HBV) prevalence is low, with around 0.1-0.2% of the population exhibiting markers suggesting they are chronic carriers. The incubation period for acute HBV infection is 3 to 6 months.

Hepatitis C: This virus circulates via blood and is other infectious than HIV. Almost 3% of the general population is infected with HCV, with over 150 million carriers globally. The transmission of HCV occurs via parenteral routes.

Hepatitis D: It can only cause an infection in patients who are simultaneously infected with HBV. It can cause acute and chronic hepatitis.

Hepatitis E: It is found in regions with inadequate sanitation, where it extends enterically and causes acute hepatitis. Its incubation period lasts for 40 days. [25]

Material and Method:

Material: black cumin, garlic, turmeric, kutki, water, fructose, sodium benzoate honey.

Apparatus: Beaker, conical flask, china dish, motar pastel, filter paper.

Instrument: water bath, heating mantle.



Methodology:

Sr. No.	Drugs	Quantities.
1.	Black Cumin.	0.38gm.
2.	Turmeric.	0.18gm.
3.	Garlic	0.74gm
4.	Kutki.	0.44gm
5.	Sodium Benzoate	0.1gm
6.	Citric Acid.	0.1gm
7.	Honey.	5ml
8.	Fructose.	66gm

Table 1: Quantities used in formulation.

Herbal drugs are used in the formulation of herbal syrup:

Preparation of fine powder from plants:

All plants part is firstly crushed with grinder and also used with pestle and mortar to prepare fine powder and sieve through sieve no 48, 80, 120 and stored in air tight closed container. To prevent bacterial growth Powder material will pass through under UV light.

Prepare extract through Decoction method:

In this process, the crude drug is boiled in a specified volume of methanol for a defined time; it is then cooled and strained or filtered. 10gm plant part taken in 50ml round bottom flask with 50ml methanol. And boiled them for up to 30min. in gentle heating 60degree. During boiling flask continuously rotating for circulation of heat after 30min. material were filtered with filter paper and collected filtrate.



5: Decoction Method.

Preparation of simple syrup:

Simple syrup is typically made by dissolving 66gm of fructose in 100ml water. Heating accelerates dissolution and can improve microbiological stability, but excessive heat may affect colour and flavour of the sample.



PHYTOCHEMICAL SCREENING:

PLANTS	<i>TURMERIC</i>	<i>BLACKSEED</i>	<i>GARLIC</i>	<i>KUTKI</i>
ALKALOIDS	Absent	Present	absent	Absent
FLAVONOIDS	Present	Present	Present	SlightlyPresent
GLYCOSIDES	Minor Present	Present	Present	Present
PHENOLIC COMPOUND	Present	Present	Present	Present
TERPENOIDS	Present	Present	Present	Present
TANNINS	Small amount is Present	Present	Absent	Present

Table 2: *Phytochemical Screening.*

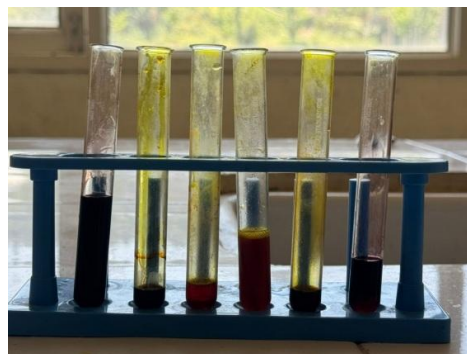


Figure 6: *Phytochemical Studies.*

Syrup Preparation:

The polyherbal extract was taken and add with simple syrup and mixed together. Then add 0.1 gm. sodium benzoate and citric acid uses as preservatives. Add 5ml of honey as a flavouring agent, mixed all ingredient together well and store into tight close container.

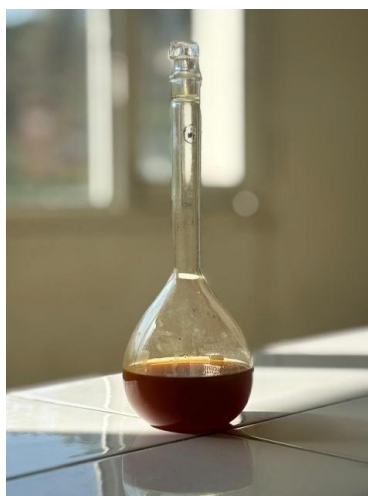


Figure 7: *Prepared Syrup*

Evaluation Parameter:

Density:

Clean the specific gravity bottle thoroughly with warm water, then rinse it 2–3times with distilled water. If necessary, rinse with acetone and dry completely. Weigh the empty dry bottle with its stopper (w1). Fill the bottle with the



unknown liquid, insert the stopper, and wipe off any excess. Weigh the filled bottle (w_2). Determine the weight of the liquid by subtracting w_1 from w_2 : $w_3 = w_2 - w_1$.

Specific gravity:

Clean the specific gravity bottle thoroughly using water, then rinse it two to three times with purified water. If necessary, rinse with acetone and dry. Weigh the empty dry bottle with stopper (w_1). Fill the bottle with distilled water, insert the stopper, wipe excess liquid, and weigh (w_2). Replace water with the test liquid after drying, repeat the procedure, and weigh again (w_3). Use the recorded weights to calculate the density of the liquid.

Viscosity:

Clean the Ostwald viscometer thoroughly with warm water, and if needed, use acetone. Mount it vertically on a stand. Fill the dry viscometer with water up to mark G. Measure the time taken for water to flow from mark A to B, repeating thrice for accuracy. Rinse the viscometer with the test liquid, fill up to mark A, and record the time to reach mark B. Determine the densities of water and test liquid as previously described.

pH:

Ensure that the pH paper and hands are clean and dry to avoid contamination and keep the colour chart ready for immediate comparison. Take a strip of pH paper and dip into the test solution but do not dip the entire strip. Then immediately remove the strip from the solution and wait for the colour stabilise and compare the colour with colour chart provided with the pH paper. (26)

Result of Evaluation parameter:

Parameter	Result
Density	1.27g/ml
Specific gravity	0.89g/ml
Viscosity	2.62cP
pH	6.7
Colour	Reddish brown
Odour	Aromatic
Taste	Sweet
Appearance	Slightly Turbid

Table 8: Result of Evaluation parameter.

II. CONCLUSION

In the present study, we have successfully formulated and evaluated a polyherbal syrup that includes black cumin, garlic, turmeric, and Kutki for its hepatoprotective activity. Phytochemical screening has shown important bioactive constituents like flavonoids, alkaloids, glycosides, tannins, and terpenoids. The formulated syrup gives satisfactory results for physicochemical and organoleptic properties. Polyherbal syrup supports liver health and is beneficial for liver health management. But further pharmacological and clinical studies are required for validation of its hepatoprotective activity.

Ethics:

This study was a primary analysis based on the currently existing data and involves human presence was just for the preparation of the syrup. Therefore, the ethics approval was not required in this paper.



ACKNOWLEDGMENT

We sincerely acknowledge the support, guidance and encouragement provided by the academic staff from our respective institutions. Their dedication to fostering a productive research environment and facilitating access to essential resources has been invaluable in the completion of this manuscript.

Conflict of interest:

The author declares that there is no conflict of interest.

Funding:

This research received no specific grant from any funding agency in the public, commercial or not for profit sectors.

Data Access:

The data that supports the finding of this study are available from the corresponding author upon reasonable individual request.

REFERENCES

- [1]. Teschke R, Wolff A, Frenzel C, Schulze J. Review article: Herbal hepatotoxicity – Ad update on traditional Chinese medicine preparations. *Aliment. Pharmacol. Ther.*
- [2]. Basnyat S, Kolasinski SL. Ayurvedic medicine for rheumatoid arthritis. *Curr. Rheumatol. Rep.* 2014;16(8)
- [3]. Tuhin Kanti Biswas SP and SC. Test on Indian and Peruvian medicinal plants for wound healing. In: Mukherjee PH and PK, editor. *Evaluation of Herbal Medicinal Products*. Pharmaceutical Press. 2009. page 228–41.
- [4]. Hussain SA, Panjagari NR, Singh RRB, Patil GR. Potential herbs and herbal nutraceuticals: food applications and their interactions with food components. *Crit Rev Food Sci Nutr.* 2015;55(1):94–122.
- [5]. Kunwar RM, Shrestha KP, Bussmann RW. Traditional herbal medicine in far-west Nepal: a pharmacological appraisal. *J Ethnobiol Ethnomed.* Leonti M, Casu L. Traditional medicines and globalization: Current and future perspectives in ethnopharmacology. *Front Pharmacol.* 2013;4 JUL.2010; 6:35.
- [6]. Leonti M, Casu L. Traditional medicines and globalization: Current and future perspectives in ethnopharmacology. *Front Pharmacol.* 2013;4 JUL.
- [7]. Ngo LT, Okogun JI, Folk WR. 21st century natural product research and drug development and traditional medicines. *Nat Prod Rep.* 2014;30(4):584–92.
- [8]. Che CT, Wang ZJ, Chow MSS, Lam CWK. Herb-herb combination for therapeutic enhancement and advancement: Theory, practice and future perspectives. *Molecules.* 2013;18(5):5125–41.
- [9]. Risberg K, Fodstad Ø, Andersson Y. Synergistic anticancer effects of the 9.2.27PE immunotoxin and ABT-737 in melanoma. *PLoS One.* 2011;6(9).
- [10]. Viswanathan V, Kesavan R, Kavitha K V., Kumpatla S. A pilot study on the effects of a polyherbal formulation cream on diabetic foot ulcers. *Indian J Med Res.* 2011;134(8):168-73.
- [11]. Tripathi YB, Singh BK, Pandey RS, Kumar M. BHUx: A patent polyherbal formulation to prevent atherosclerosis. *Evidence-based Complement Altern Med.* 2005
- [12]. "Nigella sativa" [. Germplasm Resources Information Network. Agricultural Research Se United States Department of Agriculture. Retr11 December 2017
- [13]. "Nigella sativa" [. Germplasm Resources Information Network. Agricultural Research Se United States Department of Agriculture. Retr11 December 2017
- [14]. Hala Gali-Muhtasib, Nahed El-Najjar Regine Schneider-Stock Available online 27 September 2007.[14] The medicinal potential of black seed (Nigella sativa) and its components.
- [15]. Srinivasan K. Cumin (Cuminum cyminum) and black cumin (Nigella sativa) seeds: traditional uses, chemical constituents, and nutraceutical effects. *Food quality and safety.* 2018 Mar;2(1):1-6.
- [16]. Chanda, S., & Ramachandra, T. V. (2019). Phytochemical and pharmacological importance of turmeric (Curcuma longa): A review. *Research & Reviews: A Journal of Pharmacology*, 9(1), 16-23.



- [17]. Verma, R. K., Kumari, P., Maurya, R. K., Kumar, V., Verma, R. B., & Singh, R. K. (2018). Medicinal properties of turmeric (*Curcuma longa* L.): A review. *International Journal of Chemical Studies*, 6(4), 1354-1357.
- [18]. Labban L. Medicinal and pharmacological properties of Turmeric (*Curcuma longa*): A review. *Int J Pharm Biomed Sci*. 2014;5(1):17-23.
- [19]. El-Saber Batiha G, Magdy Beshbishy A, G. Wasef L, Elewa YH, A. Al-Sagan A, Abd El-Hack ME, Taha AE, M. Abd-Elhakim Y, Prasad Devkota H. Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): A review *Nutrients*. 2020 Mar 24;12(3):872.
- [20]. Velišek J, Kubec R, Davidek J. Chemical composition and classification of culinary and pharmaceutical garlic-based products. *Zeitschrift für Lebensmitteluntersuchung und Forschung A*. 1997 Feb;204(2):161-4.
- [21]. Tesfaye A. Revealing the therapeutic uses of garlic (*Allium sativum*) and its potential for drug discovery. *The Scientific World Journal*. 2021;2021(1):8817288.
- [22]. Kumari I, Kaurav H, Chaudhary G. Ethnobotanical significance of picrorhiza kurroa (kutki), a threatened species. *International Journal of Research and Review*. 2021;8(4):363-75.
- [23]. Verma PC, Basu V, Gupta V, Saxena G, Ur Rahman L. Pharmacology and chemistry of a potent hepatoprotective compound Picroliv isolated from the roots and rhizomes of *Picrorhiza kurroa royle ex benth.* (kutki). *Current pharmaceutical biotechnology*. 2009 Sep 1;10(6):641-9.
- [24]. Zuckerman AJ. Hepatitis viruses. *Medical Microbiology*. 4th edition. 1996. 22
- [25]. Venkateswarlu K. In vitro stability testing of syrup dosage form for hepatitis. *Am J Phytomed Ther*. 2013 Sep 30;1(6):491-7.[23]
- [26]. Sheikh ZA, Zahoor A, Khan SS, Usmanghani K. Design, development and phytochemical evaluation of a poly herbal formulation linkus syrup. *Chinese Medicine*. 2014 Jun 1;5(2):104.

