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RESEARCH ARTICLE

Quality Assessment and Evaluation of a Novel Polyherbal Formulation for Antioxidant Properties

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Abstract

Background: The World Health Organization is encouraging the use of traditional medicinal plants through herbal formulations in countries around the world. **Aim:** The present investigation was focused on the development and stability study of polyherbal suspension produced from alcoholic extracts of selected medicinal plants. **Methods:** Four suspensions of Polyherbal formulation-A, Polyherbal formulation-B, Polyherbal formulation-C and Polyherbal formulation-D of different concentrations of Sodium Carboxy Methyl Cellulose 0.5%, 1.0%, 1.5 % and 2.0% g respectively formulated and evaluated to accelerated stability for 3 months. **Results:** The polyherbal formulation-1 demonstrated a pleasant appearance and texture, with no alterations observed in sedimentation, flow rate, pH, viscosity, or other physicochemical parameters after stability studies. Quality control assessments, including phytochemical analysis and High-Performance Thin Layer Chromatography, were conducted on the individual plant extracts used in the formulation, revealing the presence of various phytoconstituents. All quality control parameters for the formulated suspension were found to be stable and acceptable. **Conclusion:** In conclusion, the suspension of hydroalcoholic extracts from *Andrographis paniculata, Coriandrum sativum,* and *Azadirachta indica* combined in a therapeutic formulation, appears to be both effective and safe for use. **Major Findings:** The formulation prepared as a suspension can be used to treat liver diseases and control infections caused by microorganisms. It works through a synergistic effect, helping to regulate the organ by combating biological infections effectively.

Keywords: Antimicrobial, Antioxidant, HPTLC, Physicochemical Investigation, Stability Study

1. Introduction

Herbs are a compelling alternative to conventional medications, due to their natural healing properties[1]. When compared to many synthetic pharmaceuticals, herbs frequently have fewer side effects, making them a safer option for those seeking relief from a variety of problems. Individuals can employ polyherbal mixtures to treat numerous underlying health conditions simultaneously[2]. Approximately 25% of the active components are found in plants used as prescribed medicines[3]. Oxidative stress is a major risk factor for the development of certain chronic illnesses. An antioxidant is defined generically as a chemical that delays or prevents oxidative damage to an objective particle. An antioxidant's primary trait is its capacity to trap free radicals[4].

Polyherbal formulations have emerged as promising candidates in modern medicine due to their potential synergistic effects in treating a wide range of health issues[5]. In particular, combining multiple herbal extracts provides a comprehensive approach to healthcare by leveraging the diverse bioactive compounds found in various plant species[6]. This work focuses on the in vitro

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profile of antioxidant and antibacterial characteristics in a polyherbal formulation containing *A. paniculata* (*Kalmegh*), *A. indica* (Neem) and *C. sativum* (*Dhania*) extract.

These specific herbal extracts were chosen based on their documented pharmacological activities and traditional use in treating a variety of health conditions[7,8]. *A. paniculata*, also known as the "King of Bitters," is known for its strong antioxidant and antimicrobial properties[9]. *A. indica*, or neem is well-known for its broad-spectrum antimicrobial activity and immunomodulatory properties[10].

Coriandrum sativum, also known as coriander, is valued for its antioxidant properties[11]. To ensure the quality and efficacy of the polyherbal formulation, several analytical and experimental procedures are applied. The constituent herbal extracts are analysed qualitatively and quantitatively using High-Performance Thin-Layer Chromatography (HPTLC), providing useful information about their chemical makeup[12]. Furthermore, physiochemical investigations, preliminary studies, and stability assessments are carried out to assess formulation properties and shelf life[13].

The pharmacological evaluation of the polyherbal formulation entails determining its antioxidant and antimicrobial activities using established in vitro assays. By analysing the effects of the constituent herbal extracts individually and in combination, this study attempts to find potential synergistic interactions that boost medicinal efficacy[14]. This study, which combines analytical and experimental methods, provides useful insights into the development and evaluation of polyherbal formulations, with implications for their usage in the healthcare and pharmaceutical industries.

2. Materials and Methods

2.1 Investigational Herbs

Coriandrum sativum (Linn.) of the *Umbelliferae* family is a glabrous, sweet-smelling, herbaceous annual plant that is well recognised for its use in jaundice. It is an important component of several hepatoprotective herbal preparations. It is said to have diuretic, carminative, digestive, anthelmintic, antioxidant, and antibacterial properties[15,16]. Various phytoconstituents, fatty acids, essential oils, flavonoids, and sterols have been isolated from different sections of *C. sativum*[17].

A pharmacological study of *A. paniculata (Acanthaceae)* shows hepatoprotective and hepatostimulating activities[18]. Leaves of this plant contain several phytoconstituents like diterpene, lactones, flavonoids, and their glycosides, and several semi-synthesized derivatives suggest it is a potential herb for drug discovery[19,20]. Neem tree (*A. indica*) is used in traditional Indian medicine for its pharmacological properties such as antibacterial, hypoglycemic, anti-ulcer, antimalarial, hepatoprotective, spermicidal, and anti-inflammatory[21].

2.2 Plant Material

The raw materials were procured from Yucca enterprises, Mumbai, Maharashtra. Accurately weighed quality 100 gm of each plant parts were grinded to coarse powder and sieved though mesh no. 60. From each part equal quantity was taken and extracted by solvent apparatus using hydroalcoholic solvent in ratio 50:50 for 72 hours at 55°C. After 72 hours the extract was filtered and concentrated over the rotary evaporator. The crude extract was kept in the dedicator and used for further experimental procedures[22,23].

2.3 Evaluation of Polyherbal Formulation

2.3.1 Preliminary Studies

Samples of plant extract were tested independently to verify the presence of cardiac glycosides, saponins, alkaloids, tannin, phenolic compounds, and carbohydrates using the usual chemical tests[24].

2.3.2 Physico-chemical Investigation of Prepared Polyherbal Formulation

The physicochemical properties of individual components and polyherbal formulation were investigated and represented with standard deviation. Physicochemical assessments such as total ash, water-soluble ash, acid-soluble ash, water-soluble extractive value, and alcohol-soluble extractive value evaluated the loss of drying.

2.3.3 Formulation Development

The polyherbal formulation in the form of suspension contains the hydro-alcoholic extracts of *A. paniculata* (stem), *C. sativum* (fruit), and *A. indica* (leaves) in a ratio of 1:1:1[25]. The oral suspension was made by the trituration method using a suitable suspending agent

Table 1. Composition of polyherbal formulation (F1, F2, F3 and F4)

Ingredients	F1	F2	F3	F4
A. paniculata hydroalcoholic extract	500 mg	500 mg	500 mg	500 mg
B. sativum hydroalcoholic extract	500 mg	500 mg	500 mg	500 mg
A. indica hydroalcoholic extract	500 mg	500 mg	500 mg	500 mg
Tween 80	0.5 g	1.0 g	1.5 g	2.0 g
Sodium CMC	0.5 g	1.0 g	1.5 g	2.0 g
Sodium Saccharin	0.1%	0.1%	0.1%	0.1%
Sodium Benzoate	0.05%	0.07 %	0.1%	0.12%
Lemon oil	0.01%	0.01%	0.01%	0.01%
Purified water (q.s.)	100 ml	100 ml	100 ml	100 ml

Table 2. HPTLC quantification report

Chromatographic conditions			
	A	B	C
Mobile Phase (MP)	Chloroform: Methanol (8 : 0.5 v/v)	Toluene: Ethyl acetate: Formic acid (10: 8: 1 v/v)	Toluene: Ethyl acetate (9 : 1 v/v)
Quantification Wavelength	602 nm	220 nm	585 nm
Spray reagent	Vanillin Sulphuric acid reagent	Vanillin Sulphuric acid reagent	Vanillin Sulphuric acid reagent
Drying Mode, Temp. and Time	TLC Plate Heater Preheated at 100± 5° C for 3 minutes	30 minutes	TLC Plate Heater Preheated at 100± 5°C for 3 minutes

and other excipients[26]. From the result of stability studies, it can be concluded that formulation A was best and that was selected for further studies[25] (Table 1).

2.3.4 Quality Parameters of Polyherbal Formulations

Colour, taste, odour, texture, and other organoleptic characteristics of polyherbal formulations were evaluated, and quality parameters such as pH, sedimentation volume, particle size analysis, and re-dispersibility were studied and recorded for all formulations[27].

2.3.5 Stability Studies

The chemical stability of formulations was evaluated over three months at two different storage temperatures: 25°C/60% Relative Humidity (RH) and 40°C/75% RH. Samples (n=3) were collected at 0 and 90 days. The statistical significance of data differences was determined using analysis of variance followed by Bonferroni's test (GraphPad InStat Demo Version). P-values < 0.05 indicated statistical significance[28,29].

2.3.6 Particle Size Analysis

The distribution of particle size in suspension is an important aspect of its stability. Particle size distribution was carried out by using optical microscopy in dilute suspension.

2.3.7 HPTLC Analysis

10µl of the filtered solution of formulation extract and standard was applied on the HPTLC plate as per the conditions mentioned in Table 2.

2.3.8 Determination of Microbial Limit Test

A microbiological limit test was carried out by I.P. 2018. The polyherbal formulation did not contain microbes such as *Escherichia coli* or *Salmonella*. Aerobic plate counts showed minimal yeasts, moulds, and *Staphylococcus aureus* (<10 CFU/g)[30].

2.3.9 Determination of Heavy Metal

Heavy metals such as lead, cadmium and arsenic were estimated for polyherbal formulation as per the protocol of Inductive Coupled Plasma-Mass Spectrometry (ICP-MS) with an autosampler[31].

2.3.10 In Vitro Antioxidant Activity

2.3.10.1 DPPH Radical Activity

4.3 mg of DPPH was dissolved in 3.3 ml of methanol (covered with aluminum foil), and 150 µl of DPPH solution was added to 3 ml of methanol. The absorbance was measured at 516 nm as a control. Various concentrations of polyherbal formulation were added to a volume of 150 µl using methanol. Each sample

was diluted with methanol up to 3 ml before adding 150 µl of DPPH. After 15 minutes, absorbance was measured at 516 nm using a UV-visible spectrometer with methanol as a blank. The IC_{50} value for polyherbal formulation F_1 was determined[32,33].

2.3.10.2 Superoxide Radical Activity

The reaction mixture included 100 µl of riboflavin solution, 200 µl of EDTA solution, 200 µl of methanol, and 100 µl of NBT solution. The mixture was diluted with phosphate buffer to a volume of 3 ml. Following 5 minutes of illumination, the solution's absorbance was measured at 590 nm using phosphate buffer as a blank. This is interpreted as a control. To prepare the test tubes, 100 µl of riboflavin, 200 µl of EDTA, 200 µl of methanol, and 100 µl of NBT were added to varying amounts of polyherbal formulation. The mixture was then diluted with phosphate buffer up to 3 ml. Absorbance was determined using a UV-visible spectrophotometer after 5 minutes of irradiation at 590 nm. The IC_{50} value polyherbal formulation F1 was determined[34].

3. Results and Discussion

3.1 Formulation Development

A polyherbal suspension was developed using hydro-alcoholic extracts of *A. paniculata, A. indica,* and *C. sativum* in equal proportions. The suspension formulation was prepared using a trituration method with suitable suspending agents and excipients to ensure stability and efficacy.

3.2 Preliminary Studies

Preliminary studies assessed the presence of active constituents in individual plant extracts. Tests for cardiac glycosides, alkaloids, phenolic compounds, and carbohydrates were performed, confirming the presence of these bioactive components in the herbal extracts. Phenolic compounds and flavonoids are reported to have antioxidant and free radical scavenging activity[35,36]. They perform scavenging activity by stabilizing free radicals via their conjugated ring structures and hydroxyl groups[37]. The high quantity of phenolic contents of polyherbal formulation may contribute to its high antioxidant property. The results of preliminary studies are shown in Table 3.

3.3 Physicochemical Investigation

The polyherbal formulation underwent thorough physicochemical evaluation, including assessments of color, odor, taste, particle size, viscosity, pH, and redispersibility. The formulation exhibited desirable characteristics such as greenish-black color, characteristic odor and taste, suitable particle size distribution, optimal pH, and good redispersibility, indicating its stability and suitability for further studies. From the physicochemical asignificant results, and F1 will be employed in additional investigations. The ash content is a criterion to judge the identity and purity of crude drugs[38,39]. A high acid insoluble ash content is indicative of contamination, substitution, adulteration, or carelessness in preparing the samples for marketing. Acid-insoluble ash indicates contamination with silica,

Table 3. Preliminary phytochemical analysis

S. No.	Metabolites	Chemical tests	*A. paniculata*	*C. sativum*	*A. indica*
1	Cardiac glycoside	Keller –Killian test	Positive	Positive	Positive
2	Saponin	Foam test	Positive	Negative	Positive
3	Alkaloids	Dragendroff's test Hager's test	Positive	Negative	Positive
4	Tannins and Phenolic compound	5% $FeCl_3$ solution Acetic acid solution	Positive	Positive	Positive
5	Carbohydrate	Benedict's test Fehling's test	Positive	Positive	Positive
6	Proteins	Biuret test, Millon's test	Positive	Positive	Negative
7	Amino Acids	Ninhydrin test	Negative	Negative	Negative
8	Steroids	Liebermann's reaction	Positive	Negative	Negative

for example. earth and sand[40,41]. Therefore, the low acid insoluble ash content in plant extracts indicates high purity of the drug (Table 4 and 5).

3.4 Stability Studies

Stability tests were carried out under rapid and long-term storage circumstances to determine the formulations chemical stability. Results indicated that the formulation remained stable over 90 days, with minimal changes in sedimentation volume, particle size, viscosity, pH, and redispersibility under various storage conditions.

In polyherbal formulation F1 it was observed that, sedimentation volume ranging from (0.26 ± 0.032), pH (7.29 ± 0.34), viscosity (79.11 ± 2.22), particle size observed around (16.8± 0.68) density at 25°C in gm/ml 1.13 ± 0.2. In polyherbal formulation F2 it was observed that sedimentation volume reduced to ranging from (0.21 ± 0.041) as compared to polyherbal formulation-F1 due to the increase in the concentration of Sodium C.M.C. It also affects the viscosity (120.39 ± 7.07 centipoise), alkaline pH (7.35 ± 0.25) and particle size observed around (18.16 ± 1.14) density at 25°C in gm/ml 1.11 ± 0.19. Polyherbal Formulation F3 formulation appears like brownish- black slightly yellowish-green shade in colour with characteristic odour and texture at room temperature (RT) and 45°C. The suspension had a pleasant appearance and texture at different temperature. Sedimentation volume was observed 0.17 ± 0.041, viscosity was 146.07 ±13.81 centipoise, pH was 7.35 ± 0.72, particle size 17.52 ± 1.36, density at 25°C in gm/ml 1.1 ± 0.13. In polyherbal formulation F4 it was observed that, sedimentation volume ranging from (0.16 ± 0.022), pH (7.30 ± 0.62), viscosity (152.52 ± 3.87), particle size observed around (17.23 ± 0.56) density at 25°C in gm/ml 1.09 ± 0.71. Based on the results of the physicochemical parameters, polyherbal formulation F1 was determined to be the best as all the parameters were withing acceptable limits (Table 6).

Table 4. Physiochemical evaluation of the extracts

Parameters	*AP* extract	*CS* Extract	*AI* extract
Total Ash Value	9.5 ±1.34 %	3.52 ±0.241 %	16.22 ±0.143 %
Acid insoluble Ash	0.37 ± 1.052%	0.58 ± 0.042%	0.63 ± 0.07 %
Water soluble ash	3.48 ± 0.28 %	0.03 ± 1.02 %	3.12 ± 2.041 %
Alcohol soluble extractive value %w/w	12.92 ± 0.824 %	10.71 ± 1.031 %	16.54 ± 1.801 %
Water soluble extractive value %w/w	19.28 ± 0.34 %	18.0 ± 0.521 %	16.42 ± 0.469 %
Loss of Drying (LOD)	4.38 ± 1.034 %	3.55 ± 0.242 %	5.03 ± 0.104 %

Table 5. Physiochemical evaluation of the formulations

Parameters	Formulation 1(F1)	Formulation 2(F2)	Formulation 3 (F3)	Formulation 4(F4)
Organoleptic characters				
Colour	Greenish black	Greenish black	Greenish black	Greenish black
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Slightly bitter	Slightly bitter	Slightly bitter	Slightly bitter
Physicochemical parameters				
Sedimentation volume	0.26 ± 0.032	0.21 ± 0.029	0.17 ± 0.026	0.16 ± 0.022
Particle size (µm)	16.80 ± 0.68	18.16 ± 0.85	17.52 ± 0.92	17.23 ± 0.56
Viscosity (cP)	79.11 ± 2.22	120.39 ± 7.07	146.07 ±7.81	152.52 ± 3.87
pH	7.29 ± 0.34	7.35 ± 0.25	7.35 ± 0.72	7.3 ± 0.62
Redispersibility	Good	Good	Good	Good
Density (25°C) (gm/ml)	1.13 ± 0.20	1.11 ± 0.19	1.10 ± 0.13	1.09 ± 0.08
Flow rate	5 ml/18 sec	5ml/20 sec	5ml/20 sec	5 ml/19 sec

Table 6. Stability studies of formulation F1

S. No.	Parameters	25±2°C and 60 ± 5 % RH (long time study)		40±2°C and 75± 5% RH (accelerated study)	
		t_0	t_{90}	t_0	t_{90}
1	**Sedimentation volume**	0.26 ±0.04	0.24± 0.56	0.26 ±0.04	0.23 ± 0.39
2	**Particle size(µm)**	16.80 ± 0.68	16.47 ± 1.39	16.80 ± 0.68	16.88 ± 1.53
3	**Viscosity(cP)**	79.11 ± 2.22	75.21 ± 2.13	79.11 ± 2.22	71.36 ± 2.13
4	**pH**	7.29 ± 0.34	7.19 ± 0.27	7.29 ± 0.34	6.93 ± 0.24
5	**Re dispersibility**	Good	Good	Good	Good
6	**Density (25°C) (gm/ml)**	1.13 ± 0.20	1.13 ± 0.12	1.13 ± 0.20	1 ± 0.026
7	**Flow rate**	5 ml/18 sec	5 ml/20 sec	5 ml/18 sec	1 ± 0.026
8	**Color**	Greenish black	Greenish black (no color change)	Greenish black	Greenish black (no color change)

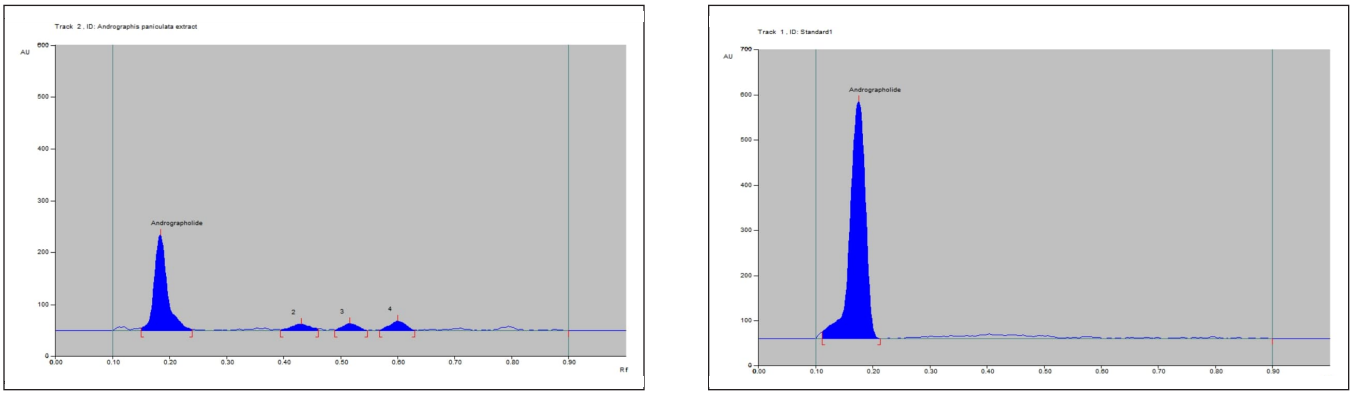


Figure 1. HPTLC chromatograms (2D) of standard (left) and different extracts (right) of andrographolide and Kalmegh extract at 602 nm.

3.5 HPTLC Analysis

The presence of active chemicals in herbal extracts was quantitatively assessed using HPTLC. The chromatographic settings were optimised and the analysis revealed the presence of marker compounds in the extract (Figures 1, 2 and 3).

3.6. Microbial and Heavy Metal Analysis

Heavy elements such as Pb, Cd, and As have not been found in polyherbal formulation. Pb, Cd, and As detection values were very low at 0.5 ppm, 0.05 ppm, and 0.05 ppm, respectively. Microbial limit tests and heavy metal level determinations were performed to confirm the safety of the polyherbal formulation. The results showed that the formulation met safety standards, with minimal microbiological contamination and undetectable quantities of heavy metals.

3.7. *In Vitro* Antioxidant Studies

Pharmacological evaluation of the polyherbal formulation included assessment of antioxidant activity using DPPH and superoxide radical assays. The formulation exhibited significant antioxidant activity, with inhibition percentages comparable to standard antioxidants, indicating its potential therapeutic efficacy (Table 7).

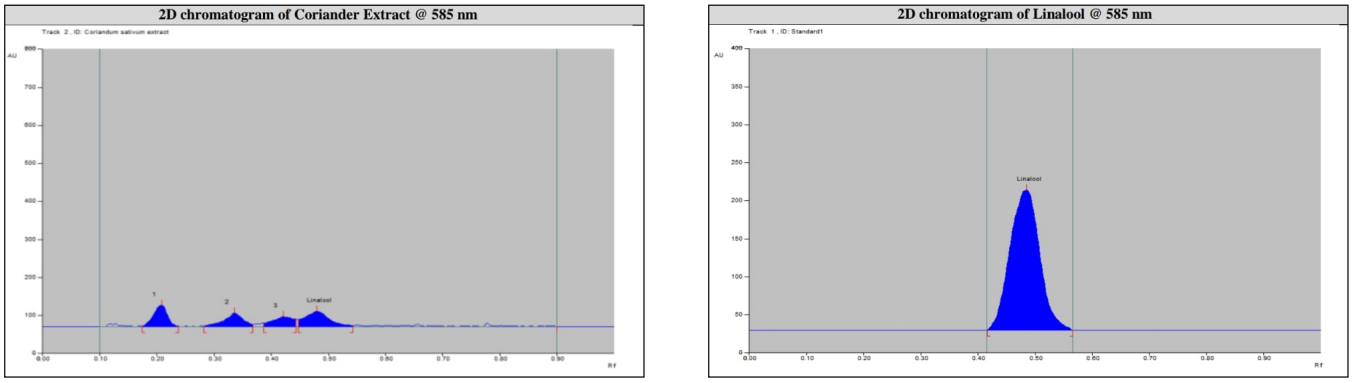


Figure 2. HPTLC chromatograms (2D) of standard (left) and different extracts (right) Linalool and Dhania extract at 585 nm.

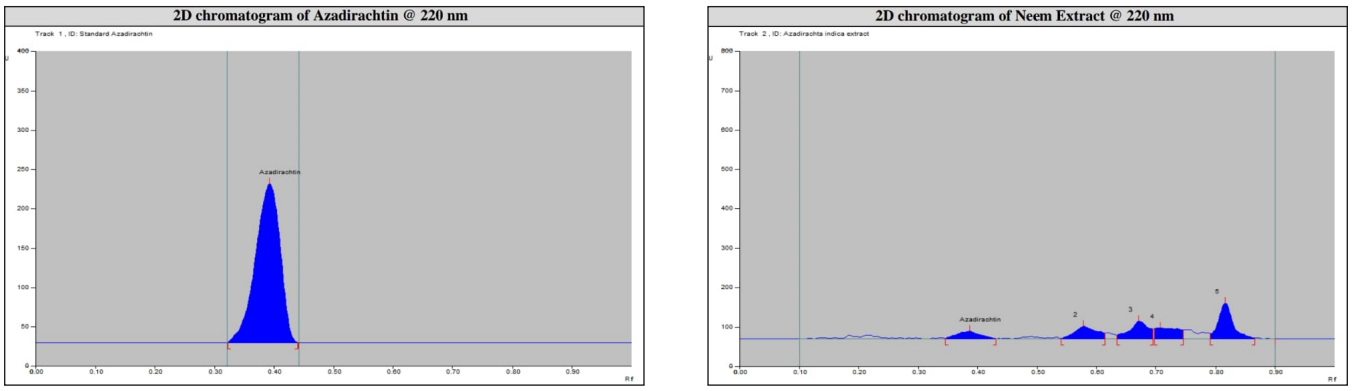


Figure 3. HPTLC chromatograms (2D) of standard (left) and different extracts (right) Azadirachtin and Neem extract at 220 nm.

Table 7. Anti-oxidant activity of polyherbal formulation (F1)

		DPPH radical activity			Superoxide radical activity		
	Concentration (µg/ml)	**% inhibition**	**IC_{50}(µg/ml)**	**R^2**	**% inhibition**	**IC_{50} (µg/ml)**	**R2**
Poly herbal formulation F1	10	20.77	27.303	0.995	16.33	3.593791	0.9971
	20	34.62			28.57		
	30	53.85			42.85		
	40	75.00			57.14		
	50	90.96			67.34		
Standard (Ascorbic Acid)	10	39.58	17.72	0.9921	44.23	4.77	0.990
	20	52.70			56.37		
	30	66.58			65.95		
	40	77.27			74.05		
	50	86.09			93.51		

4. Conclusion

Over the past few decades, researchers have extensively documented the phytochemical components of numerous medicinal plants. Among the most significant bioactive compounds found in these plants are alkaloids, flavonoids, and phenolic compounds. Additionally, antioxidants present in these extracts are known to have protective effects against various diseases, including diabetes, cancer, cardiovascular disease, Alzheimer's, Down's syndrome, Parkinson's disease, and schizophrenia. Given that this polyherbal formulation is rich in antioxidants, it holds great potential for global health benefits. In conclusion, the polyherbal formulation containing extracts of *A. paniculata, A. indica*, and *C. sativum* exhibits significant antioxidant and antimicrobial properties, indicating its potential as a versatile therapeutic agent. Further study is needed to enhance the formulation and validate its efficacy in clinical settings. However, these findings highlight the potential of polyherbal formulations to address complex health issues and improve patient care.

5. Authors Contribution

Zalak Shah: The author has conducted the experimental work; Anand Pithadiya: looked into technical aspects of the work. G. S. Chakraborthy: critically evaluated and analysed the findings and contributed to the scientific formulation and revision of the manuscript, ensuring compliance with the journal's guidelines. All authors have read and agreed to the published version of the manuscript.

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