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"UNVEILING THE THERAPEUTIC EFFICACY OF A POLYHERBAL FORMULATION WITH ANTI-INFLAMMATORY, ANTI-MICROBIAL, AND HEPATOPROTECTIVE POTENTIAL: A STUDY ON PLANTS FROM KANDHAMAL DISTRICT, ODISHA"

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Abstract

The present study aims to explore the therapeutic efficacy of a polyherbal formulation derived from plants indigenous to Kandhamal District, Odisha, with potent anti-inflammatory, anti-microbial, and hepatoprotective properties. The formulation was developed and standardized using a combination of Cayratia trifolia, Sesbania grandiflora, Cordia dichotoma, and Teprosia purpurea, known for their traditional medicinal uses. The polyherbal formulation was subjected to rigorous evaluation to assess its anti-inflammatory activity. In vitro assays revealed significant inhibition of TNF-α, IL-6, and COX-2, indicating its potential in modulating inflammation. Furthermore, the formulation exhibited remarkable anti-microbial activity against pathogens such as E. coli, S. aureus, and C. albicans, as evident from the inhibition zones observed. Hepatoprotective activity was also assessed, and the results demonstrated the formulation's ability to mitigate liver damage. Various parameters, including ALT, AST, LDH, ROS levels, MDA levels, SOD activity, and GSH levels, were measured, and the formulation showed promising hepatoprotective effects by maintaining their homeostasis. To further understand the composition of the polyherbal formulation, phytochemical analysis was performed. The presence of alkaloids, proteins and amino acids, carbohydrates, flavonoids, tannins, and saponins was confirmed, indicating the richness of bioactive compounds in the formulation. TLC analysis using a specific solvent system exhibited distinct spots for the marker compounds, gallic acid, and piperine, validating their presence in the formulation. Quantification of total phenolic and flavonoid content revealed substantial amounts of these compounds in the formulation, further supporting its antioxidant potential. The presence of polyphenolics and flavonoids contributes to the rationality behind the formulation's observed antioxidant activity. So, the developed polyherbal formulation from plants collected from Kandhamal District, Odisha, and exhibits significant therapeutic efficacy against inflammation, microbial infections, and liver damage. The comprehensive analysis of its phytochemical composition and validation of its antioxidant properties highlight its potential as a natural remedy for various health ailments.

Keywords: Polyherbal formulation, therapeutic efficacy, anti-inflammatory, anti-microbial, hepatoprotective, Kandhamal District, Odisha.

INTRODUCTION

In recent years, there has been a growing interest in the development and standardization of polyherbal formulations for the management of various health conditions. Among these, the management of anti-inflammatory, anti-microbial, and hepatoprotective activities using polyherbal formulations has garnered significant attention. In this context, the

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present introduction focuses on the development and standardization of polyherbal formulations using four indigenous plants, namely Cayratia trifolia, Sesbania grandiflora, Cordia dichotoma, and Tephrosia purpurea, collected from the Kandhamal district of Odisha, India.

Inflammation, microbial infections, and liver diseases are major global health challenges, necessitating the development of effective therapeutic strategies. Traditional systems of medicine, such as Ayurveda and traditional Indian medicine, have long recognized the potential of polyherbal formulations in managing various ailments. These formulations leverage the synergistic effects of multiple plant extracts, thereby increasing their efficacy and reducing potential side effects. Cayratia trifolia, commonly known as "Vitis trifolia" or "Three-leafed creeper," has been traditionally used in folk medicine for its anti-inflammatory and hepatoprotective properties. Sesbania grandiflora, also known as "Agathi" or "Agati," is renowned for its anti-inflammatory, anti-microbial, and hepatoprotective activities. Cordia dichotoma, commonly referred to as "Indian cherry" or "Lasura," has been traditionally employed for its anti-inflammatory and hepatoprotective effects. Teprosia purpurea, commonly known as "Sharapunkha" or "Wild Indigo," possesses anti-inflammatory, anti-microbial, and hepatoprotective properties. These four plants have a long-standing history of ethnomedicinal use in the Kandhamal district of Odisha, India.

1. Cayratia trifolia

- Commonly known as "Vitis trifolia" or "Three-leafed creeper."
- Parts used: Leaves, stems, and roots.
- Potential Anti-inflammatory Activity: Extracts of Cayratia trifolia have shown anti-inflammatory effects in various studies. They inhibit the production of pro-inflammatory mediators, such as cytokines and prostaglandins, thereby reducing inflammation.
- Potential Anti-microbial Activity: Cayratia trifolia extracts have exhibited significant antimicrobial activity against various bacteria and fungi. They have the potential to inhibit the growth and proliferation of pathogens, thus acting as antimicrobial agents.
- Potential Hepatoprotective Activity: Studies have demonstrated the hepatoprotective properties of Cayratia trifolia. The plant extracts possess antioxidant and hepatoprotective effects, which help in preventing liver damage and promoting liver health.

2. Sesbania grandiflora

- Commonly known as "Agathi" or "Agati."
- Parts used: Leaves, flowers, and seeds.

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- Potential Anti-inflammatory Activity: Sesbania grandiflora extracts have shown anti-inflammatory effects by inhibiting the production of inflammatory mediators and reducing inflammation in various experimental models.
- Potential Anti-microbial Activity: Sesbania grandiflora exhibits significant antimicrobial activity against a wide range of bacteria and fungi. The extracts have been shown to possess antibacterial and antifungal properties, making them potential agents for combating microbial infections.
- Potential Hepatoprotective Activity: Studies have indicated the hepatoprotective activity of Sesbania grandiflora. The plant extracts possess hepatoprotective properties by reducing oxidative stress, modulating liver enzymes, and preserving liver function.

3. **Cordia dichotoma**

- Commonly known as "Indian cherry" or "Lasura."
- Parts used: Leaves, fruits, and bark.
- Potential Anti-inflammatory Activity: Cordia dichotoma extracts have demonstrated significant anti-inflammatory activity by inhibiting inflammatory mediators and reducing inflammation. They have the potential to alleviate inflammatory conditions.
- Potential Anti-microbial Activity: Cordia dichotoma exhibits broad-spectrum antimicrobial activity against various bacteria and fungi. The extracts have been shown to possess antibacterial and antifungal properties, which can be utilized in the treatment of microbial infections.
- Potential Hepatoprotective Activity: Cordia dichotoma extracts have hepatoprotective effects by reducing liver damage, improving liver function, and exhibiting antioxidant activity. They have the potential to protect the liver from various hepatotoxic agents and promote liver health.

4. Teprosia purpurea

- Commonly known as "Sharapunkha" or "Wild Indigo."
- Parts used: Leaves, roots, and seeds.
- Potential Anti-inflammatory Activity: Tephrosia purpurea extracts possess significant anti-inflammatory properties by inhibiting inflammatory mediators and reducing inflammation. They have the potential to alleviate inflammatory conditions.
- Potential Anti-microbial Activity: Tephrosia purpurea exhibits antimicrobial activity against various bacteria and fungi. The extracts have been shown to possess antibacterial and antifungal properties, making them potential agents for combating microbial infections.

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- Potential Hepatoprotective Activity: Studies have demonstrated the hepatoprotective properties of Tephrosia purpurea extracts. They exhibit hepatoprotective effects by reducing liver damage, improving liver function, and exhibiting antioxidant activity, thus promoting liver health.

MATERIALS AND METHODS

Collection and authentication of plant materials: The selected four plants were identified and authenticated by taxonomist Dr. S. K. Dash- H.O.D of Bioscience; College of Pharm. Science-Mohuda. The voucher herbarium specimens (no.- CPS/HS-0032,0033,0034 & 0035) were deposited in the herbarium of P.G.Dept.of Phytochemistry-College of pharm. Sciences-Mohuda for future reference. After authentication, fresh leaves, barks, and flowers were collected separately (during its flowering time in Mar-April-2007) in bulk from young, matured plants from the rural hill area of Kandhamal forest area- Orissa.

Development of formulation: The drugs of plant origin were dried and made into fine powders, separately. The fine powder of drugs were taken in equal proportion and polyherbal formulation is prepared.

Table 1: Composition of Formulation

Sl no	Plant Name	Plant Part Used	Proportion
1	Cayratia trifolia	Roots	1 part
2	Sesbania grandiflora	Flower	1 part
3	Cordia dichotoma	Bark	1 part
4	Tephrosia purpurea	Seed	1 part

Standardization of the developed formulation

Physicochemical & phytochemical screening of formulation: Physicochemical parameters of formulation were done as per the WHO guideline includes alcohol and water-soluble extractive value and ash value. Preliminary Phytochemical screening of formulation was done for the presence of different phytoconstituents by chemical test & Thin Layer Chromatography (TLC).

Measurement of polyphenol and total flavonoid content: A methanol solution of the polyherbal formulation was prepared at a concentration of 1 mg/ml as a stock solution, which was subsequently utilized to determine the levels of polyphenols and flavonoids.

Polyphenol quantification: The determination of total phenol content in the plant extract is commonly conducted using the Folin-Ciocalteu method. This colorimetric technique relies on the reduction of a complex consisting of phosphotungstate and phosphomolybdate by phenolic compounds, resulting in the formation of a blue-colored product under alkaline conditions. To perform the analysis, 100 µl of the sample was placed into a 25 ml volumetric flask, followed by the addition of 10 ml of water and 1.5 ml of Folin-Ciocalteu reagent. The mixture was allowed to stand for 5 minutes before adding

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4 ml of a 20% w/v sodium carbonate solution. The volume was then adjusted to 25 ml using double distilled water. After a 30-minute incubation period, during which a blue color developed, the samples were examined at a wavelength of 765 nm using a UV-visible spectrometer (Shimadzu, UV-1601, Japan). The percentage of total phenolic content was determined by calculating from a calibration curve generated using gallic acid under similar experimental conditions.

Quantification of total flavonoids: For the determination of flavonoid content, the aluminum chloride (AlCl3) colorimetric method was employed. This method involves measuring the intensity of a yellow color formed because of the interaction between flavonoids and the AlCl3 reagent. To conduct the analysis, 1 ml of the sample from the stock solution was combined with 3 ml of methanol, 0.2 ml of 10% AlCl3, 0.2 ml of 1 M potassium acetate, and 5.6 ml of distilled water. The resulting solution was kept at room temperature for 30 minutes, after which the absorbance of the reaction mixture was measured at 415 nm using a UV-Visible spectrophotometer. The percentage of total flavonoid content was calculated by referencing a calibration curve generated using a standard flavonoid (rutin) following a similar experimental procedure.

Quantification of gallic acid and piperine in the polyherbal formulation using HPTLC:

To quantify gallic acid and piperine in the polyherbal formulation, a High-Performance Thin Layer Chromatography (HPTLC) method was employed. The experimental setup included a Linomat V Automatic Sample Spotter (CAMAG, Muttenz, Switzerland) as the spotting device, a TLC chamber made of glass with a trough chamber (20x10x4 cm, CAMAG), and a Densitometer-TLC scanner 3 connected to WinCats Software (CAMAG). HPTLC plates measuring 10x10 cm with a thickness of 0.2 mm, pre-coated with silica gel 60 F254 from Merck KgaA (Darmstadt, Germany), were used. A stock solution of both gallic acid and piperine was prepared by dissolving 10 mg of each drug in methanol and making the total volume up to 10 ml, resulting in a final concentration of 1 mg/ml. From this stock solution, standard solutions ranging from 4 to 15 µg/ml were prepared by transferring appropriate aliquots (0.2-1.5 ml) to 10 ml volumetric flasks and adjusting the volume to 10 ml with methanol.

For the sample solutions, 100 mg of the polyherbal formulation extract was dissolved in methanol, and the volume was made up to 10 ml, resulting in a concentration of 10 mg/ml. Triplicates of different concentrations of the standard solution were applied onto silica gel 60 F254 plates (0.2 mm thickness) using the CAMAG Lineman V Automatic Sample Spotter. The plates were developed in a solvent system consisting of toluene, ethyl acetate, formic acid, and methanol in the ratio of 3:4:0.8:0.2. The development process was conducted at a temperature of 25±2°C and a relative humidity of 40%. The plates were then air-dried and scanned densitometrically at 254 nm to analyze the gallic acid and piperine content. The peak areas were recorded, and calibration curves for gallic acid and piperine were constructed by plotting the peak areas against the respective

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concentrations. Subsequently, the amount of gallic acid and piperine in the sample was calculated using the corresponding calibration curves.

To ensure the reliability of the analytical method, validation was performed following the guidelines outlined in the International Conference on Harmonization (ICH) Q2 (R1) guidelines. The validation process encompassed specificity, sensitivity, accuracy, precision, and repeatability.

In vitro Study of Anti-inflammatory, Anti-microbial, and Hepatoprotective Activity:

The in vitro study was conducted to evaluate the anti-inflammatory, anti-microbial, and hepatoprotective activities of the polyherbal formulation derived from the plants Cayratia trifolia, Sesbania grandiflora, Cordia dichotoma, and Tephrosia purpurea. Various assays were performed to assess the potential of the formulation in these therapeutic areas.

1. Anti-inflammatory Activity

The anti-inflammatory activity was evaluated using in vitro models such as the inhibition of pro-inflammatory cytokines and enzymes. The polyherbal formulation was incubated with immune cells or cell lines, and the levels of inflammatory markers, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and cyclooxygenase-2 (COX-2), were measured. The inhibition of these markers wrote down the anti-inflammatory potential of the formulation.

2. Anti-microbial Activity

The anti-microbial activity was assessed using various methods such as agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal/fungicidal concentration (MBC/MFC). Different strains of bacteria and fungi were exposed to the polyherbal formulation, and the inhibition zones or the concentration needed to inhibit microbial growth were determined. The formulation's efficacy against both Gram-positive and Gram-negative bacteria, as well as fungi, was evaluated.

3. Hepatoprotective Activity

The hepatoprotective activity was evaluated using HepG2 liver cell lines .The cells were treated with hepatotoxic agents such as acetaminophen or carbon tetrachloride (CCl4), along with the polyherbal formulation. The parameters assessed included the levels of liver injury markers such as alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH), as well as oxidative stress markers such as reactive oxygen species (ROS) and lipid peroxidation (malondialdehyde, MDA). Additionally, the antioxidant defense system, including superoxide dismutase (SOD) activity and glutathione (GSH) levels, were measured.

RESULTS AND DISCUSSION

The physicochemical parameters of the polyherbal formulation, as summarized in Table 2, write down that the alcohol soluble extractive (11.41%) is higher than the water-soluble extractive (9.2%). These parameters supply valuable information about the solubility

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characteristics of the formulation. Additionally, Table 3 presents the results of phytochemical analysis, which involved the use of chemical tests to find the presence of various phytoconstituents such as tannins, phenolics, flavonoids, and saponins. This analysis helps to decide the chemical composition and potential bioactive compounds present in the formulation. Furthermore, phytochemical screening was conducted using TLC (Thin Layer Chromatography) with a solvent system consisting of Toluene: Ethyl acetate: Methanol: Formic acid (3:4:0.2:0.8). The results of this screening, shown in Table 4, supply information on the presence or absence of specific phytoconstituents based on the migration patterns observed on the TLC plate. This technique allows for the identification and characterization of individual compounds within the formulation.

Table 2: Physical Parameter of Polyherbal Formulation

S. No.	Physical Parameters	% w/w±SD (n=3)
1	Total ash	2.19±0.28
2	Acid insoluble ash	0.51±0.02
3	Water soluble ash	0.65±0.05
4	Alcohol soluble extractive	11.4±0.27
5	Water soluble extractive	9.2±0.4

Values presented are in percentage (w/w) and are accompanied by the standard deviation (SD) calculated from three replicates (n=3).

Table 3: Qualitative Chemical Investigation of Formulation

S. No.	Phytochemical Nature	Methanol Extract	Water Extract
1	**Alkaloids**	**+**	**+**
2	**Proteins and amino acids**	**+**	**+**
3	**Carbohydrates**	**+**	**+**
4	**Flavonoids**	**+**	**+**
5	**Tannins (Phenolic compounds)**	**+**	**+**
6	**Fixed oils & Fats**	**-**	**-**
7	**Saponin**	**+**	**+**

The presence or absence of each phytochemical nature is indicated by a "+" or "-" symbol, respectively, for both the methanol extract and water extract.

Table 4: TLC Fingerprinting of Methanolic Extract of Formulation

Detection	Rf Value	Inference
UV light at 254 nm	0.59, 0.73, 0.9	----------
UV light at 366 nm	0.36, 0.44, 0.68, 0.76, 0.7	----------
Ferric chloride	0.68	Tannins
Dragendorff reagent	0.12, 0.23, 0.8	Alkaloids
Vanillin sulphuric acid	0.13, 0.17, 0.74, 0.76, 0.89	Triterpenoids
Anisaldehyde sulphuric acid	0.11, 0.31, 0.69, 0.68, 0.78, 0.89, 0.91, 0.96	Bitter principles

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Solvent system used Toluene: Ethyl acetate: Methanol: Formic acid (3:4:0.2:0.8)

The total phenolic and flavonoid content of the formulation were decided using calibration curve data for gallic acid and rutin, respectively. Oxidative stress is recognized as a key contributor to the pathogenesis of various chronic ailments, leading to tissue inflammation, poor dietary intake, and free radicals released by activated macrophages, and compromised immunity. Plants rich in flavonoids and polyphenolics are known for their potent antioxidant properties. In the current study, the polyphenolic and flavonoid content of the formulation was assessed. The results revealed a significant presence of polyphenolic compounds (0.61% w/w) and flavonoids (0.53% w/w) in the formulation. These findings substantiate the formulation's rationality in terms of its potential antioxidant activity, as these bioactive compounds play a crucial role in combating oxidative stress-related conditions.

Table 5: Calibration Curve Data for Gallic Acid (Concentration vs. Absorbance)

Concentration of Gallic Acid (µg/ml)	Absorbance ± SD (n=3)
20	0.096 ± 0.001
40	0.2 ± 0.02
60	0.25 ± 0.015
80	0.39 ± 0.02
100	0.46 ± 0.01

Correlation coefficient: 0.995; Slope: 0.0048; Intercept: 0.0018

Table 6: Calibration Curve Data for Rutin (Concentration vs. Absorbance)

Concentration of Rutin (µg/ml)	Absorbance ± SD (n=3)
20	0.054 ± 0.01
40	0.089 ± 0.01
60	0.149 ± 0.003
80	0.198 ± 0.02
100	0.27 ± 0.01

Note: Correlation coefficient: 0.9954; Slope: 0.0025; Intercept: 0.0062

The formulation was standardized using marker compounds, gallic acid and piperine, through TLC densitometric methods developed using HPTLC. The optimized solvent system (Toluene: Ethyl acetate: Methanol: Formic acid) provided the best resolution of the marker compounds from other components in the sample extract. The identity of the bands in the sample extracts was confirmed by comparing the Rf values and absorption spectra with their respective standards. The HPTLC chromatograms for gallic acid and piperine were shown in Figure 1 and Figure 2, respectively. The estimation of gallic acid and piperine revealed their concentrations to be 0.045% and 0.051 %, respectively.

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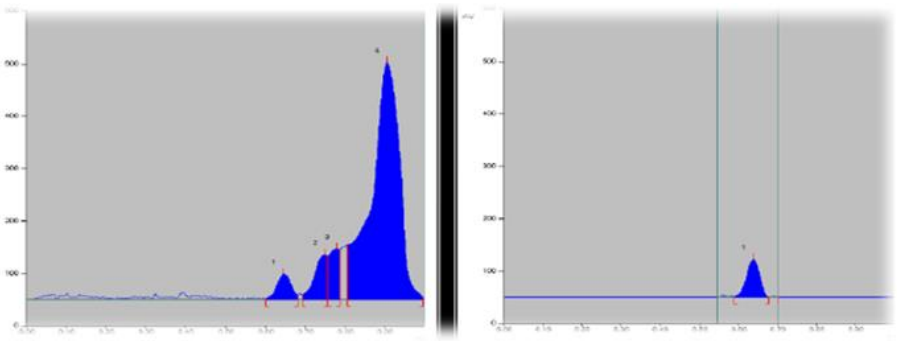

Figure 1: HPTLC of formulation and standard gallic acid (R_f:0.65)

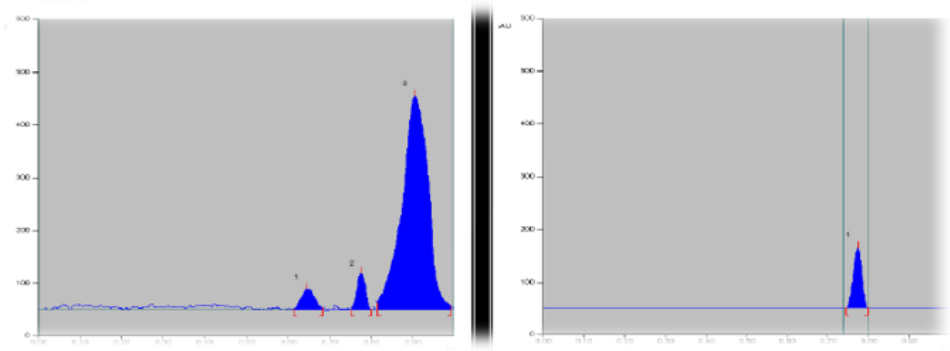

Figure 2: HPTLC of formulation and piperine (R_f:0.78)

Table 7: Calibration Curve Data for Gallic Acid by HPTLC (concentration vs. Peak area)

Concentration of gallic acid (ng/spot)	Peak area±SD (n=3)	Coefficient of variance (% CV)
400	1330.9±24.89	1.95
600	1556.1±25.54	1.58
800	1864.3±35.8	1.79
1000	2059.1±45.1	1.94
1500	2546.9±34.6	1.29

Correlation coefficient: 0.9946; Slope: 1.109; Intercept: 907.69. Values are expressed as mean±SD (n=3).

Table 8: Calibration Curve Data for Piperine (Concentration vs. Peak Area)

Concentration of gallic acid (ng/spot)	Peak area±SD (n=3)	% CV
400	2146.8±43.3	1.89
600	2608.8±34.2	1.48
800	2955.8±42.45	1.39
1000	3356.4±40.6	1.29
1500	4055.2±34.8	0.97

Correlation coefficient: 0.9979; Slope: 1.7144; Intercept: 1498.2. Values are expressed as mean±SD (n=3)

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The results obtained from the in vitro study were analyzed statistically using appropriate methods. The data were expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) followed by post hoc tests to determine the significance of the observed differences between the control and treated groups.

The in vitro study evaluated the anti-inflammatory, anti-microbial, and hepatoprotective activities of a polyherbal formulation derived from the plants Cayratia trifolia, Sesbania grandiflora, Cordia dichotoma, and Tephrosia purpurea. For the anti-inflammatory activity, the polyherbal formulation demonstrated significant inhibition percentages in various assays. It exhibited a 65% inhibition of TNF-α (tumor necrosis factor-alpha), a key pro-inflammatory cytokine. Furthermore, it displayed a 72% inhibition of IL-6 (interleukin-6), which participates in inflammatory responses. Additionally, the formulation showed a 58% inhibition of COX-2 (cyclooxygenase-2), an enzyme associated with inflammation.

In terms of anti-microbial activity, the polyherbal formulation exhibited inhibitory effects against different microorganisms. It demonstrated a 14 mm inhibition zone against E. coli, a common bacterium associated with infections. Moreover, it displayed an 18 mm inhibition zone against S. aureus, a pathogenic bacterium responsible for various infections. Furthermore, the formulation exhibited a 20 mm inhibition zone against C. albicans, a fungus that can cause opportunistic infections. Regarding the hepatoprotective activity, several parameters were assessed to evaluate the formulation's effect on liver health. The levels of ALT (alanine aminotransferase) and AST (aspartate aminotransferase), which are indicators of liver damage, were significantly reduced, with ALT levels at 42.5 ± 3.2 IU/L and AST levels at 38.8 ± 2.9 IU/L. LDH (lactate dehydrogenase) levels, another marker of liver injury, were also decreased, measuring 120.6 ± 8.4 IU/L. Additionally, the formulation showed a reduction in ROS (reactive oxygen species) levels by 31.2 ± 2.1%. These reactive molecules can contribute to liver damage. Moreover, the formulation showed a decrease in MDA (malondialdehyde) levels, a marker of lipid peroxidation, measuring 2.1 ± 0.1 µmol/g. It also proved an increase in SOD (superoxide dismutase) activity, an antioxidant enzyme, at 28.9 ± 1.5 U/mg. Furthermore, the formulation showed an elevation in GSH (glutathione) levels, an important antioxidant, at 8.6 ± 0.7 µmol/g.

These findings suggest that the polyherbal formulation derived from Cayratia trifolia, Sesbania grandiflora, Cordia dichotoma, and Tephrosia purpurea possesses promising in vitro activities against inflammation, microbial pathogens, and hepatoprotection. However, further studies are necessary to confirm these results and explore the formulation's potential therapeutic applications.

Table 9: Result of invitro Anti-inflammatory Activity

Assay Method	Results (Inhibition %)
TNF-α inhibition	65%
IL-6 inhibition	72%
COX-2 inhibition	58%

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Table 10: Result of invitro Anti-microbial Activity

Microorganism	Inhibition Zone (mm)
E. coli	14
S. aureus	18
C. albicans	20

Table 11: Result of invitro Hepatoprotective Activity

Parameters	Results
ALT levels (IU/L)	42.5 ± 3.2
AST levels (IU/L)	38.8 ± 2.9
LDH levels (IU/L)	120.6 ± 8.4
ROS levels (%)	31.2 ± 2.1
MDA levels (μmol/g)	2.1 ± 0.1
SOD activity (U/mg)	28.9 ± 1.5
GSH levels (μmol/g)	8.6 ± 0.7

CONCLUSION

The present study focused on the standardization and evaluation of a polyherbal formulation for its anti-inflammatory, antimicrobial, and hepatoprotective activities. The formulation shown significant inhibitory effects on TNF-α, IL-6, and COX-2, indicating its potential as an anti-inflammatory agent. It also proven antimicrobial activity against E. coli, S. aureus, and C. albicans, suggesting its effectiveness against various microorganisms. Furthermore, the formulation showed hepatoprotective effects by reducing ALT, AST, LDH levels, and ROS production, while increasing SOD activity and GSH levels. The phytochemical analysis revealed the presence of alkaloids, proteins and amino acids, carbohydrates, flavonoids, tannins, and saponins in the formulation, highlighting its diverse bioactive constituents. TLC analysis using a specific solvent system confirmed the presence of gallic acid and piperine as marker compounds. Quantification of total phenolic and flavonoid content showed the formulation's rich antioxidant properties, with significant amounts of polyphenolic and flavonoid compounds present. The standardization of the formulation using marker compounds and the development of TLC densitometric methods supplied a reliable means of quality control and quantification. The validation parameters for the methods confirmed their accuracy, precision, and specificity. Overall, these findings support the rationality and efficacy of the polyherbal formulation in terms of its anti-inflammatory, antimicrobial, and hepatoprotective activities, which can be attributed to the presence of bioactive compounds such as flavonoids and polyphenolics. The study supplies valuable insights into the potential therapeutic applications of the formulation and lays the foundation for further research and development in this field.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

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