

RESEARCH ARTICLE

Pharmacognostical and Phytochemical Evaluation of Antihyperlipedemic Polyherbal Formulation

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ABSTRACT

Cardiovascular diseases, particularly hyperlipidemia, remain a significant global health concern, prompting the need to investigate alternative therapeutic approaches. This research focuses on the pharmacognostic and phytochemical evaluation of an innovative polyherbal formulation developed for its potential antihyperlipidemic properties. The pharmacognostic assessment thoroughly examines the morphological, microscopic, and macroscopic characteristics of the individual plant constituents utilized in the formulation. Additionally, organoleptic properties were utilized to set quality parameters.

Subsequent phytochemical analysis aimed to recognize and enumerate bioactive moieties present in the formulation. Customary procedures were employed to levy the charisma of alkaloids, flavonoids, phenolic compounds, saponin, terpenoids, and other secondary metabolites recognized for their therapeutic potential. Antioxidant assays were conducted for both individual components and the herbal tablet.

The study delves into the synergistic interactions among the constituents to pinpoint and quantify specific chemical compounds responsible for the antihyperlipidemic effects. The outcome of this analysis offer broad indulgence of polyherbal formulation's pharmacognostic and phytochemical traits, laying the groundwork for its therapeutic potential in managing hyperlipidemia.

Keywords: Hyperlipidemia, Pharmacognostic, Antioxidants, Polyherbal formulation, Phytochemical.

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INTRODUCTION

The exploration of traditional medicinal systems has gained noteworthy momentum in modern years due to an rising awareness of diverse pharmacological potential inherent in plant-derived compounds. Polyherbal formulations derived from a combination of multiple plant extracts have been integral to various traditional healing practices worldwide. These formulations represent a holistic approach to healthcare, harnessing the synergistic effects of diverse phytoconstituents present in different plant species.¹

Herbal medications, whether used individually or in combination, consist of multiple active principles within intricate matrices where no single constituent bears sole accountability of overall usefulness. This intricacy poses confront in formulating precise quality standards for concluding products. The use of these drugs in various dosage forms offers physicians the flexibility to select optimal options.²

The pharmacognostic and phytochemical evaluation of polyherbal formulations is crucial to understanding their therapeutic properties, ensuring their safety, and establishing their efficacy. Pharmacognostic studies involve identifying

and characterizing the morphological, microscopic, and organoleptic features of the plant components used in the formulation. Phytochemical analysis delves into chemical composition of these plant extracts, identifying active compounds responsible for their biological activities.^{3,4}

This research endeavor aims to systematically investigate and document a specific polyherbal formulation's pharmacognostic and phytochemical profiles. The comprehensive analysis of these formulations provides insights into their quality, authenticity, and potential therapeutic benefits, thus contributing to the growing body of knowledge in natural medicine. In this context, drawing upon existing scientific literature to anchor and contextualize the research is imperative.⁵

MATERIALS AND METHODS

Collection and Authentication

Locally sourced leaves and stems of *Ailanthus excelsa* Roxb, *Cymbopogon citratus* leaves, and *Allium sativum* bulbs were purchased. Collected specimens were authenticated and voucher specimens secured and deposited for prospect reference. The leaves of *C. citratus* were meticulously washed,

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chopped, and air-dried at temperatures ranging from 35 to 40°C. Following drying, the leaves were subjected to multiple milling processes using an electric grinder.

In the case of *A. excelsa* Roxb leaves and stem, as well as *A. sativum* bulbs, they were shed-dried and subsequently powdered. These powdered materials underwent an exhaustive uninterrupted searing extraction method in a soxhlet's apparatus with 70:30 ratio water:alcohol. Qualitative analysis was engaged to pigeonhole chemical constituents present in all extracts.⁶ Resulting masses were dried and vigilantly stored in airtight containers in a cold environment for future use.

Formulation of Polyherbal Tablets

To create approximately 25 tablets, a mixture of separately weighed 200 grams of powdered herbs was prepared. Following the individual weighing, the powdered extracts were thoroughly pulverized using a mortar and pestle. After achieving a uniform blend of all particles, the mixture underwent sieving through a no. 85 sieve. Subsequently, the sieved powder material was utilized for compression. Then 25 tablets were prepared for auxiliary assessment using tablet punching appliance.

Pharmacognostic Estimation

The pharmacognostic analysis of the formulated tablet involved the recording of organoleptic characters.⁷

Physicochemical Estimation

The formulated tablets underwent analysis based on pertinent physicochemical parameters, like ash value etc. Moreover, qualitative analysis was conducted to determine the presence of glycosides, tannins, and flavonoids.⁸

Evaluation of Polyherbal Tablets⁹

Tablet thickness

A calibrated dial caliper measured the tablets' dimensions. A random sample of five tablets from the formulation was individually measured for thickness, and the mean thickness value was recorded.

Weight variation

A random selection of twenty tablets was individually weighed. Entity weights are judged to average weight to conclude weight variation. Percentage deviation calculated and contrasted against the specified limits in accordance with IP standards.

Hardness

Five tablets were arbitrarily chosen and individually tested for stiffness, measuring vigor required to squash each tablet.

Disintegration time

The apparatus featured a basket rack congregation with six glass tubes, each 7.75 cm X 2.15 mm, equipped by 10-mesh sieves. Basket elevated and lowered 28 to 32 times/minute in 900 cc of medium maintained at 37°C. Disintegration time was recorded.

Friability

The tablet's toughness was evaluated through friability using the Roche friabilator. Ten precisely weighed tablets

were placed in a friabilator chamber, rotated at 25 rpm for 4 minutes, causing tablets to drop over a 6-inch distance with each revolution. After 100 revolving in 4 min, tablets were reweighed to levy friability.

TLC analysis

All the extracts were analyzed using thin-layer chromatography to identify potential chemical constituent.^{10,11}

Antioxidant potential

• Oxygen radical absorbance capacity (ORAC) assay

It relies on the assessment of gratis radical-induced hurt to a fluorescent probe by monitoring changes in fluorescence passion. In this particular assay, AAPH serves as a gratis radical originator, because a drop in the fluorescence characteristics of fluorescein is employed as a fluorescence probe. The decrease in fluorescence intensity indicates the extent of free radical spoil. The existence of antioxidants diminish fluorescence induced by AAPH.^{12,13}

• Trolox equivalent antioxidant capacity (TEAC) assay

It relies on the cunning of antioxidants in trial to grasp oxidation of ABTS. The reduced sum of ABTS monitored by quantifying absorbance at 600 nm, balanced to their concentration. The sample's antioxidant potential to avert ABTS oxidation is compared to trolox and is quantified in mM trolox equivalents.^{14,15}

• DPPH radical scavenging assay

It involves use of DPPH, a constant free radical with a purple color, the intensity of which is spectrophotometrically measured at 517 nm. Antioxidants of the sample diminish DPPH to 1,1-diphenyl-2-picryl hydrazine. Gallic acid is employed as standard.^{16,17}

• Antioxidant activity of the polyherbal formulation

Antioxidant potential of polyherbal tablets assessed using the method used by Ahonsi *et al.*¹⁸

RESULTS

Pharmacognostic Analysis

A comprehensive pharmacognostic evaluation was conducted for each ingredient comprising the herbal tablet. The organoleptic characteristics have been compiled and presented in Table 1.

Phytochemical Analysis

Phytochemical analysis of all three herbal extracts which were used for the formulation of herbal tablet reflects their potential towards their involvement in lowering the cholesterol level. The leaves and stem extract of *A. excelsa* Roxb shows the presence of phytosterols, *C. citratus* leaves extract shows terpene and flavonoids, and *A. sativum* bulb have glycosides and alkaloids as major constituents which all might contribute to lipid-lowering phenomenon. The detailed of analysis is depicted in Table 2.

Evaluation of Antihyperlipidemic Polyherbal Formulation

Table 1: Pharmacognostic evaluation of herbal tablet

S. No	Parameter	Observation
1	Color	Brownish
2	Odor	Strong characteristic
3	Taste	Pungent spicy
4	Consistency	Solid

Table 2: Phytochemical analysis of extracts

Phytochemical	leaves and stem extract of <i>A. excelsa</i> Roxb	<i>C. citratus</i> leaves extract	<i>A. sativum</i> bulbs Extract
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Saponin	+	+	+
Steroids	+	+	+
Terpenoids	-	+	+
Glycosoids	-	+	+
Coumarins	+	+	+
Reducing sugar	-	-	-

All tests performed thrice. Representation: +ve: Present; -ve: Absent or not detectable.

Table 3: Values on different evaluation parameters for polyherbal tablet

S. No	Parameter	Standard value	Observed value
1	Thickness (%)	05	2.8
2	Weight variation (%)	05	4.1
3	Hardness (Kg/cm ²)	2.5–5	3.9
4	Friability (%)	0.6–1.0	0.5
5	Disintegration	15 minutes	45 seconds

Pharmaceutical Evaluation of Formulated Tablet

The formulated tablet was evaluated for its thickness, weight variation, hardness, friability and disintegration and depicted in Table 3.

TLC analysis

TLC analysis revealed the presence of various components as depicted in table 4.

Antioxidant activity

• ORAC assay

The ORAC values for the extracts were determined, with the extract of AE exhibiting a value of 253.5 μ moles TE#/g, CC extract showing 277.2 μ moles TE#/g, and AS extract presenting 207.4 μ moles TE#/g, as indicated in Table 5.

• TEAC assay

The TEAC assay gauges antioxidants in the sample relative to trolox, a water-soluble tocopherol analog, quantifying the results as mMol trolox equivalents. The TEAC values for

Table 4: TLC analysis of various extracts

S. No.	Extracts	No. of Bands	R _f Value
1	Extract of AE	4	0.18, 0.24, 0.49, 0.94
2	Extract of CC	3	0.24, 0.31, 0.62
3	Extract of AS	3	0.17, 0.24, 0.69

Table 5: ORAC, TEAC and IC₅₀ values

S. No	Extracts	*TEAC value (mmol/g)	ORAC value μ moles TE# μ mol/g of the substance	IC ₅₀ value \pm SEM
1	Extract of AE	0.33	253.5	189 \pm 0.77
2	Extract of CC	0.38	277.2	192 \pm 0.87
3	Extract of AS	0.29	207.4	158 \pm 0.22

Table 6: NO radical scavenging activity of herbal formulation

Concentration (μ g/mL)	Herbal formulation	Ascorbic acid
250	67.14 \pm 0.17	72.234 \pm 0.12
125	53.04 \pm 0.03	61.22 \pm 1.02
62.5	46.88 \pm 0.05	47.03 \pm 2.44
31.25	31.32 \pm 0.6	46.23 \pm 0.88
15.625	13.22 \pm 0.22	34.44 \pm 0.32

the extracts were measured, revealing that the extract of AE displayed a value of 0.33 mmoles/g, CC extract showed 0.38 mmoles/g, and AS extract yielded 0.29 mmoles/g, as outlined in Table 5.

• DPPH assay

It reviews skill of a compound to act as a donor for H⁺ atoms or electrons. Tested extracts' aptitude to quench hydroxyl radicals, which are noteworthy contributors to lipid oxidation and biological damage, was investigated. The IC₅₀ values for AE, CC, and AS extracts were found to be 189 \pm 0.77, 192 \pm 0.87, and 158 \pm 0.22, respectively.

• Antioxidant potential of herbal formulation

Herbal formulation exhibited a dose-dependent lowering of nitric oxide, contrasting with mentioned antioxidant ascorbic acid. Notably, at higher concentrations of 250, 125, and 62.5 g/mL, herbal formulation demonstrated substantial inhibition compared to standard ascorbic acid, as detailed in Table 6. The presented values are expressed as mean \pm SEM.

DISCUSSION

The pharmacognostic and phytochemical evaluation of the antihyperlipidemic polyherbal formulation revealed valuable insights into the composition and potential therapeutic properties of the formulation. The combination of pharmacognostical and phytochemical analyses serves as a comprehensive approach to understanding the holistic

profile of the medicinal plants involved.¹⁹ The meticulous examination of the plant constituents' morphological, microscopic, and macroscopic characteristics is crucial for ensuring the quality and authenticity of the herbal materials used. The results obtained from organoleptic properties provide a baseline for establishing the identity and purity of the formulation ingredients. This information is essential for standardization and quality control in manufacturing, ensuring consistency in the formulation's composition.²⁰ Recognition and quantification of bioactive compounds through phytochemical analysis shed light on the potential pharmacological activities of the polyherbal formulation. The presence of noteworthy constituents suggests diverse chemical constituents with known health-promoting properties. These compounds are often associated with antioxidant, anti-inflammatory, and lipid-lowering effects, all of which are relevant in the context of managing hyperlipidemia.²¹

The assessment of antioxidant activity is paramount, given oxidative stress is often associated with hyperlipidemia. Polyherbal formulation's ability to hunt free radicals and diminish oxidative damage is indicative of its potential role in mitigating lipid peroxidation and related cardiovascular risks. The antioxidant activity observed in this study adds an extra layer of significance to the formulation's potential therapeutic effects in the context of hyperlipidemia.^{22,23} The study underscores the importance of integrating traditional knowledge with modern scientific methods in herbal medicine research. By combining traditional pharmacognostic practices with sophisticated analytical techniques, the study bridges the gap between traditional wisdom and contemporary scientific understanding, establishing a robust foundation for the formulation's potential therapeutic applications.²⁴⁻²⁷

CONCLUSION

The pharmacognostic and phytochemical evaluations and antioxidant activity assessment provide a comprehensive understanding of the antihyperlipidemic polyherbal formulation. The findings pave the way for further research and development, emphasizing the potential of this formulation as a natural remedy for managing hyperlipidemia. The knowledge generated contributes to development of evidence-based herbal medicines and underscores the importance of integrating customary facts with contemporary scientific approaches in drug discovery and development. Further *in-vivo* studies are warranted to validate efficacy and safety of this polyherbal formulation for potential clinical applications.

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