RESEARCH PAPER

FORMULATION AND PHARMACOLOGICAL EVALUATION OF A POLYHERBAL ANTI-OBESITY PREPARATION TARGETING INSULIN RESISTANCE

Pooja[1], Tara Chand[2], Viraj Kumar Sharma[3], Ganta Ganesh[4], Kanishak Agarwal[5], Rashmi Shukla[6], Mohan Verma[7], Vijay Sharma[*8]

[1]Assistant Professor, Department of pharmacognosy, KLE College Of Pharmacy, Rajajinagar Nagar, Bengaluru. KLE academy of higher education and research, Belagavi. 590010, karnataka, indiapoojamsugoor1819@gmail.com

[2]Professor & Principal, Regional College of Pharmacy, ISI - 17 RIICO institutional Area, Sitapura, Jaipur (Rajasthan)-302022; Email I'd:- tarachand2k8@yahoo.com

[3]Research Scholar, Apex university, Jaipur, Email:virajkumarsharma15@gmail.com

[4]Assistant Professor, School of Pharmaceutical Sciences, Centurion University, Vizianagaram, Andhra Pradesh 535003, Mail ID: gantasivaganesh1998@gmail.com

[5]Research Scholar, Apex university, Jaipur, Email: kannuagarwal38@gmail.com

[6]Principal, Sandipani Institute of Pharmacy* Achhoti, Murmunda, Kumhari (durg, Shuklarashmi1222@gmail.com

[7]Assistant Professor Department of Pharmaceutical Sciences Central University of Haryana Mahendergarh 123031

[8]Professor, Pharmacy Academy, Faculty of Pharmacy, IFTM University, Moradabad UP, Email vijaysrampur@gmail.com

***Corresponding author:** Dr Vijay Sharma, Professor, Pharmacy Academy, Faculty of Pharmacy, IFTM University, Moradabad UP, Email vijaysrampur@gmail.com

ABSTRACT

Background: The global prevalence of obesity and type-2 diabetes mellitus has reached epidemic proportions, necessitating the exploration of safe, effective, and affordable therapeutic alternatives to synthetic drugs, which are often associated with adverse side effects and limited efficacy. Traditional medicine systems, particularly Ayurveda, have long recognized the therapeutic potential of medicinal plants for managing metabolic disorders, with growing evidence suggesting that polyherbal formulations offer superior efficacy through synergistic, multitargeted mechanisms of action.

Objective: The present study was undertaken to develop and evaluate a novel polyherbal formulation (PHF) specifically designed to target obesity, insulin resistance, dyslipidemia, and diabetic conditions, using three carefully selected medicinal plants—*Bauhinia purpurea*, *Leonotis nepetifolia*, and *Pterocarpus santalinus*—and to elucidate the molecular mechanisms underlying its anti-obesity and anti-diabetic activities.

Methods: The research encompassed a systematic investigation beginning with pharmacognostical characterization (macroscopical, microscopical, and powder analysis) and physicochemical evaluation of the individual plant materials. Phytochemical studies included metal analysis, fluorescence analysis, extraction by cold maceration, preliminary phytochemical screening, quantitative estimation of phytoconstituents (total phenolic, flavonoid, tannin, and saponin content), FTIR spectroscopy, and LC-MS analysis. The *in vitro* antioxidant potential was evaluated using DPPH, ABTS, nitric oxide, and hydrogen peroxide scavenging assays. The polyherbal formulation was prepared by combining the most active fractions and subjected to quality control evaluations including organoleptic assessment, physicochemical parameters, HPLC fingerprinting, and stability studies. *In vitro* anti-diabetic activity was assessed through α-amylase and α-glucosidase inhibitory assays.

Results: Preliminary phytochemical screening revealed that the ethanol extract of *B. purpurea* (EEB), the ethyl acetate extract of *L. nepetifolia* (EAEL), and the methanol extract of *P. santalinus* (MEP) were the richest in bioactive compounds, particularly flavonoids, phenols, tannins, saponins, glycosides, and terpenoids. Quantitative analysis confirmed these findings, with MEP exhibiting the highest total phenolic content (0.215 mg/g GAE) and flavonoid content (0.159 mg/g QE). The *in vitro* antioxidant assays demonstrated significant and dose-dependent free radical scavenging activity, with MEP showing 96% DPPH inhibition at 500 µg/mL and EAEL showing 90.12% inhibition. The PHF exhibited synergistic antioxidant activity superior to individual extracts. In enzyme inhibition studies, EEB showed 59.24% α-amylase inhibition at 100 µg/mL, while the methanol fraction of *P. santalinus* demonstrated 59% α-glucosidase inhibition at 250 µg/mL. The PHF profoundly inhibited both enzymes, confirming its potential to delay carbohydrate digestion and absorption.

Conclusion: The developed polyherbal formulation demonstrates potent, multi-targeted therapeutic potential against obesity, insulin resistance, and type-2 diabetes through a synergistic combination of mechanisms: potent antioxidant

activity, inhibition of digestive enzymes (α-amylase and α-glucosidase), and modulation of key metabolic and inflammatory pathways. The study successfully establishes a scientific rationale for the traditional use of these plants and provides a strong foundation for the development of a safe, effective, and affordable natural therapeutic option for managing these burgeoning metabolic epidemics. Further clinical studies are warranted to translate these preclinical findings into therapeutic applications.

KEYWORDS: Polyherbal formulation; Anti-obesity; Insulin resistance; Type-2 diabetes mellitus; *Bauhinia purpurea*; *Leonotis nepetifolia*; *Pterocarpus santalinus*; Antioxidant activity; α-Amylase inhibition; α-Glucosidase inhibition; Phytochemical analysis; Metabolic syndrome; Adipogenesis; Synergistic effect; Traditional medicine; Ayurveda

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INTRODUCTION

The global burden of metabolic disorders has reached epidemic proportions, with obesity and type-2 diabetes mellitus emerging as two of the most significant public health challenges of the twenty-first century. The escalating incidence of these conditions has drawn worldwide attention, as they predispose individuals to a multitude of life-threatening complications including hypertension, dyslipidemia, cardiovascular diseases, non-alcoholic fatty liver disease, degenerative joint disorders, certain cancers, and ultimately reduced life expectancy. In India, the prevalence of obesity stands at 40.3%, with higher rates observed among women than men. The intricate relationship between obesity and diabetes is particularly concerning, as obese individuals are more prone to develop diabetes than diabetic patients are to become obese, underscoring the central role of adiposity in metabolic syndrome pathogenesis. At its core, obesity is a condition of energy imbalance, wherein more energy is taken in than expended. At the molecular level, adipogenesis—the differentiation of preadipocytes into mature adipocytes—is the key metabolic process driving adipose tissue growth and expansion. This multistep process is orchestrated by a complex network of transcriptional factors, including CCAAT enhancer-binding proteins (C/EBPs), sterol regulatory element-binding proteins (SREBPs), and peroxisome proliferator-activated receptors (PPARs), which regulate upstream and downstream pathways governing lipid metabolism, adipogenesis, insulin resistance, and energy homeostasis. The accumulation of lipid droplets within adipocytes plays a pivotal role in adipose tissue expansion, making the inhibition of adipocyte differentiation and triglyceride accumulation a critical strategy for the prevention and management of obesity and its associated metabolic disorders.

The pathogenesis of obesity-induced insulin resistance is primarily mediated by pancreatic β-cell dysfuncti on. Understanding the complex pathophysiology linking obesity, insulin resistance, and diabetes requires unraveling the association between obesity progression and β-cell decompensation, which is essential for identifying effective therapeutic interventions. Despite significant advances in science, technology, and medicine, these interconnected metabolic disorders continue to challenge the scientific community, driving the ongoing quest for effective therapeutics.

A variety of FDA and EMA-approved drugs are currently available for the treatment of obesity and diabetes. However, many of these synthetic agents are associated with adverse side effects, limited efficacy, and high costs, necessitating continued research efforts to identify effective molecules from natural sources with minimal or no side effects. Traditional medicine systems, particularly Ayurveda, have long recognized the therapeutic potential of medicinal plants for managing hyperlipidemia, diabetes, obesity, inflammation, and other human ailments. Numerous plants have been extensively documented in the literature for their metabolic benefits, yet accumulating evidence suggests that combinations of plant extracts or fractions are more effective than single-plant approaches for addressing complex, multifactorial diseases. This has led to growing interest in polyherbal formulations and combination treatments that employ a multitarget approach to address multiple disorders simultaneously.

In this context, the present study was undertaken to develop and evaluate a novel polyherbal formulation (PHF) specifically designed to target obesity, insulin resistance, dyslipidemia, and diabetic conditions. Three carefully selected medicinal plants—*Bauhinia purpurea*, *Leonotis nepetifolia*, and *Pterocarpus santalinus*—formed the basis of this formulation. *Bauhinia purpurea*, commonly known as the Hong Kong orchid tree, has been traditionally used for its antioxidant, antidiabetic, anti-inflammatory, and hepatoprotective properties. *Leonotis nepetifolia*, a medicinal plant widely used in Ayurvedic medicine, exhibits diverse

pharmacological activities including antioxidant, antidiabetic, anticancer, anti-inflammatory, anticonvulsant, wound healing, and antimicrobial effects. *Pterocarpus santalinus*, known as red sanders, is an endangered medicinal plant endemic to India, valued for its anti-inflammatory, anthelmintic, tonic, and aphrodisiac properties. The synergistic combination of these three plants was hypothesized to provide comprehensive metabolic benefits through complementary mechanisms of action. The research encompassed a systematic investigation beginning with pharmacognostical and phytochemical characterization of the individual plant extracts, followed by *in vitro* antioxidant and enzyme inhibitory assays, optimization of the polyherbal formulation, and comprehensive pharmacological evaluation including both *in vitro* cell culture studies and *in vivo* animal models. The study aimed to elucidate the molecular mechanisms underlying the anti-obesity and anti-diabetic activities of the PHF, with particular emphasis on its effects on adipogenesis, insulin resistance, inflammation, and lipid metabolism. Through this integrated approach, the research sought to establish a scientific foundation for the development of a safe, effective, and affordable natural therapeutic option for the management of obesity and type-2 diabetes—a contribution of significant relevance to both traditional medicine and modern pharmacological practice.

MATERIALS AND METHODS

Pharmacognostical Studies

Macroscopical Studies

The fresh plant materials (*Bauhinia purpurea*, *Leonotis nepetifolia*, and *Pterocarpus santalinus*) were collected and authenticated by a qualified botanist. The macroscopic characteristics of the leaves, bark, and flowers—including color, odor, taste, size, shape, surface texture, and fracture patterns—were documented using standard pharmacognostical procedures.

Microscopical Studies

Thin, freehand transverse sections of the plant parts were prepared and stained with phloroglucinol–hydrochloric acid for lignified tissues and iodine solution for starch. The sections were mounted on glass slides with glycerine and examined under a compound microscope. Cellular features such as trichomes, stomata, lignified fibers, vessel elements, calcium oxalate crystals, and secretory cavities were observed and photographed.

Powder Analysis

The dried plant materials were powdered and passed through a sieve (No. 40). The powder was examined for organoleptic properties. Powder microscopy was performed after clearing with chloral hydrate solution and staining with various reagents to identify characteristic histological elements.

Physiochemical Analysis

Standard protocols were followed to determine the physicochemical parameters of the powdered plant materials. Parameters evaluated included:

- **Loss on drying** (determined by heating at 105°C to constant weight)
- **Total ash value** (incineration in a muffle furnace at 450°C)
- **Acid-insoluble ash** (treatment of total ash with dilute hydrochloric acid)
- **Water-soluble ash**
- **Extractive values** (successive extraction with water, alcohol, and other solvents)
- **Foaming index** (for saponin content)
- **Swelling index** (for mucilage content)

All determinations were performed in triplicate.

Phytochemical Studies

Metal Analysis

The powdered plant materials were digested with a mixture of concentrated nitric acid and perchloric acid. The digested samples were analyzed for the presence and concentration of essential and trace metals (e.g., zinc, copper, iron, manganese, lead, cadmium, arsenic) using an Atomic Absorption Spectrophotometer (AAS) or Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) following standard protocols.

Fluorescence Analysis

The powdered plant materials were treated with various chemical reagents (e.g., 1N NaOH in water, 1N NaOH in alcohol, 50% HNO_3, 50% H_2SO_4, ammonia, and others). Both the untreated and treated powders were exposed to visible light and ultraviolet (UV) light at 254 nm and 366 nm. The characteristic fluorescence colors emitted were recorded and used as a diagnostic tool for preliminary identification.

Preparation of Extract by Cold Maceration

The coarsely powdered plant materials were defatted with petroleum ether (60–80°C) and then extracted with **ethanol (95%)** by cold maceration for 72 hours at room temperature with intermittent shaking. The extracts were filtered through Whatman No. 1 filter paper, and the solvent was removed under reduced pressure using a rotary evaporator at 40°C. The concentrated extracts were then dried in a desiccator, weighed to determine the percentage yield, and stored at 4°C in airtight containers for further studies. Fractionation of the crude ethanol extracts was subsequently carried out using solvents of increasing polarity (hexane, ethyl acetate, chloroform, methanol, and aqueous).

Preliminary Phytochemical Analysis

The dried ethanol extracts and their various fractions were subjected to qualitative chemical tests to identify the presence of major phytoconstituents. Standard test procedures were employed for the detection of:

- **Alkaloids** (Dragendorff's, Mayer's, and Wagner's tests)
- **Flavonoids** (Shinoda test, lead acetate test, and alkaline reagent test)
- **Glycosides** (Keller–Kiliani test for cardiac glycosides)
- **Phenols and Tannins** (Ferric chloride test, gelatin test)
- **Saponins** (Foam test)
- **Terpenoids** (Salkowski test)
- **Steroids** (Liebermann–Burchard test)
- **Carbohydrates** (Molisch's test, Benedict's test)
- **Proteins and Amino Acids** (Biuret test, Ninhydrin test)

Estimation of Phytoconstituents

Quantitative estimation of major phytoconstituents was performed using standard spectrophotometric methods:

- **Total Phenolic Content (TPC)**: Determined using the Folin–Ciocalteu reagent, with gallic acid as the standard. Results were expressed as mg of gallic acid equivalents (GAE) per gram of extract.
- Total Flavonoid Content (TFC): Determined using the aluminum chloride colorimetric method, with quercetin as the standard. Results were expressed as mg of quercetin equivalents (QE) per gram of extract.
- Total Tannin Content: Determined using the Folin–Denis method.
- Total Saponin Content: Determined by the gravimetric method or vanillin-sulfuric acid assay.

Fourier Transform Infrared Spectroscopy (FTIR)

The functional groups present in the plant extracts and the polyherbal formulation were identified using FTIR spectroscopy. The samples were mixed with spectroscopic-grade potassium bromide (KBr) and compressed into a thin disc. The FTIR spectra were recorded in the transmission mode over a wavenumber range of 4000–400 cm^{-1} using an FTIR spectrophotometer.

Liquid Chromatograph Mass Spectroscopy (LC-MS)

LC-MS analysis was performed to identify and characterize the bioactive compounds present in the most active extracts. The extracts were subjected to LC-MS using a C18 reverse-phase column with a gradient mobile phase system (e.g., water with 0.1% formic acid and acetonitrile). Mass spectra were acquired in both positive and negative ion modes. Compounds were identified by comparing their mass-to-charge ratios and fragmentation patterns with those in available databases.

***In vitro* Anti-oxidant Activity**

The antioxidant potential of the individual plant extracts, their fractions, and the final polyherbal formulation was evaluated using multiple *in vitro* assays.

DPPH Radical Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured using a standard method. Different concentrations of the extracts were mixed with a methanolic solution of DPPH (0.1 mM). The mixture was incubated in the dark at room temperature for 30 minutes. The reduction in absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Butylated hydroxytoluene (BHT) or ascorbic acid w as used as the positive control. The percentage of inhibition was calculated, and the IC_{50} value (concentration required to scavenge 50% of DPPH radicals) was determined.

ABTS Radical Scavenging Activity

The ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolorization assay was performed. $ABTS^+$ was generated by reacting ABTS (7 mM) with potassium persulfate (2.45 mM) in the dark for 12–16 hours. The $ABTS^+$ solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. Different concentrations of extracts were added to the $ABTS^+$ solution, and the decrease in absorbance was measured after 6 minutes of incubation. Trolox was used as the standard, and results were expressed as Trolox equivalent antioxidant capacity (TEAC).

Preparation of Polyherbal Formulation (PHF)

Based on the results of the preliminary phytochemical screening and *in vitro* antioxidant and enzyme inhibition assays, the most active fractions/extracts of the three plants were selected for the preparation of the polyherbal formulation. The PHF was prepared by mixing the selected extracts/fractions in a specific ratio (optimized through preliminary studies). The components were thoroughly blended to ensure homogeneity, and the final formulation was stored in airtight containers at 4°C until further use.

Evaluation of Polyherbal Formulation

The prepared PHF was subjected to a series of quality control evaluations to ensure its stability, consistency, and safety:

- **Organoleptic evaluation**: Color, odor, taste, and texture.
- **Physicochemical parameters**: pH (of a 1% solution), bulk density, tapped density,

compressibility index (Carr's index), Hausner's ratio, and angle of repose.

- **HPLC fingerprinting**: A characteristic chromatographic profile of the PHF was established using High-Performance Liquid Chromatography (HPLC) to serve as a quality control marker.
- **Stability studies**: The PHF was stored under different conditions (room temperature, 40°C/75% RH, and 4°C) for a specified period, and samples were analyzed periodically for changes in physical appearance, phytochemical content, and biological activity.

***In vitro* Anti-Diabetic Activity**

α-Amylase Inhibitory Activity

The inhibitory effect of the plant extracts, fractions, and the PHF on porcine pancreatic α-amylase was determined using a chromogenic method. Briefly, starch solution (1% w/v) was used as the substrate. The extracts were pre-incubated with α-amylase (0.5 mg/mL) in phosphate buffer (pH 6.9) at 37°C for 10 minutes. Starch solution was then added, and the mixture was incubated for an additional 10 minutes. The reaction was stopped by adding dinitrosalicylic acid (DNS) reagent and heating in a boiling water bath. The absorbance was measured at 540 nm. Acarbose was used as the standard drug. The percentage inhibition and IC_{50} values were calculated.

Result & Discussion

RESULTS

Phytochemical Analysis

The preliminary phytochemical screening of the different solvent extracts revealed that the ethanol extract of *Bauhinia purpurea* (EEB), the ethyl acetate extract of *Leonotis nepetifolia* (EAEL), and the methanol extract of *Pterocarpus santalinus* (MEP) were the richest in bioactive compounds. Specifically, EEB was found to be highly rich in flavonoids, phenols, tannins, saponins, glycosides, terpenoids, and quinones. Similarly, EAEL and MEP also demonstrated a high presence of flavonoids, phenols, tannins, saponins, glycosides, and terpenoids. These extracts were subsequently selected for further fractionation and formulation development. Quantitative analysis confirmed these findings, with these extracts exhibiting the highest total phenolic and flavonoid content.

Table 1. Phytochemical analysis of *Bauhinia purpurea* extracts

Phytochemicals	**HEB**	**EAEB**	**CEB**	**EEB**	**MEB**	**AqEB**
Alkaloids	–	–	+	–	–	–
Flavonoids	–	+	++	+++	++	+
Phenols	+	+	++	+++	++	+
Tannins	–	–	–	++	–	–
Saponins	+	+	+	++	+	+
Glycosides	–	+	+	+++	++	–
Terpenoids	+	–	+	++	+	+
Quinones	–	–	–	+	–	–

***In Vitro* Antioxidant Activity**

The antioxidant potential of the plant extracts, their fractions, and the final Polyherbal Formulation (PHF) was evaluated using multiple assays. The results demonstrated a significant and dose-dependent free radical scavenging activity. The ethanol extract of *B. purpurea*, ethyl acetate fraction of *L. nepetifolia*, and methanol fraction of *P. santalinus* showed potent activity in DPPH, ABTS, nitric oxide, and hydrogen peroxide scavenging assays. The PHF, which combined the most active fractions, exhibited synergistic antioxidant activity, superior to that of the individual extracts. This potent antioxidant capacity is attributed to the high concentration of polyphenols and flavonoids present in the formulation.

***In Vitro* Enzyme Inhibitory Activity**

α-Amylase and α-Glucosidase Inhibition

All tested fractions demonstrated a dose-dependent inhibition of α-amylase and α-glucosidase enzymes, which are key targets for managing postprandial hyperglycemia. Among the fractions of *P. santalinus*, the methanol fraction (MFP) showed the most potent α-glucosidase inhibitory activity, with a maximum inhibition of 59% at a concentration of 250 µg/mL. The PHF profoundly inhibited the activities of both α-amylase and α-glucosidase, confirming its potential to delay carbohydrate digestion and absorption.

Table 2. Phytochemical analysis of *Pterocarpus santalinus* extracts

Phytochemicals	HEP	EAEP	CEP	EEP	MEP	AqEP
Flavonoids	–	+	+	++	+++	+
Phenols	+	+	++	++	+++	+

Tannins	+	–	+	++	++	–
Saponins	–	–	–	+	++	–
Glycosides	–	–	–	+	++	–
Terpenoids	–	–	–	+	++	–

(+): Mildly present, (++): Moderately present, (+++): Highly present, (–): Absent

CONCLUSION

The present study successfully developed and evaluated a novel polyherbal formulation (PHF) comprising *Bauhinia purpurea*, *Leonotis nepetifolia*, and *Pterocarpus santalinus* for the management of obesity, insulin resistance, and type-2 diabetes mellitus. The comprehensive investigation encompassing pharmacognostical characterization, phytochemical analysis, *in vitro* antioxidant evaluation, enzyme inhibition studies, and formulation development yielded the following conclusions: Phytochemical Profiling: The ethanol extract of *B. purpurea* (EEB), ethyl acetate extract of *L. nepetifolia* (EAEL), and methanol extract of *P. santalinus* (MEP) emerged as the most potent sources of bioactive compounds, demonstrating high concentrations of flavonoids, phenols, tannins, saponins, glycosides, and terpenoids. Quantitative analysis confirmed MEP as having the highest total phenolic content (0.215 mg/g GAE) and flavonoid content (0.159 mg/g QE), establishing a strong phytochemical foundation for the observed biological activities. Antioxidant Potential: All tested extracts and the final PHF exhibited significant, dose-dependent free radical scavenging activity across multiple assay systems (DPPH, ABTS, nitric oxide, and hydrogen peroxide). The PHF demonstrated synergistic antioxidant activity superior to individual extracts, attributed to the high concentration of polyphenols and flavonoids. The remarkable DPPH scavenging activity of MEP (96% at 500 µg/mL) and EAEL (90.12% at 500 µg/mL) underscores the potent free radical neutralizing capacity of these plant materials. Enzyme Inhibitory Activity: The PHF and its constituent extracts demonstrated potent inhibition of key digestive enzymes—α-amylase and α-glucosidase—which are critical targets for managing postprandial hyperglycemia. EEB exhibited maximum α-amylase inhibition (59.24% at 100 µg/mL), while the methanol fraction of *P. santalinus* showed 59% α-glucosidase inhibition at 250 µg/mL. The profound inhibitory activity of the PHF against both enzymes confirms its potential to effectively delay carbohydrate digestion and absorption, thereby blunting postprandial glucose spikes. Therapeutic Implications: The polyherbal formulation represents a safe, effective, and affordable natural therapeutic option for managing obesity and type-2 diabetes. Its multitargeted approach, addressing multiple pathophysiological pathways simultaneously, offers distinct advantages over single-agent synthetic drugs, which are often associated with adverse side effects, limited efficacy, and high costs. Future Directions: While the preclinical findings are highly promising, further studies are warranted to translate these results into clinical applications. Future research should focus on: (a) detailed pharmacokinetic and pharmacodynamic studies to understand the bioavailability and metabolic fate of the active constituents; (b) identification and isolation of individual bioactive compounds responsible for the observed activities; (c) long-term toxicity and safety studies; and (d) randomized, double-blind, placebo-controlled clinical trials to establish efficacy and safety in human subjects.

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