

Exploration of Pharmacognostic Attributes, Phytochemicals Constituents, and In Vitro Antioxidant Activity of Selected Indian Medicinal Plants

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Abstract

The objective of this study is to examine the pharmacognostic properties, antioxidant activity in vitro, phytochemical constituents, and significant Indian medicinal botanicals, such as Tulsi, Turmeric, Ginger, Cinnamon, and Cardamom. After obtaining the plant components from nearby sources, they were desiccated and then ground into minuscule particulates. Subsequently, a sequence of extractions was conducted utilizing Soxhlet machines and solvents progressively gaining polarity, including petroleum ether, chloroform, and methanol. To evaluate the qualitative attributes of the plants, a number of physicochemical constants were calculated: total ash, acid-insoluble ash, water-soluble ash, and extractive values corresponding to alcohol and water solubility. Furthermore, a preliminary examination was undertaken in order to ascertain the existence of a number of phytochemical compounds. The assessment of the herbal extracts' antioxidant activity was performed utilizing DPPH and ABTS radical scavenging assays, in addition to the determination of Total Antioxidant Capacity (TAC). The results revealed that the plants exhibited a diverse array of physicochemical features, indicating that they held different chemical compositions and potential applications. The plant extracts were analyzed using phytochemical analysis, which detected alkaloids, glycosides, tannins, flavonoids, steroids, proteins, carbohydrates, lipids, oils, phenols, diterpenes, and saponins. The in vitro antioxidant experiments confirmed the concentration-dependent antioxidant effectiveness of the polyherbal extract against DPPH and ABTS radicals, indicating its potential for effective scavenging of free radicals. A rise in the concentration of the extract resulted in a conspicuous upsurge in the overall antioxidant capacity, showing a noteworthy potential for antioxidants. This study aims to improve understanding of the antioxidant capabilities, chemical profiles, and quality criteria of the native medicinal plants found in India. By doing this, it lays the foundation for future research and possible practical uses in therapy and product development.

Keywords: Pharmacognostic, phytochemical, antioxidant activity, Indian plants, polyherbal extract, quality parameters, in vitro evaluation, medicinal herbs.

INTRODUCTION:

Herbal formulations have a rich historical background in traditional medicinal practices, spanning numerous cultures, due to their perceived therapeutic advantages and comparatively minimal adverse effects.[1,2]

This has generated a current fascination with the investigation of herbal remedies and their possible implementations in contemporary medicine. Recently, there has been increased interest in polyherbal formulations, which combine multiple plant extracts to exploit their synergistic effects and collective bioactive compounds. [3,4]

The application of polyherbal formulations to the management of inflammatory conditions has garnered significant interest among emerging fields of study, primarily because of the demand for anti-inflammatory agents that are both efficacious and have minimal detrimental effects. A number of distinct botanical extracts

have exhibited intrinsic anti-inflammatory characteristics. These include Tulsi (*Ocimum sanctum*), Turmeric (*Curcuma longa*), Cinnamon (*Cinnamomum verum*), Ginger (*Zingiber officinale*), and Cardamom (*Elettaria cardamomum*). Nevertheless, a significant research void persists regarding the investigation of polyherbal gel formulations that are specifically engineered to elicit anti-inflammatory responses.[4,5]

The current body of literature provides limited and fragmented perspectives on the specific anti-inflammatory properties exhibited by these herbal extracts. Notwithstanding this, there is a conspicuous lack of exhaustive research examining their potential for synergy within an anti-inflammatory gel matrix.[6,7] By combining these potent extracts in the formulation and evaluation of a polyherbal gel, it is possible that a novel therapeutic agent with enhanced anti-inflammatory properties could be produced, superseding the capabilities of each constituent herb. The lack of comprehensive research examining the physicochemical properties, stability, and in vivo anti-inflammatory effectiveness of these polyherbal gel formulations represents a significant shortcoming in present-day scientific pursuits. [8,9]

To determine the viability and therapeutic potential of these polyherbal formulations, a comprehensive investigation is necessary, which should include in-depth physicochemical characterization, in vitro tests, preclinical evaluations, and stability assessments.[10,11]

Therefore, the purpose of this research is to fill the gap in information within the scientific community by conducting a systematic inquiry. The investigation will involve comprehensive examinations, including physicochemical characterization, preliminary phytochemical profiling, in vitro antioxidant assays, and subsequent in vivo anti-inflammatory assessments. This study is expected to address a gap in the existing literature by providing strong scientific evidence that supports the use of these polyherbal gel formulations for managing inflammatory conditions, both in terms of their development and potential clinical application. These findings offer substantial potential to strengthen pharmacological treatments, meet unmet needs in the treatment of inflammation-related illnesses, and minimize unwanted effects. As a result, they contribute to the improvement of current therapeutic techniques.[12]

Experimental procedures and techniques:

The plant materials, including Tulsi, turmeric, cinnamon, ginger, and cardamom, were harvested and extracted. These plants were identified locally under the supervision of an expert and preserved for future use. The desiccated leaves were pulverized into a fine powder and subsequently extracted using a series of solvents in an Otterlet apparatus, with the order of polarity of the solvents increasing from ether to methanol, until the eluent became colorless. The prepared extract will be retained in an airtight container, shielded from direct sunlight, and concentrated under reduced pressure.[13]

Extraction

Tulsi, turmeric, cinnamon, ginger, and cardamom were gathered and allowed to dry in the shade before being ground and sealed in an impermeable receptacle. Soxhlet's extractor was utilized to defatten the desiccated, pulverized plants with petroleum ether at 60-80 °C after weighing them. The marc underwent a second 72-hour extraction with methanol in Soxhlet's extractor after being desiccated. By means of a rotary evaporator, the ethanolic extract was evaporated.[14]

The physicochemical constants of the powdered botanicals were determined.

Moisture Ratio

The value of moisture content that is present in a given plant material. Through loss on drying, the moisture content of the pulverized sample will be ascertained. Each pulverized sample was precisely weighed at 3.0g and subsequently transferred into a set of sterile, dehydrated evaporating containers with known weights. The samples underwent a one-hour heating procedure at a temperature of 105°C inside an oven. Subsequently, they

were cooled in a desiccator and then re-weighed. The procedure of repeatedly heating and weighing was continued until a consistent and unchanging weight was attained.[15]

Ash content

After accurately measuring the weight, an additional 2 grams of the ground plant material was placed into a crucible with a known mass. It experienced a gradual rise in temperature from a low level to a high level, ultimately becoming white, indicating the lack of carbon. The specimen was weighed subsequent to cooling in a desiccator, and this procedure was repeated until a consistent weight was attained.[16]

Ash intractable in acid

This is the residue that remains after dilution hydrochloric acid is used to evaporate the total ash. This information pertained to the pulverized botanical substance. 25 mL of diluted hydrochloric acid was introduced into the ash-filled crucible. The liquid that resulted from cleansing the watch glass for 5ml with heated water was added to the crucible. The insoluble substances were collected on ash-free filter paper, and the filtrate obtained was subjected to rinsing with hot water until it achieved a neutral pH. Reintroducing the filter paper containing the insoluble substance into the initial crucible, desiccated in an oven, and ignited at a constant weight. [17]

Ash soluble in water

Twenty-five milliliters of water were introduced into the crucible containing the complete ash, and the mixture was brought to a simmer for five minutes. The intractable substance was gathered within a crucible made of sintered glass. After being rinsed with heated water, it was ignited in a crucible at 105oC for 15 minutes. A deduction of the residual quantity was applied to the total weight of ash. The quantification and recording of the water-soluble ash content per air-dried powder sample were performed. [18]

Value of Alcohol-Soluble Extract

This value signifies the proportion of a botanical specimen that underwent alcohol extraction. An individual 4g of each of the plant materials was weighed in a conical vial. Following the addition of 100 mL of ethanol and 24 hours of maceration, the mixture underwent vigorous agitation for the initial six hours using a mechanical agitator. After filtration, 25 mL of the filtrate was transferred to an evaporating vessel of known weight and desiccated in a water bath. The plant's percentage of alcohol-soluble extractive value was subsequently calculated after it had been desiccated to a constant weight.[19]

Preliminary Phytochemical Evaluation of herbalExtract

A variety of assays are employed during the preliminary phytochemical analysis of herbal extract to determine the presence of the compound. Alkaloids are examined using the Mayer's Test, Wagner's Test, Raymond's Test, Killer Killani Test, and Legal Test. In order to analyze glycosides, the Legal Test, Killer Killani Test, and Raymond's Test are all applied. [19]

The ferric chloride test, the turbidity test, the libermann-burchard test, and the Salkowski reaction are all utilized to examine resins. The Sudan III stain, spot test, and sponification test are utilized to examine fats. The product of the ferric chloride test is dark blue or greenish-black in color, whereas the Diterpenes Test yields an emerald green hue.[19]

Saponins are evaluated by means of the Froth and Foam Tests. In a graduated cylinder, attenuated extracts are agitated for 15 minutes during the Froth Test, whereas 0.5 grams of extract mixed with water is shaken for ten minutes during the Foam Test. In order to analyze lipids, the Sudan Red Test, Spot Test, and Senponification Test are applied. The assessment of saponins is conducted via the Frost Test and Foam Test, whereas the evaluation of diterpenes is conducted via the Copper Acetate Test.[20].

In-Vitro anti-oxidant activity

Determination of DPPH scavenging assay:

The DPPH radical scavenging assay is a method that is employed to evaluate the antioxidant potential of compounds or extracts. Following the preparation of the DPPH solution, the methodology involves the amalgamation of dissolved test compounds or extracts in solvents with the DPPH solution in distinct containers. By subjecting the reaction mixtures to darkness for a duration of 30 minutes to one hour, the DPPH radical is able to facilitate efficient interaction with the samples. The quantification of the compounds' absorbance at a specific wavelength is performed, and the percentage of DPPH radical scavenging activity is calculated using a formula. Antioxidant activity is discernible with the aid of a graph that illustrates scavenging activity versus concentration. To assure the reliability of the experiment, appropriate controls and replicates are incorporated. The results can be interpreted to understand and compare the antioxidant activities of difference.

The outcomes may be analyzed in order to comprehend and contrast the antioxidant capacities of various specimens, thereby enhancing their potential as antioxidant compounds. Potential adjustments might be required in consideration of the particular attributes of the extracts or compounds being studied.[21]

ABTS assay for radical scavenging

The ABTS radical scavenging assay is a method by which the antioxidant capacity of compounds or extracts is evaluated. Incubate the ABTS radical cation with potassium persulfate for a duration of 12 to 16 hours, or until the formation of a stable blue-green radical cation occurs. At 734 nanometers, the absorbance of the solution is modified to approximately 0.70. In suitable solvents, various concentrations of test compounds or extracts are dissolved before being added to the ABTS radical cation solution. The reaction is then incubated for 6 to 10 minutes at ambient temperature. At 734 nm, the absorbance of the reaction mixtures is quantified by means of a spectrophotometer or microplate reader. Using the formula, the percentage of ABTS radical scavenging activity is determined. The results are interpreted so as to fathom the antioxidant capacity of the samples and their concentration-dependent effects on ABTS radical scavenging. [22]

Total antioxidant capacity is denoted as TAC.

To determine the Total Antioxidant Capacity (TAC), various assays are employed, including FRAP, ORAC, and CUPRAC. To determine TAC in milligrams of ascorbic acid equivalents per gram (mg AAE/g), prepare a FRAP reagent by combining acetate buffer, TPTZ solution, and FeCl₃ solution. To achieve different concentrations, dilute test compounds or extracts in solvents. Transfer aliquots to tubes using a pipette. Subsequently, add the FRAP reagent to each tube containing test samples. Maintain the incubation temperature constant for a duration of 30 minutes to one hour. The absorbance of the reaction mixtures should be determined using a microplate reader or spectrophotometer at the designated wavelength. Utilize known concentrations of ascorbic acid and the calibration curve, which converts absorbance measurements to equivalent ascorbic acid concentrations, in order to ascertain the overall antioxidant capacity of the test samples. [23]

RESULTS AND DISCUSSION:

The physicochemical constants of the powdered botanicals were determined.

For analysis, the physicochemical constants of the following pulverized herbs were determined: basil, black pepper, giloy, amla, ginger, cinnamon, and cinnamon.

Table 2: Determination of Physicochemical Constants of the powdered herbs

Parameter	Values (% w/w)					
	Tulsi	Termeric	Amla	Cinnamon	Ginger	Cardmom
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 insoluble 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 herbalExtract

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	Tulsi	Termeric	Amla	Cinnamon	Ginger	Cardmom
1	Alkaloids	a. Mayer test b. Wagner test	++ve +ve	++ve +ve	++ve ++ve	+ve +++ve	-ve +++ve	-ve +ve

2	Glycosides	a. Legal test	++ve	++ve	+ve	++ve	+ve	+ve
		b. Libbermanb uchard test	-ve	+ve	+ve	-ve	+ve	+ve
		c. salkowski test	+ve	+ve	+ve	+ve	+ve	+ve
		d. keller killani test	+ve	+ve	+ve	-ve	+ve	+ve
3	Tannins	a. Vanillin- HCL test	+ve	-ve	+ve	+ve	-ve	+ve
		b. Gelatin test	-ve	-ve	-ve	-ve	+ve	--ve
4	Flavanoids	a. Shinoda test	+ve	+ve	+ve	+ve	+ve	+ve
		b. Lead acetate test	+ve	-ve	-ve	+ve	-ve	-ve
		c. Alkaline test	++ve	++ve	++ve	+ve	+ve	-ve
5	Steroids	a. Salkowski test	-ve	-ve	-ve	-ve	+ve	+ve
		b. Libermann - reaction	+ve	+ve	+ve	+ve	+ve	+ve
6	Amino- acids	a. Ninhydrin test	-ve	-ve	-ve	-ve	-ve	+ve
		b. Cysteine test	-ve	-ve	+ve	-ve	-ve	-ve
7	Proteins	a. Precipitate test	+ve	+ve	+ve	+ve	+ve	+ve
		b. Biuret Test	+ve	+ve	+ve	+ve	+ve	+ve
8	Carbohydra te	a. Molish test	+ve	+ve	++ve	+ve	+ve	+ve
		b. Benedict test	+++ve	+++ve	+++ve	+++ve	+++ve	+++ve
9	Phenol test	a. ferric chloride test	++ve	++ve	+++ve	+ve	++ve	+ve
10	Diterpens	a. cooper acetate test	+ve	+ve	++ve	+ve	+ve	+ve
11	saponins test	a. forth test	++ve	++ve	++ve	++ve	++ve	++ve
		b. foam test	-ve	-ve	-ve	-ve	-ve	-ve

The antioxidant activity of a polyherbal extract in vitro, as determined by DPPH radical scavenging

In order to evaluate the antioxidant capacity of a poly-herbal extract in vitro, the DPPH radical scavenging assay was employed to assess its efficacy at different concentrations. The percentages of DPPH radical scavenging activity for the poly-herbal extract at concentrations of 0.5, 1.5, and 2.5 mg/mL are shown in Table 4.

Table 4: Radical DPPH Scavenging (percent)

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

At a concentration of 0.5 mg/mL, the DPPH radical scavenging activity of the extract was quantified to be 32.21% ± 1.58, suggesting a moderate antioxidant capacity. Following this, the scavenging activity significantly increased to 70.92% ± 3.15 and 57.76% ± 2.03 at concentrations of 1.5 mg/mL and 2.5 mg/mL, respectively, indicating that the antioxidant's efficacy increased in a dose-dependent manner. 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 assay for radical scavenging

As shown in Table 5, the in-vitro antioxidant activity of a polyherbal extract at different concentrations was determined using the ABTS radical scavenging assay. The poly-herbal extract exhibited a progressive increase in the percentage of ABTS radical scavenging as the concentrations increased (0.5 mg/mL, 1.5 mg/mL, and 2.5 mg/mL). The extract demonstrated a moderate ABTS radical scavenging activity of 41.87% ± 2.17 at a concentration of 0.5 mg/mL, suggesting a significant antioxidant impact. Following this, there was a significant rise in the scavenging activity as the concentrations increased to 1.5 mg/mL and 2.5 mg/mL, representing increases of 81.15% ± 2.92 and 67.92% ± 3.46, respectively.

Table 5: ABTS Radical Scavenging (%)

	Concentration (mg/mL)	ABTS Radical Scavenging (%)
<i>Poly herbal extract</i>	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 correlation between the concentration of the extract and its overall antioxidant capacity. The extract exhibited a moderate level of antioxidant potential; at a concentration of 0.5 mg/mL, it possessed a total antioxidant capacity of 27.65 mg AAE/g $\pm$ 1.12.

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

	Concentration (mg/mL)	Capacity of total antioxidants (mg AAE/g)
<i>Poly herbal extract</i>	0.5	27.65 $\pm$ 1.12
	1.5	41.81 $\pm$ 1.41
	2.5	54.36 $\pm$ 2.76

The findings indicate that the concentration of the extract correlates positively with its overall antioxidant capacity. The extract demonstrated a moderate degree of antioxidant capacity, with a total antioxidant capacity of 27.65 mg AAE/g $\pm$ 1.12 at a concentration of 0.5 mg/mL. 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.

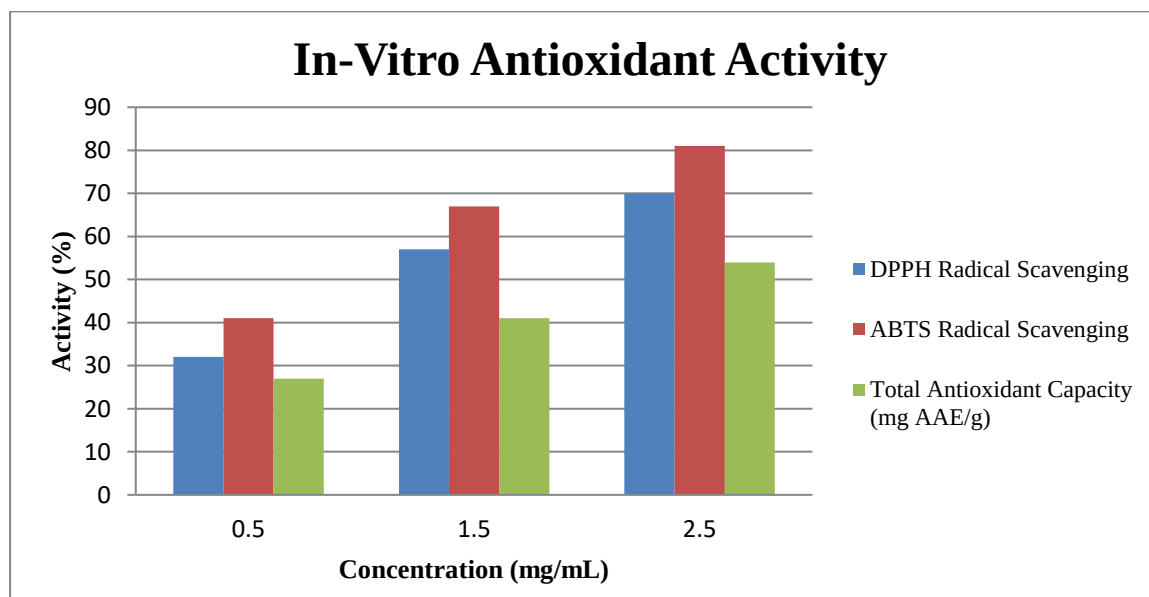


Fig 1: Visual depiction of the antioxidant activity measured in vitro

Conclusion:

A comprehensive investigation was undertaken by the researchers to analyze the pharmacognostic parameters, antioxidant capacity, and phytochemical components of polyherbal extracts derived from indigenous medicinal plants in India. The findings illuminated the prospective therapeutic uses of Tulsi, Turmeric, Cinnamon, Ginger, and Cardamom extracts by showcasing their varied physicochemical properties and bioactive constituents. The polyherbal extract exhibited noteworthy scavenging capabilities against DPPH and ABTS radicals in in vitro antioxidant assays, thereby indicating its potential as a natural antioxidant. The results establish a strong foundation for subsequent investigations that aim to identify the precise bioactive compounds that are accountable for the observed antioxidant effects, investigate the underlying mechanisms of their effectiveness, and conduct in vivo trials to determine their therapeutic merit. Furthermore, the creation of original combinations of therapies, dosage forms, and formulations utilizing these polyherbal extracts may facilitate the development of groundbreaking pharmaceuticals that target a wide range of health conditions associated with inflammation and oxidative stress. Further research and clinical trials are crucial in order to authenticate these results and advance the integration of these natural remedies into conventional healthcare procedures, potentially presenting more secure and efficacious options for the management of diseases.

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