PHYTOCHEMICAL CHARACTERIZATION OF HERBAL FORMULATION CONTAINING *NIGELLA SATIVA*, *TERMINALIA ARJUNA*, *WITHANIA SOMNIFERA* AND *LINUM USITATISSIMUM*.

ABSTRACT

Hyperlipidemia, a metabolic disorder characterized by elevated plasma lipid and lipoprotein levels, is a major risk factor for cardiovascular diseases. Although synthetic drugs such as statins and fibrates are widely used for treatment, their long-term use is often associated with adverse effects, prompting the search for safer alternatives. Medicinal plants rich in bioactive phytoconstituents offer promising therapeutic potential in managing dyslipidemia. The present study was conducted to perform phytochemical screening of ingredients in a herbal formulation comprising *Nigella sativa*, *Terminalia arjuna*, *Withania somnifera* and *Linum usitatissimum*. The aqueous extract was prepared by cold maceration and analyzed for major phytochemical constituents using standard qualitative tests. Phytochemical analysis revealed the presence of alkaloids, tannins, terpenoids, saponins, phenols, glycosides, flavonoids, and coumarins. Overall, the findings validate the traditional use of these herbal formulations as safe and effective alternatives for the prevention and management of hyperlipidemia.

Keywords: Phytochemical screening, Herbal formulation, *Nigella sativa*, *Terminalia arjuna*, *Withania somnifera*, *Linum usitatissimum*, Hyperlipidemia.

INTRODUCTION

Hyperlipidemia is a metabolic disorder characterized by elevated levels of lipids and lipoproteins in the bloodstream (Jani, 2011). It may occur due to inherited abnormalities in lipoprotein metabolism or develop secondary to other systemic diseases (Barriga and Fonturbel, 2011). Dietary factors also play a significant role, as consumption of high-fat and cholesterol-rich foods increases the risk of developing this condition. The incidence of hyperlipidemia has been rising steadily, largely attributed to poor dietary habits and sedentary lifestyles, both of which adversely affect overall health and quality of life (Dyer *et al.,* 2014).

Conventional treatment of hyperlipidemia primarily involves the use of statins and fibrates. Statins lower cholesterol levels by inhibiting its biosynthesis, while fibrates enhance the removal of triglyceride-rich lipoproteins from circulation (Jani,

2011). However, prolonged or excessive use of these synthetic agents often results in adverse effects, limiting their suitability for long-term therapy (Boden *et al.,* 2011).

As a result, there has been a growing interest in the use of herbal therapies as safer alternatives. Medicinal plants contain bioactive phytoconstituents that exhibit lipid-lowering, antioxidant, and hepatoprotective activities with minimal side effects. Herbal medicines are increasingly being integrated with modern pharmacotherapy due to their safety, efficacy, and better tolerance. Consequently, the therapeutic potential of herbal drugs in managing hyperlipidemia has gained significant scientific and clinical attention in recent years (Jani, 2011).

Nigella sativa, commonly known as black seed, is recognized as a remarkable medicinal plant with deep historical, cultural, and religious significance. Its seeds and oil have been traditionally used for thousands of years across various civilizations to treat numerous ailments. In India, *Nigella sativa* holds an important place in traditional systems of medicine, including Unani and Ayurveda. The seeds are known to exhibit a diverse spectrum of pharmacological and therapeutic properties, such as anti-diabetic, anticancer, immunomodulatory, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renoprotective, gastroprotective and antioxidant effects (Maheswari *et al.,* 2022).

Terminalia arjuna, commonly known as Arjuna, is a large deciduous tree extensively distributed across India. The bark of *T. arjuna* has been traditionally employed in the management of various ailments, including cardiovascular disorders, bone fractures, skin diseases, polyuria, vertigo, fever and parasitic infections. Administration of *T. arjuna* bark extract markedly decreased plasma total cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol levels in hypercholesterolemic rats, indicating its potent lipid-lowering activity (Patil *et al.,* 2011).

Withania somnifera, popularly known as Ashwagandha, has been a cornerstone of Ayurvedic and traditional medicine for more than 3000 years. The plant exhibits a wide spectrum of pharmacological effects, including antibacterial, antifungal, antidiabetic, and antitumor activities. It is traditionally valued as an adaptogen, helping to alleviate nervous exhaustion, insomnia, and stress-induced debility, and is also used as an immunostimulant for individuals with reduced white blood cell counts (Sharma *et al.,* 2018).

Linum usitatissimum, commonly known as flaxseed, offers numerous health-promoting benefits owing to its rich composition of biologically active compounds. It is an excellent source of omega-3 fatty acids, particularly alpha-linolenic acid, as well as lignans, which contribute to its therapeutic potential. Flaxseed exhibits a variety of pharmacological activities, including anti-inflammatory, antioxidant, diuretic, anticancer and antidiabetic effects (Qureshi *et al.,* 2018).

The study aimed to phytochemical characterization of active phytoconstituents present in the formulation, thereby establishing a correlation between their bioactive composition and therapeutic efficacy against hyperlipidemia.

MATERIALS AND METHODS

Location of work

The present research work was conducted in the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh.

Collection and processing of plant

Fresh plant material including the seeds of *Nigella sativa*, bark of *Terminalia arjuna*, roots of *Withania somnifera* and seeds of *Linum usitatissimum* were obtained from the Department of Plant Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya (J.N.K.V.V.), Jabalpur (M.P.). The collected plant parts were shade-dried and ground into fine powder using a grinder. The powdered material were combined in specific proportions to formulate a herbal mixture, which was subsequently subjected to aqueous extraction as per the method described by El-Desouky (2021), with slight modifications.

Preparation of aqueous extract of herbal formulation

Briefly, the aqueous extract was prepared by mixing the dry seed powders of *Nigella sativa* and *Linum usitatissimum* in a fixed proportion with 650 ml of distilled water. The mixture was placed in conical flasks, sealed with cotton plugs and aluminum foil, and incubated in a shaker incubator at 37°C with gentle agitation at 120 rpm for 24 hours. After incubation, the mixture was filtered using Whatman filter paper No. 1 and the filtrate was collected. The filtrate was then concentrated using a rotary evaporator at 40°C until complete solvent evaporation. The resulting dried extract was weighed and stored in airtight containers for subsequent experimental analysis.

Preliminary phytochemical analysis of plant extracts

The aqueous extract prepared from seeds of *Nigella sativa* and *Linum usitatissimum* were subjected to phytochemical screening. The presence of bioactive constituents such as alkaloids, tannins, glycosides, flavonoids, saponins, phenols, carbohydrates, proteins and terpenoids was evaluated using standard procedures described by Jeevalatha *et al.* (2022).

Test for Phenol

Ferric chloride test

To 1 ml of the extract, 3 ml of distilled water was added followed by few drops of 10 per cent aqueous ferric chloride solution. The appearance of a green coloration confirmed the presence of phenolic compounds.

Test for Flavonoids

Shinoda test

To 2 ml of the extract, 1 ml of 1 per cent ammonia solution was added. Appearance of yellow colour signifies the presence of flavonoids.

Test for Tannins

Ferric chloride test

To 1 ml of the extract, 1 ml of 0.008 M potassium ferricyanide was added and then 1 ml of 0.02 M ferric chloride containing 0.1 N HCl was added. Appearance of blue-black colour indicates the presence of tannins.

Test for Alkaloids

Wagner's reagent test

Approximately, 1 ml of crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colour precipitate indicates the presence of alkaloids.

Test for carbohydrates

Benedict's test

1 ml of crude extract was mixed with 2 ml of Benedict's reagent and boiled. A reddish-brown precipitate was formed which indicates the presence of the carbohydrates.

Test for proteins

Millon's test

1 ml of crude extract was mixed with 2 ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Test for Glycosides

Keller-Kilian test

5 ml of the extract were mixed with 2 ml of glacial acetic acid containing a drop of ferric chloride solution, and the mixture was carefully underlaid with 1 ml of concentrated sulfuric acid. The formation of a brown ring at the interface indicated the presence of deoxy sugars characteristic of cardenolides. Additionally, a violet ring could appear beneath the brown layer, while a greenish coloration might gradually develop in the acetic acid layer.

Test for Saponins

Foam test

2 ml of crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam is taken as an indication for the presence of saponins.

Test for Coumarin

Coumarins test

10 per cent Sodium hydroxide was added to the extract and chloroform was added. Formation of yellow colour shows the presence of Coumarin.

Test for Terpenoids

Salkowski test

5 ml of extract was mixed with 2 ml of chloroform and 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish-brown colouration of the inter face was formed which indicates the presence of terpenoids.

Test for Steroids

Salkowski test

2 ml of acetic anhydride was added to 0.5 ml of crude extract containing 2 ml of sulphuric acid. The colour changed from violet to blue or green in samples indicates the presence of steroids.

Test for Quinones

Quinone test

Diluted sodium hydroxide was added to the 1 ml of crude extract. Blue green or red coloration indicates the presence of quinones.

RESULTS AND DISCUSSION

In the present study, preliminary phytochemical screening of various ingredients of *Nigella sativa* seeds, *Terminalia arjuna* bark, *Withania somnifera* roots and *Linum usitatissimum* seeds were carried out to identify the presence of phytoconstituents like alkaloids, tannins, flavonoids, phenols, carbohydrates, proteins, glycosides, saponins, coumarins, terpenoids and quinones in aqueous extracts of these plants.

Preliminary Phytochemical Analysis

Hyperlipidemia is a major risk factor for cardiovascular diseases and medicinal plants have gained considerable attention for their role in managing lipid disorders owing to their rich bioactive phytoconstituents. Phytochemical screening plays a vital role in detecting these constituents within plant extracts, as they are largely responsible for the pharmacological and therapeutic effects of the plants (Shree *et al.,* 2018). In the present investigation, aqueous extract of *Nigella sativa*, *Terminalia arjuna*, *Withania somnifera* and *Linum usitatissimum* were analyzed for the presence of phytoconstituents such as alkaloids, tannins, flavonoids, phenols, carbohydrates, proteins, glycosides, saponins, coumarins, terpenoids and quinones, with the findings summarized in Tables 01.

Analysis of aqueous extracts showed that *Nigella sativa* seeds contained tannins, alkaloids, saponins and terpenoids, while phenols, flavonoids, carbohydrates, proteins, glycosides, coumarins and quinones were not detected. Shrivastava (2023) reported comparable findings, noting tannins, steroids, terpenoids and saponins, but absence of phenols, flavonoids, glycosides and amino acids in aqueous seed extracts of *N. sativa*.

Similarly, the aqueous extract of *Terminalia arjuna* bark demonstrated the presence of phenols, tannins, alkaloids, carbohydrates, glycosides, saponins, terpenoids and quinones. Vijayalakshmi and Rajendran (2023) also reported the occurrence of alkaloids, tannins, saponins, phenols, flavonoids, carbohydrates and glycosides in aqueous bark extracts of *T. arjuna*.

Table 01: Preliminary phytochemical analysis of aqueous extract of different component of herbal formulation

S.No	Phytochemicals	Tests	Results			
			Nigella sativa	***Terminalia arjuna***	***Withania somnifera***	***Linum usitatissimum***
1	Flavonoids	Shinoda test	-	-	+	-
2	Tannins	Ferric chloride test	+	+	+	+
3	Alkaloids	Wagner's reagent test	+	+	+	+
4	Carbohydrates	Benedict's test	-	+	+	-
5	Proteins	Millon's test	-	-	+	-
6	Glycosides	Keller-Kiliani test	-	+	+	-
7	Saponins	Foam test	+	+	-	+
8	Phenols	Ferric chloride test	-	+	+	+
9	Coumarin	Coumarins test	-	-	-	+
10	Terpenoids	Salkowski test	+	+	+	+
11	Quinones	Quinone test	-	+	-	-

The preliminary phytochemical screening of the aqueous root extract of *Withania somnifera* demonstrated the presence of phenols, flavonoids, proteins,

tannins, alkaloids, carbohydrates, glycosides and terpenoids in varying amounts, whereas saponins, coumarins, and quinones were absent. These findings align with those of Kumar *et al.* (2013), who reported the presence of steroids, alkaloids, terpenoids, flavonoids, tannins and phenols, along with the absence of coumarins, saponins and quinones in soxhlet-extracted aqueous root preparations of *W. somnifera*. Conversely, Saika *et al.* (2024) observed carbohydrates, tannins, phenols, flavonoids, proteins, amino acids and saponins in the aqueous root extract of the same plant, highlighting some variation in constituent profiles depending on extraction methods.

The aqueous extract of *Linum usitatissimum* seeds was found to contain tannins, alkaloids, saponins, phenols, coumarins and terpenoids in different concentrations, while flavonoids, carbohydrates, proteins, glycosides and quinones were absent. Hussien *et al.* (2020) reported the presence of alkaloids, phenols, tannins, flavonoids and saponins, but absence of glycosides, in aqueous extracts of *L. usitatissimum* seeds. On the other hand, Hanaa *et al.* (2017) documented the presence of steroids, terpenoids, anthocyanins, emodins, flavonoids and phenols and absence of tannins, saponins, alkaloids or glycosides in aqueous seed extracts of *L. usitatissimum*.

The anti-hyperlipidemic potential of the herbal formulation containing *Nigella sativa*, *Terminalia arjuna*, *Withania somnifera* and *Linum usitatissimum* can be attributed to their diverse phytochemical composition. *N. sativa* provides flavonoids, tannins and alkaloids that modulate lipid metabolism by lowering LDL-C and triglycerides while enhancing HDL-C levels. *W. somnifera*, rich in withanolides, flavonoids and terpenoids, supports lipid regulation through antioxidant activity and improved fat metabolism. *T. arjuna*, containing phenols, tannins, flavonoids and saponins, exerts hypolipidemic effects by reducing intestinal cholesterol absorption, stimulating bile acid excretion and minimizing lipid peroxidation. Similarly, *L. usitatissimum*, a source of lignans, omega-3 fatty acids and flavonoids, aids in triglyceride reduction and strengthens antioxidant defenses. Together, these botanicals act synergistically by enhancing antioxidant protection, limiting lipid absorption and promoting lipid utilization, thereby establishing their potential as effective natural agents in the management of hyperlipidemia.

CONCLUSION

The present investigation successfully established the phytochemical profile of an aqueous herbal formulation containing *Nigella sativa, Terminalia arjuna, Withania somnifera* and *Linum usitatissimum.* Preliminary phytochemical analysis revealed the presence of key bioactive constituents such as tannins, alkaloids, terpenoids, phenols and glycosides, which are known to contribute to lipid regulation and antioxidant defense. The synergistic action of these phytochemicals is likely responsible for the formulation's potential to lower lipid levels, enhance fat metabolism and protect against oxidative stress. These findings support the traditional use of these medicinal plants and provide a scientific basis for their application as safe, natural and effective agents in the management of hyperlipidemia.

REFERENCES

Barriga, C.V. and Fontúrbel, F.E. (2011). Cholesterol, glucose and triglycerides role in the prevalence of hyperlipidemia in dogs at higher elevations. *Revista Cientifica-Facultad De Ciencias Veterinarias*, 21(1): 22-26.

Boden, W.E., Probstfield, J.L., Anderson, T., Chaitman, B.R., Desvignesnickens, P., Koprowicz, K. and Weintraub, W. (2011). Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *New England Journal of Medicine,* 365(1): 2255–2267.

Dyer, S. M., Gomersall, J. S., Smithers, L. G., Davy, C., Coleman, D. T. and Street, J. M. (2017). Prevalence and characteristics of overweight and obesity in indigenous Australian children: A systematic review. *Critical Reviews in Food Science and Nutrition*, **57**(7): 1365-1376.

El-Desouky, T.A. (2021). Evaluation of effectiveness aqueous extract for some leaves of wild edible plants in Egypt as anti-fungal and anti-toxigenic. *Heliyon*, **7**(2):1-6.

Hanaa, M.H., Ismail, H.A., Mahmoud, M.E. and Ibrahim, H.M. (2017). Antioxidant activity and phytochemical analysis of flaxseeds *(Linum usitatisimum*). *Minia Joumal of Agricultural Research and Development,* 37(1): 129-140.

Hussien, Z.G., Azize, R.A. and Al-Mousawi, A.T. (2020). The effect of flaxseed (Linum usitatissimum) consumption in biochemical parameters of rats. *International Journal of Pharmaceutical Research,* 12(2): 3759-3764.

Jani Dilip, K. (2011). A study of anti-hyperlipidemic activity of polyherbal formulation using various experimental animal models. Inventi Rapid: *Ethnopharmacology,* 2(1): 3.

Jeevalatha, A., Kalaimathi, R.V., Basha, A.N., Kandeepan, C., Ramya, S., Loganathan, T. and Jayakumararaj, R. (2022). Profile of bioactive compounds in Rosmarinus officinalis. *Journal of Drug Delivery and Therapeutics,* 12(1): 114-122.

Kumar, R., Garipatti, V., Hazra, K., Mangal, A.K. and Sannd, R. (2013). Preliminary phytochemical evaluation of root of natural grown Withania somnifera of northern part

of India. International Journal of Pharmacognosy and Phytochemical Research, 5(1): 15-18.

Maheswari, K. U., Dilara, K., Vadivel, S., Johnson, P., & Jayaraman, S. (2022). A review on hypo-cholesterolemic activity of Nigella sativa seeds and its extracts. *Bioinformation*, *18*(4), 343.

Patil, R. H., Prakash, K., & Maheshwari, V. L. (2011). Hypolipidemic effect of Terminalia arjuna (L.) in experimentally induced hypercholesteremic rats. *Acta Biologica Szegediensis*, *55*(2), 289-293.

Qureshi, J.A., Zahida, M., Mirza, K.M., Agha, S., Fizza, S. and Fatima, S. (2018). Anti-hyperglycemic and anti-hyperlipidemic activity of Linum usitatissimum and Glycyrrhiza glabra extracts in streotozotocin induced diabetic rats. *Asian Journal of Research in Medical and Pharmaceutical Sciences,* 5(2): 1-10.

Saika, V., Saleem, M., Kumar, J.S., Raju, V. and Mounika, C. (2024). Evaluation of anti-obese activity of Withania somnifera in high fat diet-induced obese male wistar rats. Journal for Innovative Development in Pharmaceutical and Technical Science, 7(6): 33-39.

Sharma, S., Singh L, S.B. and Das, M.K. (2018). Evaluation of antihyperlipidemic activity of ethanolic extract of *Withania somnifera* in Triton X-100 induced hyperlipidemic rats. *International Journal of Pharmacy and Biological Sciences*, **8**(2): 639-646.

Shree, R., Ghosh, A.K. and Bharti, S.K. (2018). Effect of dietary Withania somnifera and Commiphora weightii on induced hyperlipidemic Wistar rats. *The Pharma Innovation Journal,* 7(4): 302-313.

Shrivastava, A.K. Shrestha, L. Pokhrel, B.R.. Joshi, B., Lamichhane, G., Vidovic, B. and Koirala, N. (2023). LC-MS based metabolite profiling, in-vitro antioxidant and in-vivo anti-hyperlipidemic activity of *Nigella sativa* extract, *eFood*, 4(4): 107.DOI: https://doi.org/10.1002/efd2.107

Vijayalakshmi, K. and Rajendran, K. (2023). Histological evaluation of aqueous extracts of Phyllanthus emblica fruit and Terminalia arjuna bark on body weight, hepatic and renal structures in high fat diet induced albino Wistar rats. Journal of Complementary Medicine Research, 13(4): 82.