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Comprehensive Pharmacognostic and Phytochemical Analysis of Select Indian Herbs: In Vitro Anti-oxidant Assessment

Ms. Swati Laxman Khedekar[1] and Dr. Kailash R. Biyani[2]
Anuradha College of Pharmacy, Chikhli, Maharashtra, India
swatikhedekar1990@gmail.com

Abstract: *This groundbreaking study aims to thoroughly investigate specific Indian herbs, including Giloy, Black Pepper, Amla, Ginger, Cinnamon, and Basil leaves, using a systematic evaluation of their medicinal properties and analysis of their chemical components. The study involves the methodical gathering, conservation, and extraction of these herbs using solvents with different polarity to determine their physicochemical properties. The moisture content, ash value, acid-insoluble ash, water-soluble ash, and water and alcohol-soluble extractive values were accurately measured to gain important information about the quality characteristics of these herbs. In addition, initial phytochemical analyses using various identification assays identified a wide range of components in the extracts, including as alkaloids, glycosides, tannins, resins, flavonoids, steroids, amino acids, proteins, carbohydrates, fats & oils, phenols, diterpenes, and saponins. Furthermore, the research examined the antioxidant capabilities of a poly-herbal extract obtained from these herbs in a laboratory setting. It clarified the extract's effectiveness in neutralising free radicals by conducting DPPH and ABTS assays. Significantly, the poly-herbal extract had antioxidant activity that increased in proportion to its concentration, as indicated by larger scavenging percentages at increasing concentrations. The extract also exhibited a significant overall antioxidant capacity, suggesting its potential in counteracting free radicals and addressing oxidative stress. Moreover, this study resulted in the creation of rapidly dissolving tablets enhanced with these herbs, demonstrating a new method for using their immunomodulatory capabilities. This study's findings emphasise the varied phytochemical composition of the chosen Indian herbs, as well as their promising antioxidant capabilities and suitability for medicinal use. This research enables further investigations into the precise bioactive compounds found in these herbs, providing valuable knowledge for their use in pharmacological, nutraceutical, or therapeutic formulations. This contributes significantly to the field of herbal medicine and the development of drugs based on natural products.*

Keywords: Comprehensive pharmacognostic analysis, phytochemical evaluation, antioxidant assessment, Indian herbs, bioactive compounds, medicinal plants, therapeutic potential.

I. INTRODUCTION

The utilization of natural products derived from medicinal plants has garnered significant interest in contemporary pharmacology and healthcare. Indian herbs, renowned for their diverse phytochemical compositions, have been traditionally employed for therapeutic purposes owing to their rich bioactive constituents. The exploration of these botanical resources offers a promising avenue for developing novel pharmaceutical agents and nutraceuticals. However, despite extensive use and historical significance, a comprehensive and integrated investigation into the pharmacognostic profiles, phytochemical content, and antioxidant potential of select Indian herbs remains a critical research gap.

Contemporary scientific advancements advocate for a detailed understanding of the qualitative and quantitative phytochemical constituents within these herbal sources to decipher their therapeutic relevance. Moreover, bridging the gap between traditional knowledge and modern scientific validation can provide insights into their medicinal efficacy, paving the way for innovative formulations and therapeutic interventions. This manuscript aims to address this research

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lacuna by conducting a thorough pharmacognostic assessment and phytochemical analysis of Giloy, Black Pepper, Amla, Ginger, Cinnamon, and Basil leaves.

The amalgamation of traditional wisdom with scientific scrutiny will facilitate a holistic comprehension of these herbal entities, potentially unraveling their pharmacological significance. This investigation endeavors to bridge the existing knowledge gap, providing a scientific foundation for the therapeutic potential of these Indian herbs, and may pave the way for the development of efficacious pharmaceutical formulations with enhanced bioactivity and therapeutic benefits.

II. MATERIALS AND METHODS

Collection and extraction of plant material:

Giloy, black pepper, amla, ginger, cinnamon and basil leaves were collected locally identified under expert guidance and preserved for future reference. The herbs were dried and ground to a very fine powder and subjected to successive extraction by using different solvent in increasing the order of polarity (pet.ether, chloroform and methanol) in soxhlet apparatus until the eluent became colourless. The prepared extract will be concentrated under reduced pressure and stored in air tight container away from direct sunlight.

Extraction

Giloy, black pepper, amla, ginger, cinnamon and basil leaves were collected and dried under the shade condition, crushed with the help of grinder and stored in the airtight container. The dried crushed herbs were weighed and defatted with petroleum ether (60-80 °C) in Soxhlet's extractor. The marc was dried and again extracted with methanol for 72hrs in Soxhlet's extractor. The ethanolic extract was evaporated using rotary evaporator.

Determination of Physicochemical Constants of the powdered herbs

Moisture Content

This is the quantity of moisture present in a plant material. Moisture content of the powdered sample will be determined by loss on drying method. 3.0g each of the powdered sample was accurately weighed and placed in some clean, dried evaporating dishes of known weights. They were placed in an oven and heated at a temperature of 105ºC for 1 hour, then cooled in a desiccator and re-weighed. Heating and weighing were repeated until a constant weight was obtained.

Total Ash Value

2g of powdered plant materials was accurately weighed and placed separately in a crucible of known weight. It was heated gently and the heat gradually increased until it is white indicating the absence of carbon. It was allowed to cool in a desiccator and weighed; this was repeated until a constant weight was obtained.

Acid-insoluble ash

This is the residue that remains after boiling the total ash with dilute hydrochloric acid. This was determined for the powdered plant material. 25ml of dilute hydrochloric acid was added to the crucible containing ash. It was covered with a watch glass and gently boiled for 5mins. The watch glass was rinsed with 5ml of hot water and the liquid added to the crucible. The insoluble matter was collected on an ash less filter-paper and washed with hot water until the filtrate is neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, dried in an oven and ignited to a constant weight

Water soluble ash

To the crucible containing the total ash, 25ml of water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered glass crucible. It was then be washed with hot water and ignited in a crucible for 15 minutes at 105^{o}C. The weight of the residue was subtracted from the weight of the total ash. The content of water soluble ash per air dried powdered sample was calculated and recorded (WHO, 2011).

Alcohol-Soluble Extractive Value

This is the amount of extraction in percentage of a plant sample with alcohol. 4g of each of the plant material was separately weighed in a conical flask. 100ml of ethanol was added and macerated for 24 hours, during which the mixture was frequently shaken within the first 6hours using a mechanical shaker. It was filtered and 25ml of the filtrate transferred into an evaporating dish of known weight and evaporated to dryness on a water bath. It was dried to a constant weight, the percentage of alcohol-soluble extractive value was then determined for the plant.

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Water-Soluble Extractive Value

This is the amount of extraction in percentage of a plant sample with water. Same procedure as in alcohol-soluble extractive value was repeated here for the two plants, but solvent for extraction here was water.

Preliminary Phytochemical Evaluation of herbal Extract

The preliminary phytochemical evaluation of herbal extract involves various tests to determine its presence. Alkaloids are tested using Mayer's Test, Wagner's Test, Raymond's Test, Killer Killani Test, and Legal Test. Glycosides are tested using Raymond's Test, Killer Killani Test, and Legal Test. Carbohydrate tests include Molsch's Test, Benedict's Test, Vanillin-HCl Test, Gelatin Test, Flavanoids Test, Alkaline Reagent Test, Resins Test, Ferric chloride Test, Turbidity Test, Steroids Test, Libermann-Burchard Test, and Salkowski Reaction.

Resins are tested using Ferric chloride Test, Turbidity Test, Libermann-Burchard Test, and Salkowski Reaction. Proteins and amino acids are tested using Biuret Test, Precipitation Test, Ninhydrin Test, Cysteine Test, and Sudan Red Test. Fats are tested using Sudan III stain, Spot test, and Senponification Test. Ferric chloride Test results in a dark blue or greenish black product, while Diterpenes Test shows an emerald green color.

Saponins are tested using Froth Test and Foam Test. Froth Test involves diluted extracts being shaken in a graduated cylinder for 15 minutes, while Foam Test involves shaking 0.5 gm of extract with water for ten minutes. Flavonoids are tested using Lead Acetate Test, Shinoda Test, and Alkaline Reagent Test. Flavanoids are tested using Lead Acetate Test, Alkaline Reagent Test, and Salkowski Reaction. Proteins and amino acids are tested using Biuret Test, Precipitation Test, Ninhydrin Test, and Cysteine Test. Fats are tested using Sudan Red Test, Spot Test, and Senponification Test. Diterpenes are tested using Copper Acetate Test, and saponins are tested using Frost Test and Foam Test.

In summary, the preliminary phytochemical evaluation of herbal extract includes various tests to determine its presence of alkaloids, glycosides, tannins, diterpenes, saponins, and other compounds.

In-Vitro anti-oxidant activity

Determination of DPPH scavenging assay:

The DPPH radical scavenging assay is a method used to evaluate the antioxidant potential of compounds or extracts. It involves preparing a DPPH solution, dissolved test compounds or extracts in solvents, and mixing them with the DPPH solution in separate tubes. The reaction mixtures are incubated in darkness for 30 minutes to an hour, allowing effective interaction between the samples and the DPPH radical. The absorbance of the mixtures is measured at a specific wavelength, and the percentage of DPPH radical scavenging activity is calculated using a formula. A graph plotting scavenging activity against different concentrations helps determine antioxidant activity. The experiment's reliability is ensured by including suitable controls and replicates. The results can be interpreted to understand and compare the antioxidant activities of different samples, contributing to their potential as antioxidants. Adjustments may be necessary based on the specific characteristics of the compounds or extracts under investigation.

% of inhibition = absorbance of control – absorbance of sample / absorbance of control ×100

ABTS radical scavenging assay

The ABTS radical scavenging assay is a method used to evaluate the antioxidant capacity of compounds or extracts. It involves preparing the ABTS radical cation by mixing it with potassium persulfate and incubating it for 12-16 hours until a stable blue-green radical cation forms. The absorbance of the solution is adjusted to around 0.70 at 734 nm. Different concentrations of test compounds or extracts are prepared in appropriate solvents and added to the ABTS radical cation solution. The reaction is then incubated at room temperature for 6-10 minutes. The absorbance of the reaction mixtures is measured at 734 nm using a microplate reader or spectrophotometer. The percentage of ABTS radical scavenging activity is calculated using the formula. The results are interpreted to understand the antioxidant potential of the samples and their concentration-dependent effects on scavenging ABTS radicals.

Total Antioxidant Capacity (TAC)

The Total Antioxidant Capacity (TAC) is determined using assays like FRAP, ORAC, or CUPRAC. To determine TAC in milligrams of ascorbic acid equivalents per gram (mg AAE/g), prepare a FRAP reagent by mixing acetate buffer, TPTZ solution, and FeCl3 solution. Dilute test compounds or extracts in solvents to obtain various concentrations, pipette aliquots into tubes, add the FRAP reagent to each tube containing test samples, and incubate at a constant

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temperature for 30 minutes to an hour. Measure the absorbance of the reaction mixtures at the appropriate wavelength using a microplate reader or spectrophotometer. Calculate the total antioxidant capacity of the test samples using the calibration curve generated from known concentrations of ascorbic acid, which converts absorbance readings into equivalent ascorbic acid concentrations.

III. RESULTS AND DISCUSSION

Determination of Physicochemical Constants of the powdered herbs

The physicochemical constants of powdered herbs, including Giloy, Black Pepper, Amla, Ginger, Cinnamon, and Basil leaves were evaluated and tabulated for analysis. These constants serve as crucial indicators influencing the quality and applicability of these herbal materials across diverse industries.

Table 2: Determination of Physicochemical Constants of the powdered herbs

Parameter	Values (% w/w)					
	Giloy	*Black Pepper*	*Amla*	*Ginger,*	*Cinnamon*	*Basil*
Moisture content	6.25	12.36	5.54	14.77	7.47	11.45
Ash content	7.82	6.20	5.59	8.52	5.65	9.20
Acid in soluble content	6.19	8.55	7.88	8.80	5.45	8.57
Water soluble content	5.34	4.18	5.10	6.21	3.94	4.20
Water extractive value	18.15	16.28	14.27	21.35	17.50	22.70
Ethanol extractive	22.43	24.12	25.73	20.46	21.34	23.28

Moisture content, a pivotal factor affecting shelf life and stability, varied among the herbs, with Giloy and Amla exhibiting lower moisture content (6.25% and 5.54%, respectively) compared to Ginger (14.77%), possibly indicating differing susceptibility to microbial degradation. Ash content, representing the inorganic residue after combustion, showcased diversity among the herbs, with Basil and Giloy having higher ash content (9.20% and 7.82%, respectively), potentially indicating a greater mineral concentration. Acid insoluble content, revealing non-digestible materials, varied notably, with Black Pepper and Ginger showing higher values (8.55% and 8.80%, respectively). The water-soluble content, reflective of dissolved constituents, ranged with Ginger exhibiting a higher value (6.21%). Water extractive values varied, suggesting the potential for diverse extraction capabilities; Ginger and Basil displayed higher extractive values (21.35% and 22.70%, respectively). Ethanol extractive values indicated a greater solubility in ethanol, with Amla and Black Pepper exhibiting higher values (25.73% and 24.12%, respectively). These physicochemical constants collectively elucidate the distinctive chemical compositions and potential applications of these herbs, forming a foundation for selecting suitable extraction methods and understanding their potential bioactive compounds for medicinal or industrial purposes.

Preliminary Phytochemical Evaluation of herbal Extract

The preliminary phytochemical evaluation of herbal extracts involves the identification of various phyto-constituents present in Giloy, Black Pepper, Amla, Ginger, Cinnamon, and Basil, performed through specific identification tests for different classes of compounds.

Table 3: Preliminary Phytochemical Evaluation of herbalExtract

S. No	*Phyto-constituents*	*Identification Test*	*Giloy*	*Black Pepper,*	*Amla*	*Ginger,*	*Cinnamon*	*Basil*
1	***Alkaloids***	*Mayer test*	*++ve*	*++ve*	*++ve*	*+ve*	*-ve*	*-ve*
		Wagner test	*+ve*	*+ve*	*++ve*	*+++ve*	*+++ve*	*+ve*
2	***Glycosides***	*Legal test*	*++ve*	*++ve*	*+ve*	*++ve*	*+ve*	*+ve*
		Libberman buchard test salkowski test	*-ve*	*+ve*	*+ve*	*-ve*	*+ve*	*+ve*
		keller	*+ve*	*+ve*	*+ve*	*+ve*	*+ve*	*+ve*
		killani test	*+ve*	*+ve*	*+ve*	*-ve*	*+ve*	*+ve*

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3	Tannins	Vanillin-HCL test Gelatin test	+ve -ve	-ve -ve	+ve -ve	+ve -ve	-ve +ve	+ve --ve
4	Resins	Turbidity test Ferric- Cl test	-ve -ve	+ve -ve	-ve -ve	-ve -ve	-ve +ve	+ve -ve
5	Flavanoids	Shinoda test Lead acetate test Alkaline test	+ve +ve ++ve	+ve -ve ++ve	+ve -ve ++ve	+ve +ve +ve	+ve -ve +ve	+ve -ve -ve
6	Steroids	Salkowski test Libermann - reaction	-ve +ve	-ve +ve	-ve +ve	-ve +ve	+ve +ve	+ve +ve
7	Amino-acids	Ninhydrin test Cysteine test	-ve -ve	-ve -ve	-ve +ve	-ve -ve	-ve -ve	+ve -ve
8	Proteins	Precipitate test Biuret Test	+ve +ve	+ve +ve	+ve +ve	+ve +ve	+ve +ve	+ve +ve
9	Carbohydrate	Molish test Benedict test	+ve +++ve	+ve +++ve	++ve +++ve	+ve +++ve	+ve +++ve	+ve +++ve
10	Fats & Oil	Sudan red spot test saponificati on test	+ve ++ve +ve	+ve ++ve +ve	+ve ++ve +ve	+ve ++ve +ve	++ve ++ve +ve	+ve ++ve +ve
11	Phenol test	a. ferric chloride test	++ve	++ve	+++ve	+ve	++ve	+ve
12	Diterpens	a. cooper acetate test	+ve	+ve	++ve	+ve	+ve	+ve
13	saponins test	forth test foam test	++ve -ve	++ve -ve	++ve -ve	++ve -ve	++ve -ve	++ve -ve

The preliminary phytochemical evaluation of herbal extracts revealed a diverse spectrum of phyto-constituents across Giloy, Black Pepper, Amla, Ginger, Cinnamon, and Basil. Alkaloids, identified via the Mayer and Wagner tests, exhibited positive outcomes in Giloy, Black Pepper, and Amla extracts. However, Ginger displayed mixed results, while Basil showed negativity in both tests. Glycosides, assessed through Legal, Libermann Buchard, Salkowski, and Keller Killani tests, demonstrated positive outcomes in most extracts except for Black Pepper, which indicated negative results in Legal and Keller Killani tests. Tannins, evaluated using Vanillin-HCl and Gelatin tests, displayed diverse patterns across extracts; Giloy and Cinnamon exhibited positive reactions in Vanillin-HCl and negative in Gelatin, while Amla showed the opposite. Ginger and Basil yielded negative results in both tests. Black Pepper demonstrated positivity in the Ferric Chloride test, indicating a possible presence of resins. Flavonoids showed varying outcomes; Giloy, Amla, and Cinnamon revealed positive reactions in some tests, while Ginger, Basil, and Black Pepper exhibited mixed or negative results. Steroids, analyzed through Salkowski and Libermann-Reaction tests, indicated negative responses in Giloy and Cinnamon, while other extracts displayed varied patterns suggesting the potential presence of steroids. The tests conducted for Amino Acids, Proteins, Carbohydrates, Fats & Oils, Phenols, Diterpenes, and Saponins depicted diversified results among the herbal extracts, delineating the varying presence or absence of these phyto-constituents. These preliminary findings provide a glimpse into the qualitative composition of the herbal extracts, suggesting the potential presence of specific phytochemical compounds that may impact their medicinal or nutritional properties. Further quantitative analysis and characterization of these constituents would offer a more comprehensive understanding of their potential health benefits or applications.

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In-vitro antioxidant activity of poly-herbal extract

DPPH Radical Scavenging assay

The in-vitro antioxidant activity of a poly-herbal extract was assessed using the DPPH radical scavenging assay, presenting its efficacy at various concentrations. The results depicted in Table 4 showcase the percentage of DPPH radical scavenging activity at concentrations of 0.5, 1.5, and 2.5 mg/mL of the poly-herbal extract.

Table 4: DPPH Radical Scavenging (%)

	Concentration (mg/mL)	DPPH Radical Scavenging (%)
Poly herbal extract	0.5	32.21 ± 1.58
	1.5	57.76 ± 2.03
	2.5	70.92 ± 3.15

At 0.5 mg/mL concentration, the extract exhibited a DPPH radical scavenging activity of 32.21% ± 1.58, indicating a moderate antioxidant potential. Subsequently, at higher concentrations of 1.5 mg/mL and 2.5 mg/mL, the scavenging activity notably increased to 57.76% ± 2.03 and 70.92% ± 3.15, respectively, showcasing a dose-dependent improvement in antioxidant efficacy. The escalating trend in radical scavenging activity with increasing concentrations implies a potential concentration-dependent antioxidant effect of the poly-herbal extract, suggesting its capability to neutralize free radicals effectively. These findings suggest the promising antioxidant properties of the poly-herbal extract, particularly at higher concentrations, which could be beneficial for various applications requiring potent antioxidant activity. Further investigations into the extract's specific constituents and their respective contributions to its antioxidant potential could offer deeper insights into its efficacy and suitability for therapeutic or functional purposes.

ABTS radical scavenging assay

The ABTS radical scavenging assay was employed to evaluate the in-vitro antioxidant activity of a poly-herbal extract across various concentrations, as presented in Table 5. At concentrations of 0.5 mg/mL, 1.5 mg/mL, and 2.5 mg/mL, the poly-herbal extract demonstrated escalating ABTS radical scavenging percentages. At 0.5 mg/mL, the extract exhibited a moderate ABTS radical scavenging activity of 41.87% ± 2.17, indicating a notable antioxidant effect. Subsequently, as the concentration increased to 1.5 mg/mL and 2.5 mg/mL, the scavenging activity substantially improved to 67.92% ± 3.46 and 81.15% ± 2.92, respectively.

Table 5: ABTS Radical Scavenging (%)

	Concentration (mg/mL)	ABTS Radical Scavenging (%)
Poly herbal extract	0.5	41.87 ± 2.17
	1.5	67.92 ± 3.46
	2.5	81.15 ± 2.92

This trend illustrates a concentration-dependent increase in antioxidant efficacy, highlighting the extract's ability to effectively neutralize ABTS radicals, further supported by the enhanced scavenging percentages at higher concentrations. These findings underscore the potent antioxidant properties of the poly-herbal extract, particularly at elevated concentrations, suggesting its potential utility in combating oxidative stress and supporting its application in various therapeutic or functional contexts. Further exploration to identify specific constituents contributing to its antioxidant effects would augment the understanding of its efficacy and potential mechanisms of action.

Total Antioxidant Capacity

The results demonstrate a concentration-dependent relationship between the extract's concentration and its total antioxidant capacity. At a concentration of 0.5 mg/mL, the extract exhibited a total antioxidant capacity of 27.65 mg AAE/g ± 1.12, indicating its moderate antioxidant potential.

Table 6: Total Antioxidant Capacity (mg AAE/g)

	Concentration (mg/mL)	Total Antioxidant Capacity (mg AAE/g)
Poly herbal extract	0.5	27.65 ± 1.12
	1.5	41.81 ± 1. 41
	2.5	54.36 ± 2. 76

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Table 6 illustrates the total antioxidant capacity of a poly-herbal extract at varying concentrations, determined in milligrams of ascorbic acid equivalents per gram (mg AAE/g).With an increase in concentration to 1.5 mg/mL and 2.5 mg/mL, there was a notable enhancement in the total antioxidant capacity, reaching values of 41.81 mg AAE/g ± 1.41 and 54.36 mg AAE/g ± 2.76, respectively. This upward trend signifies an escalating antioxidant efficacy with higher concentrations of the poly-herbal extract, implying its ability to neutralize free radicals effectively. The observed concentration-dependent increase underscores the extract's potential for combating oxidative stress and suggests its suitability for applications requiring potent antioxidant properties. Further exploration of the extract's specific constituents and their contribution to its overall antioxidant capacity could provide deeper insights into its mechanisms and support its potential therapeutic or functional uses.

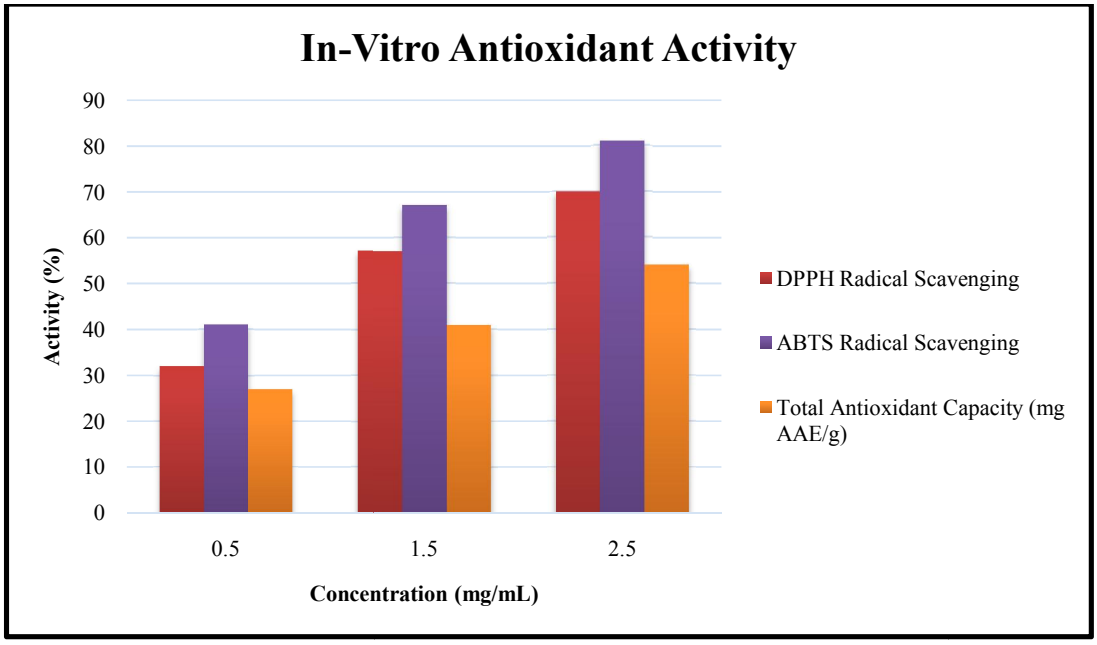

Fig 1: Graphical representation of In-vitro antioxidant activity

IV. CONCLUSION

This groundbreaking study aims to thoroughly investigate specific Indian herbs, including Giloy, Black Pepper, Amla, Ginger, Cinnamon, and Basil, using a systematic evaluation of their medicinal properties and analysis of their chemical components. The study involves the methodical gathering, conservation, and extraction of these herbs using solvents with different polarity to determine their physicochemical properties. The moisture content, ash value, acid-insoluble ash, water-soluble ash, and water and alcohol-soluble extractive values were accurately measured to gain important information about the quality characteristics of these herbs. In addition, initial phytochemical analyses using various identification assays identified a wide range of components in the extracts, including as alkaloids, glycosides, tannins, resins, flavonoids, steroids, amino acids, proteins, carbohydrates, fats & oils, phenols, diterpenes, and saponins. Furthermore, the research examined the antioxidant capabilities of a poly-herbal extract obtained from these herbs in a laboratory setting. It clarified the extract's effectiveness in neutralising free radicals by conducting DPPH and ABTS assays. Significantly, the poly-herbal extract had antioxidant activity that increased in proportion to its concentration, as indicated by larger scavenging percentages at increasing concentrations. The extract also exhibited a significant overall antioxidant capacity, suggesting its potential in counteracting free radicals and addressing oxidative stress. Moreover, this study resulted in the creation of rapidly dissolving tablets enhanced with these herbs, demonstrating a new method for using their immunomodulatory capabilities. This study's findings emphasise the varied phytochemical composition of the chosen Indian herbs, as well as their promising antioxidant capabilities and suitability for medicinal use. This research enables further investigations into the precise bioactive compounds found in these herbs, providing valuable

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knowledge for their use in pharmacological, nutraceutical, or therapeutic formulations. This contributes significantly to the field of herbal medicine and the development of drugs based on natural products.

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